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Influence of distillery effluent on germination and growth of mung bean (*Vigna radiata*) seeds

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Abstract

Distillery effluent or spent wash discharged as waste water contains various toxic chemicals that can contaminate water and soil and may affect the common crops if used for agricultural irrigation. Toxic nature of distillery effluent is due to the presence of high amounts of organic and inorganic chemical loads and its high-acidic pH. Experimental effects of untreated (Raw) distillery effluent, discharged from a distillery unit (based on fermentation of alcohol from sugarcane molasses), and the post-treatment effluent from the outlet of conventional anaerobic treatment plant (Treated effluent) of the distillery unit were studied in mung bean (*Vigna radiata*, L.R. Wilczek). Mung bean is a commonly used legume crop in India and its neighboring countries. Mung bean seeds were presoaked for 6 h and 30 h, respectively, in different concentrations (5–20%, v/v) of each effluent and germination, growth characters, and seedling membrane enzymes and constituents were investigated. Results revealed that the leaching of carbohydrates and proteins (solute efflux) were much higher in case of untreated effluent and were also dependent to the presoaking time. Other germination characters including percentage of germination, speed of germination index, vigor index and length of root and embryonic axis revealed significant concentration-dependent decline in untreated effluent. Evaluation of seedlings membrane transport enzymes and structural constituents (hexose, sialic acid and phospholipids) following 6 h presoaking of seeds revealed concentration-dependent decline, which were much less in treated effluent as compared to the untreated effluent. Treated effluent up to 10% (v/v) concentration reflected low-observed adverse effect levels.

Keywords: Distillery effluent; Mung bean; Germination; Membrane; Enzymes

1. Introduction

Increasing number of distilleries in India has resulted into substantial increase in industrial pollutant load. Most of these distillery units are based on fermentation of alcohol from sugarcane molasses. Untreated (Raw) distillery effluent or spent wash are well known to cause pollution in the natural streams by lowering of pH value, increase in organic load, depletion of oxygen content, discoloration and destruction of aquatic life. It has also been shown that untreated (Raw) distillery effluent contains excess amounts of organic and inorganic loading and has high-acidic pH [1,2]. It can pose serious threats on the welfare and health of the adjoining aquatic and terrestrial habitats, if discharged inadvertently or advertently, into nearby water courses or used for irrigation purpose [3]. Therefore, conventional anaerobic treatment of raw distillery effluent is essential before its release into the environment. Distillery effluent contains many constituents, which are phyto-toxic at higher concentration to plants. However, some of the constituents at lower concentrations are also beneficial for growth and development of plants. The effect of different concentrations of distillery effluent on germination of three multi-useful tree species (Areca/Betel nut (*Acacia catechu*), Shisham (*Dalbergia sissoo*) and Mulberry (*Morus alba*) which grow abundantly under tropical and subtropical climatic conditions) were found to enhance the germination at low-effluent concentrations. However, higher concentrations (>10%) were found to inhibit the germination [4]. Deleterious effects on the growth and productivity of Mung bean or Green gram (*Vigna radiata*) crop have been assessed with higher concentrations of distillery effluent [5]. Distillery effluents concentration-dependent decrease in germination of maize

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(*Zea mays*), black gram (*Vigna mungo*), green gram/mung bean (*V. radiata*), pigeon pea (*Cajanus cajan*), soy bean (*Glycine max*) and chick pea or Bengal gram (*Cicer arietinum*) have also been reported [6]. Mung bean (*V. radiata*) is a common and widely cultivated legume crop in tropical countries including India. Studies under various stress conditions using mung bean as a model have been documented [7–11]. The present study investigates comparative effects of untreated (Raw) distillery effluent discharged as waste water, and that of the post-treated distillery effluent (from the outlet of conventional anaerobic treatment plant of the distillery unit) on seed germination, growth characteristics and seedlings membrane enzymes and constituents of mung bean.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (A-7638) and mannose (M-6020) were purchased from Sigma, St. Louis, MO, USA; *p*-nitro phenyl phosphate (144816) and ATP (014014) were purchased from SRL, Mumbai, India. Other chemicals used were of Analytical grade, purchased from Sigma–Aldrich, New Delhi, India or Qualigens Fine Chemicals, Mumbai, India.

2.2. Collection of distillery effluents

Distillery effluent or spent wash discharged from a distillery unit (hereby termed as untreated effluent) and the post-treatment effluent from the outlet of conventional anaerobic treatment plant of the distillery unit (hereby termed as treated effluent) were collected from Unnao Distilleries, Unnao (Uttar Pradesh), India. This industrial distillery unit is based on fermentation of alcohol from sugarcane molasses. Untreated and treated effluents were freshly diluted to 5%, 10% and 20% (v/v) with distilled water for experimental studies. These concentrations were selected on the basis of our preliminary studies showing that 10% (v/v) concentration, in general, declines seed germination up to 50%. Untreated and treated distillery effluents were analysed for different physico-chemical parameters as described in standard methods for examination of waste water and water by American Public Health Association (APHA) [12].

2.3. Germination character studies

Mung bean (*V. radiata*, L.R. Wilczek, Var. K-851) seeds (Catalog No. 244) were procured from State Government Seed Cooperative Store, Lucknow, Uttar Pradesh, India. Twenty-five seeds for each group were pretreated (presoaked) with 10.0 mL each of 5%, 10% and 20% untreated and treated effluents for 6 h and 30 h, respectively. Seeds of control group were presoaked in distilled water. Following presoaking, it was filtered through a single layer of cheese cloth (Garaha Bhandhar, Lucknow, India) and the seeds were collected. The supernatant containing seed diffusates were centrifuged (Remi Equipments Model R8C, Bombay, India) at 1000 rpm for 5 min to remove the debris, if any and the final supernatant (leachates) was collected. Carbohydrate and protein contents of different concentration of effluents and respective leachates were analysed separately. Seeds were allowed to germinate at 25 ± 2 °C on Petri dishes of 9 cm diameter containing a double layer Whatman No. 1 filter paper and irrigated with respective concentrations of effluents [13]. Germination percentage and length of embryonic axis and root were measured after every 24 h interval for 7 days. The speed or duration of germination index was calculated according to the method of Carley and Watson [14] and germination vigor index by paper towel method of Abdul-Baki and Anderson [15]. Membrane fraction of seedlings, following 6 h presoaking of seeds, was prepared according to the method described by Hodges and Leonard [16].

2.4. Biochemical estimations

Alkaline phosphatase (EC 3.1.3.1) was determined according to Weiser [17] and Ca^{2+}–Mg^{2+}-ATPase (EC 3.6.1.3) as described by Hidalgo et al. [18]. Enzyme units were defined as micromoles of product formed or liberated per minute under the assay conditions. Specific activity was expressed as units per milligram protein. Protein was determined according to Lowry et al. [19] using bovine serum albumin as standard. Carbohydrates (total hexose) were estimated by the anthrone reagent method [20]. For the estimation of sialic acid content, samples were hydrolysed with 0.1N H_2SO_4 at 80 °C for 1 h and color was developed according to the method of Warren [21] using *N*-acetyl neurominic acid as standard. In order to quantitate phospholipids, total lipids were extracted according to Folch et al. [22] and digested in the presence of 70% perchloric acid (PCA) in a sand bath brought up to 180 °C. Phospholipids were quantified according to the method of Wagner et al. [23].

2.5. Statistical analysis

The results were expressed as mean ± S.D. and comparisons were made with appropriate controls employing Student's *t*-test. Probability values of less than 0.05 were considered significant. Analysis of variance (ANOVA) was performed considering untreated and treated effluents with various concentrations as independent variables using statistical software SYSTAT 9.0 (SPSS, Chicago, IL, USA).

3. Results

The physico-chemical parameters of untreated and treated distillery effluents are shown in Table 1. The color of untreated effluent was dark brown, whereas it was light brown when collected from the outlet of Treatment plant. The pH of untreated effluent was acidic, whereas it was slightly alkaline in case of treated effluent. The other physico-chemical parameters of untreated effluent had higher values than the standard permissible limits as prescribed by Central Pollution Control Board of India. In case of treated effluent, the values were within the permissible limits.

Table 1
Physico-chemical parameters of untreated and treated distillery effluents

Parameters	Untreated distillery effluent	Treated distillery effluent
Color	Dark brown	Light brown
pH	4.5	7.15
Suspended solids	4578	26
Dissolved solids	16,866	150
Total solids	21,444	176
Oil	52	4.5
BOD	7752	28.7
COD	13,824	73.4
Total nitrogen	604.8	12
Chlorides	1300	100
Sulfates	120	12
Phenol	34	10
Phosphates	2.0	0.75
Cadmium	1.2	0.40
Copper	1.71	0.35
Lead	0.48	0.16

Except pH and color, all values are in mg/L.

3.1. Effect of distillery effluents on germination characters of mung bean seeds

The leaching of protein and carbohydrates from mung bean seeds following 6 h and 30 h presoaking with different concentrations of untreated and treated effluents are shown in Table 2. In comparison to the distilled water controls, 30 h pre-treatment revealed a significant concentration-dependent increase in protein and carbohydrate contents. As compared to the treated effluent, the solute effluxes were much higher in case of untreated effluent in all the tested concentrations. On the other hand, 6 h pre-treatment revealed only significant increase in efflux with 20% (v/v) untreated effluent.

Other germination characters such as percentage of germination, speed of germination index and vigor index are shown in Table 3. As compared to distilled water controls, presoaking of seeds for 6 h with untreated effluent resulted in 64%, 36% and 20% germination at 5%, 10% and 20% (v/v) effluent concentrations, respectively. There was no significant decline in percentage of germination when the seeds were presoaked with treated effluents for 6 h. However, only 20% and 8% seeds could germinate following 30 h presoaking in 5% and 10% (v/v) of untreated effluents concentrations, respectively, and no seed germination was observed at 20% (v/v) concentration. Conversely, in case of 30 h presoaking in treated effluents, the percentage of germination in the highest tested concentration (20%, v/v) was 60%. The calculated speed of germination index and vigor index further substantiated these findings.

Length of root and embryonic axis of germinated seeds with various concentrations of untreated and treated effluents are shown in Table 4. Presoaking of seeds with untreated effluent for 6 h significantly reduced the length of embryonic axis and the root in a concentration-dependent manner. Presoaking with 5% (v/v) untreated effluent for 6 h revealed significant reductions of 84% and 73% in the length of embryonic axis and root, respectively. Further marked reductions were evident with tested higher concentrations of untreated effluent. On the other hand, insignificant growth of seedling was observed when the seeds were presoaked for 30 h with all the tested concentrations of untreated effluent (Table 4). In case of treated effluent, the lowest tested concentration of 5% (v/v) did not cause any significant reduction in the overall mean length of embryonic axis and root following presoaking of seeds either for 6 h or 30 h. Simultaneously, there was no significant reduction in the mean length of embryonic axis following 6 h presoaking of seeds in 10% (v/v) treated effluent. However, the mean length of root showed a significant reduction of 78% as compared to the distilled water control. Treated effluent at 20% (v/v) concentration and 6 h presoaking showed significant length reduction of 27% and 87%, in embryonic axis and root, respectively. This indicated that the growth of root is markedly affected with effluent concentrations higher than 5% (v/v). Following germination, it is the root which continuously remains in direct contact with water/effluent; hence, the higher concentration of effluent could affect cell multiplication or the growth. However, a detailed study is needed to elucidate the mechanism. Furthermore, it was observed that higher concentrations of treated effluent following 30 h presoaking of seeds

Table 2
Effect of distillery effluents on solute efflux in mung bean seeds following 6 h and 30 h presoaking

Concentration of effluent (%)	Protein (mg/mL)		Carbohydrate (mg/mL)	
	6 h	30 h	6 h	30 h
Control	0.36 ± 0.030	0.36 ± 0.036	0.51 ± 0.049	0.5 ± 0.04
Untreated effluent				
5.0	0.39 ± 0.04	1.44 ± 0.13*	0.62 ± 0.06	2.64 ± 0.26*
10.0	0.53 ± 0.06	3.20 ± 0.32*	0.75 ± 0.08	3.20 ± 0.32*
20.0	0.73 ± 0.07*	6.00 ± 0.60*	1.02 ± 0.10*	3.84 ± 0.37*
Treated effluent				
5.0	0.38 ± 0.04	0.60 ± 0.06*	0.53 ± 0.05	1.28 ± 0.13*
10.0	0.41 ± 0.04	0.56 ± 0.06*	0.62 ± 0.06	1.32 ± 0.13*
20.0	0.53 ± 0.06	2.92 ± 0.29*	0.70 ± 0.08	2.32 ± 0.23*

Values are mean ± S.D. from three experiments. The amount of carbohydrate and protein present in different concentrations of effluents were deducted to obtain the net values of solute efflux.
* $P < 0.05$ as compared to the respective controls.

Table 3
Effect of distillery effluents on germination parameters of mung bean seedlings

Concentration of effluent (%)	Percentage of germination	Speed of germination index	Vigor index
Presoaking (6 h)			
Control	100 ± 10	594 ± 50	1270 ± 130
Untreated effluent			
5.0	64 ± 8	160 ± 16*	128 ± 13*
10.0	36 ± 5	80 ± 8*	43 ± 5*
20.0	20 ± 4	44 ± 4*	11 ± 2*
Treated effluent			
5.0	100 ± 9	594 ± 50	1250 ± 120
10.0	100 ± 10	594 ± 50	1010 ± 101
20.0	80 ± 9	320 ± 31*	744 ± 75*
Presoaking (30 h)			
Control	100 ± 10	594 ± 50	1270 ± 130
Untreated effluent			
5.0	20 ± 2	116 ± 11*	2.4 ± 0.2*
10.0	8 ± 0.8	20 ± 2*	0.0 ± 0.0
20.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Treated effluent			
5.0	100 ± 10	594 ± 51	1220 ± 126
10.0	84 ± 8.5	506 ± 51	318 ± 31*
20.0	60 ± 6	480 ± 48*	162 ± 17*

Values are mean ± S.D. from three experiments.
* $P < 0.05$ as compared to the respective controls.

caused marked reductions in the overall mean length of both; the embryonic axis and the root (Table 4).

3.2. Effect of distillery effluents on mung bean seedling membrane enzymes and constituents

A concentration-dependent decrease in activities of important membrane transport enzymes namely alkaline phosphatase and Ca^{2+}–Mg^{2+}-ATPase was observed in case of untreated effluent (Table 5). Highest tested concentration of 20% (v/v) showed 88% and 70% decline in alkaline phosphatase and Ca^{2+}–Mg^{2+}-ATPase activities, respectively. However, the same effluent concentration in case of treated effluent caused only 42% and 27% decline in two enzyme activities, respectively. Alkaline phosphatase and Ca^{2+}–Mg^{2+}-ATPase activities at 10% (v/v) concentration of untreated effluent revealed decline of 45% and 37%, respectively. On the other hand, treated effluent at 10% (v/v) concentration showed a decline of only 35% in alkaline phosphatase with no significant inhibition in basal level of Ca^{2+}–Mg^{2+}-ATPase activity. Subsequently, the untreated effluent at 5% (v/v) concentration revealed a decline of 25% in alkaline phosphatase with no significant change in Ca^{2+}–Mg^{2+}-ATPase activity. The exposure of treated effluent at 5% (v/v) concentration had no influence on both enzymes activities (Table 5).

Effect of distillery effluents on membrane structural constituents of mung bean seedlings is shown in Table 6. All the tested concentrations of untreated effluent revealed significant

Table 4
Effect of distillery effluents on the length of embryonic axis and root (cm) of mung bean seedlings ($N = 25$) following pretreatment of seeds for 6 h and 30 h

Effluent concentration (%)	Embryonic axis		Root	
	6 h	30 h	6 h	30 h
0.0	12.7 ± 1.3	12.1 ± 1.2	3.80 ± 0.40	4.2 ± 0.4
Untreated				
5.0	2.0 ± 0.11*	0.12 ± 0.0*	1.01 ± 0.22*	0.0
10.0	1.2 ± 0.10*	0.0	0.63 ± 0.04*	0.0
20.0	0.51 ± 0.10*	0.0	0.31 ± 0.09*	0.0
Treated				
5.0	12.5 ± 0.43	12.2 ± 0.36	3.70 ± 0.12	3.3 ± 0.20
10.0	10.1 ± 0.32	3.78 ± 0.36*	0.84 ± 0.04*	0.65 ± 0.07*
20.0	9.3 ± 0.64*	2.70 ± 0.31*	0.50 ± 0.05*	0.40 ± 0.05*

Values are mean ± S.D. from three separate experiments.
* $P < 0.05$ as compared to the respective controls.

Table 5
Effect of distillery effluents on mung bean seedlings membrane enzymes

Effluent concentration (%)	Specific activity (units/mg protein)			
	Alkaline phosphatase		Ca^{2+}–Mg^{2+}-ATPase	
	Untreated	Treated	Untreated	Treated
0.0	0.570 ± 0.047	0.570 ± 0.047	0.164 ± 0.012	0.164 ± 0.012
5.0	0.424 ± 0.012*	0.474 ± 0.05	0.133 ± 0.012	0.162 ± 0.017
10.0	0.312 ± 0.01*	0.370 ± 0.012*	0.103 ± 0.02*	0.151 ± 0.015
20.0	0.066 ± 0.08*	0.33 ± 0.012*	0.050 ± 0.002*	0.119 ± 0.02*

Values are mean ± S.D. from three experiments.
* $P < 0.05$ as compared to the respective controls.

concentration-dependent decline in total hexose, sialic acid and phospholipids contents compared to their respective controls. Significant decline in membrane constituents at higher concentrations of treated effluent were also noticed. However, in comparison to the untreated effluent, the respective declines were much less. The exposure of 5% (v/v) treated effluent did not cause any marked change in membrane constituents.

4. Discussion

During normal germination in legumes, carbohydrates are formed in excess amount to produce energy for various metabolic activities and for the process of intensive differentiation and growth under relatively steady state conditions [24]. Under the environmental stress conditions, the energy forming molecules may be disturbed and subsequently carbohydrate and protein metabolites of the membrane are altered. Sufficient water absorption is essential for proper seed germination, without which seedling growth and development is severely affected [25,26]. The absorbed water in the seed is used for activation of hydrolytic enzymes, which in turn break the seed reserves in to the simple molecules needed for various metabolic activities including cell division, differentiation and cell elongation [27,28]. These factors may also lead to the reduction in the overall length of shoot and root of seedlings as evident in the present study. The untreated distillery effluent has an acidic pH, high-organic load and heavy metal contents. The presence of most of these constituent of effluent are not compatible with the seedling growth and agricultural requirements in general [29]. Specific effects of distillery effluent on seed germination and growth of some vegetable crops (tomato, chili, bottle gourd, cucumber and onion) have also been reported and the observations revealed that low concentrations of distillery effluent (up to 5%) do not show marked inhibitory effect on seed germination. Furthermore, the reduction in germination and development process in the present study was also found to be dependent to the concentration of the effluent.

Significant influence of untreated distillery effluent even in the lowest tested dilution resulted in higher leaching of carbohydrate and proteins, coupled with low-germination rate, reduced shoot and root length, compared to the treated effluent. During imbibition, seed membrane lipids become transiently leaky. This leakage depends on the water potential, which relies on the organic load of the water. Defects at the boundary level between crystalline to gel phase are responsible for membrane permeability. In seeds, there is a chance of up to 50% leakage of endogenous solutes to which seeds can restore the viability and vigor [30,31]. We also observed that the water potential in untreated distillery effluent was very low as compared to the treated effluent (data not shown). This reflects less water absorption by seeds in case of untreated effluent. The limit of leakage of endogenous solutes from seed imbibition is a major limiting factor of germination, which is due to the defective water potential of water absorbed [32,33]. Furthermore, germination character studies also indicated, that in addition to the dilution of effluent, the duration of mung bean seeds exposure, particularly with untreated distillery effluent, is another important factor for germination and growth. Therefore, proper anaerobic treatment

Table 6
Effect of distillery effluents on mung bean seedlings membrane constituents

Effluent concentration (%)	Hexose (μg/mg protein)	Sialic acid (μg/mg protein)	Phospholipid (μg/mg protein)
0.0	1427 ± 123	13 ± 1.4	16.4 ± 1.5
Untreated			
5.0	630 ± 60* (−55%)	9.9 ± 1.8* (−24%)	12.3 ± 1.3* (−25%)
10.0	548 ± 56* (−62%)	7.4 ± 0.8* (−45%)	9.9 ± 0.9* (−40%)
20.0	297 ± 30* (−79%)	6.7 ± 0.6* (−53%)	7.4 ± 0.8* (−55%)
Treated			
5.0	1430 ± 140	10.9 ± 1.0	13.5±1.2
10.0	809 ± 80* (−43%)	8.4 ± 0.8* (−35%)	10.3 ± 1.0* (−37%)
20.0	703 ± 80* (−51%)	7.7 ± 0.7* (−41%)	9.4 ± 0.9* (−43%)

Values are mean ± S.D. from three experiments. Values in parenthesis are percent decline.
* $P < 0.05$ as compared to the respective controls.

of untreated distillery effluent is necessary to obtain a safe, stable and easily manageable end product.

In order to evaluate the stress conditions caused by distillery effluents, we have studied two membrane bound enzymes, alkaline phosphatase, responsible for phosphorylation; and Ca^{2+}–Mg^{2+}-ATPase, responsible for transport of calcium and its balance inside the cell. In addition, total hexose, sialic acid and phospholipids, responsible for structural integration of membrane and membrane fluidity, were also monitored. Significant concentration-dependent decline in mung bean seedlings membrane transport enzymes and structural constituents reflected the membrane damage, which was much higher in untreated effluent as compared to the treated effluent. The decline in two enzyme activities caused by 20% (v/v) untreated effluent, was abolished by about 50%, consequent to effluent treatment. The data is indicative of the substantial removal of effector substance(s), which caused such an inhibition. Furthermore, the effective load of inhibitory substances in 10% and 5% (v/v) effluents seems to be correspondingly lower vis-à-vis that in 20% (v/v) concentration. This fact is strengthened by only 45% decline in alkaline phosphatase on exposure to 10% (v/v) effluent and only 25% decline on exposure to 5% (v/v) untreated effluent. The inhibitory effect of 10% (v/v) untreated effluent and the ameliorating effect of effluent treatment were also seen on other membrane enzyme Ca^{2+}–Mg^{2+}-ATPase. Treated effluent at 5% (v/v) concentration seems to be relatively safe, not influencing membrane enzymes and membrane structural constituents. These findings correlate well with other germination and growth parameters studied. Alterations in the activity of membrane bound ATPase and plasma membrane constituents under various stress conditions have been reported in *V. radiata* [7,8,34,35].

5. Conclusion

The present study concludes that the untreated distillery effluent is highly toxic for mung bean seed germination and plant growth even at low concentration of 5% (v/v) and may not be suitable for irrigation. On the other hand, treated effluent up to 10% (v/v) concentration reflected low-observed adverse effect levels. Therefore, the treated distillery effluent if used for irrigation of legume crops needs to be diluted over 10 times. Furthermore, it is also necessary to evaluate the quality of crop and levels of heavy metals taken up by plants/pulses.

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