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Kirti Pandita and Piyush Malaviya

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RESEARCH ARTICLE

IMPACT OF COMBINED BREWERY-DISTILLERY EFFLUENT (CBDE) ON GERMINATION, SEEDLING GROWTH AND PIGMENT CONTENT OF *CICER ARIETINUM* L. VAR. GNG26054

Kirti Pandita and Piyush Malaviya*

Department of Environmental Sciences, University of Jammu, Jammu

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ABSTRACT

The aim of the present experiment was to study the impact of different concentrations of untreated, primary treated and secondary treated Combined Brewery-Distillery Effluent (CBDE) on various parameters of seed germination and growth, chlorophyll and carotenoid content of *Cicer arietinum* L. variety GNG26054. The 100% untreated effluent showed very low pH (3.51) and very high COD (5280 mg L^{-1}). The study showed the maximum values of positive germination parameters viz. speed of germination (8), peak value (20), germination index (114.5), percent germination (80) and minimum values of negative germination parameters viz. delay index (3) and percent inhibition (0) at 25% untreated effluent. The maximum values of root length (8.2 cm) and shoot length (12.83 cm) were found at 25% primary treated CBDE whereas, minimum values for root length (0.66 cm) and shoot length (1.5 cm) were found at 50% untreated CBDE. Fresh and dry seedling weight also followed the above trend. The chlorophyll a and b, total chlorophyll and carotenoid content showed a gradual decline with increase in effluent concentration with maximum value at 25% primary treated CBDE.

INTRODUCTION

Environmental pollution constitutes a great health hazard to human, animals and plants (Ali *et al*., 2015). Among the various kinds of pollution, the water pollution is the serious problem in India. Water is at first place in the priority list of basic necessities of life on our planet earth.

The available fresh water to man is hardly 0.3 to 0.5 per cent of the total water (2.4 %) supply on earth and therefore, its judicious use is important. In India there are about 7500 medium to large industrial units, which generate lot of wastewater, which is difficult and costly to treat. Wastewater characteristics and levels of pollutants vary significantly from industry to industry.

Distilleries are one of the 17 most polluting industries listed by the Central Pollution Control Board (CPCB). At present, there are 319 distilleries in India with an installed capacity of 3.29 billion liter of alcohol (Malaviya and Sharma, 2011a). In the distillery industry, for every liter of alcohol produced, about 15 litres of spentwash is released as wastewater. The spentwash is acidic (pH 3.94 - 4.30), dark brown liquid with high BOD and COD and emits abnoxious odour (Mane *et al*., 2006). The wastewater of various industries used for irrigation, spoil the seed germination and seedling growth of various crops but their effects varies from crops to crops.

Various workers have carried out many investigations to find the effect of different types of effluents on germination and growth of different crops (Malaviya and Sharma, 2011a; 2011b; Narain *et al*., 2012; Malaviya *et al*., 2012; Kathirvel, 2012; Rath *et al*. 2013). The present investigation was carried out to monitor the impact of CBDE on the various germination, growth and pigment parameters of *Cicer arietinum* L. variety GNG26054.

MATERIALS AND METHODOLOGY

Seeds of *Cicer arietinum* L. variety GNG26054 were used in the present experiment. The untreated, primary treated and secondary treated CBDE (Combined Brewery Distillery Effluent) used in the present study were collected in pre cleaned containers on weekly basis from M/S Dewans Breweries Ltd. (Brewers and Distillers) located at Talab Tillo, Jammu. The effluent was allowed to settle overnight in a shallow and large trough to minimize the possibility of clogging. Various physiochemical characteristics of the

Corresponding author:* **Piyush Malaviya
Department of Environmental Sciences, University of Jammu, Jammu

effluent samples were analyzed using standard methods (APHA, 1998).

Experimental Set-up and Experiment Process

The experimental set up was designed in the Department of Environmental Sciences, University of Jammu, Jammu. Five treatment sets were made, Set-1 was taken as control in which tap water was used for irrigation and for Set 2, 3, 4 and 5 different concentrations of untreated, primary treated and secondary treated CBDE viz. 25%, 50%, 75% and 100%, respectively were used. Petri plates were prepared by placing sterilized absorbent cotton layer in it. The cotton was moistened with 50 ml of tap water for control and with the same quantity of various concentrations of CBDE effluent (25%, 50%, 75% and 100%) made in tap water. Seeds were treated in antifungal solution and washed thoroughly with distilled water before using for experiment. The ten seeds of *Cicer arietinum* L. variety GNG26054 were sown in the petri plates in triplicate.

The Petri plates were incubated at 25 ± 1^0C in a BOD incubator. Germination was recorded daily at a fixed hour and the emergence of the radical was taken as a criterion of germination. Number of seeds germinated per day was counted until the germination of seeds became constant. Germination Index (GI) was calculated by the formula given by Zucconi *et al*. (1981), Delay Index (DI) by following the methodology modified after Kaushik *et al*. (2005), and Vigour Index by Abdul Baki and Anderson (1973). Similarly, some other parameters like percent inhibition, germination value, peak value, speed of germination time were also estimated using formulae adopted from Rao *et al*. (1993) and Czabator (1962). Root and shoot length were measured by using a scale. Root/ Shoot Ratio were calculated in terms of biomass (dry weight). Pigment content like Chlorophyll a, Chlorophyll b and Total Chlorophyll were measured by the methodology described by Arnon (1949) and carotenoid content was calculated by the equation given by Duxbury and Yentsch (1956).

Effluent Characterization

Various physicochemical characteristics like colour, odour, pH, electrical conductivity, turbidity, total suspended solids (TSS), chemical oxygen demand (COD), chloride, sodium, nitrate andcalcium of untreated, primary treated and secondary treated CBDE used in the present study were compared with CPCB discharge standards (CPCB, 1998). It was found that the colour of untreated effluent was dark brown (1375 CU) whereas, it was light brown (1170.45 CU) in primary treated and almost colourless (1128.79 CU) in secondary treated effluent. The pH of untreated and primary treated effluent was acidic (3.51 and 4.53, respectively) and slightly alkaline (7.46) in secondary treated effluent.

Turbidity of untreated, primary treated and secondary treated effluent was 240 NTU, 057 NTU and 004 NTU, respectively. For untreated effluent, the values of TSS (1899 mg L^{-1}) and COD (5280 mg L^{-1}) exceeded CPCB discharge standards (1998) while Chloride (454 ppm) was found to be within permissible limits. The values of all other parameters were found to be higher in untreated as compared to primary treated effluent and for the secondary treated effluent all the parameters were within the permissible limits except the COD which exceeded the permissible limits of CPCB discharge standards (CPCB, 1998).

Germination parameters

The effects of CBDE (Combined Brewery-Distillery Effluent) on various germination parameters of *Cicer arietinum* L. variety GNG26054 are shown in Table-1. It was observed that maximum values for positive germination parameters like cumulative percent germination (80), peak value (20), germination value (1600) and speed of germination (8) was found at 25% concentration of untreated CBDE while maximum values for vigour index (1320) and germination index (114.5) were found in seeds irrigated with 25% primary treated effluent.

Table 1 Effect of different concentrations of untreated, primary treated and secondary treated CBDE on different germination parameters of *Cicer arietinum* L variety GNG26054.

Treatments	Germination percentage	Peak value	Germination value	Germination index	Speed of germination	Vigour index	Delay index	Percent Inhibition
Control (E_0)	80	25	1600	-	8	1470	-	-
			Untreated Effluent (UE)[1]					
UE_{25}	80	20	1600	24.75	8	404.8	3	0
UE_{50}	40	5.71	206.8	5	2	892	-	40
UE_{75}	0	-	-	-	-	-	-	80
UE_{100}	0	-	-	-	-	-	-	80
			Primary Treated Effluent (PE)[2]					
PE_{25}	70	20	1250	114.4	**7**	1320	3	10
PE_{50}	50	18	1225	17.98	5	245	5	30
PE_{75}	43	17.5	648	12	2	172	5	37
PE_{100}	36	8.6	369.8	-	2	-	-	44
			Secondary Treated Effluent (SE)[3]					
SE_{25}	73	19	1332	37.41	3.5	474.5	6	7
SE_{50}	57	18.25	1083	31.5	6	450.3	5	23
SE_{75}	53	10.6	562.33	25	5	360.4	3	27
SE_{100}	37	9.5	342.5	9.5	4	111	4	43

[1] UE_{25}, UE_{50}, UE_{75}, UE_{100}: 25, 50, 75 and 100% untreated CBDE, respectively
[2] PE_{25}, PE_{50}, PE_{75}, PE_{100}: 25, 50, 75 and 100% primary treated CBDE, respectively
[3] SE_{25}, SE_{50}, SE_{75}, SE_{100}: 25, 50, 75 and 100% secondary treated CBDE, respectively

Table 2 Effect of different concentrations of untreated, primary treated and secondary treated CBDE on morphological parameters (root length, shoot length, fresh wt., dry wt., root-shoot ratio (in terms of dry biomass) of *Cicer arietinum* L variety GNG26054.

Treatments	Root length (cm)	Shoot length (cm)	Plant length (cm)	Fresh seedling wt.(g)	Dry seedling wt.(g)	Dry root wt.(g)	Dry shoot wt.(g)	Root-shoot ratio
Control (E_0)	5.7	10.7	16.5	0.893	0.070	0.007	0.009	0.77
				Untreated Effluent (UE)[1]				
UE_{25}	1.3	3.7	5.06	0.249	0.081	0.001	0.004	0.21
UE_{50}	0.66	1.5	2.2	0.12	0.05	0.001	0.002	0.5
UE_{75}	-	-	-	-	-	-	-	-
UE_{100}	-	-	-	-	-	-	-	-
				Primary Treated Effluent (PE)[2]				
PE_{25}	8.2	12.83	21.03	0.472	0.134	0.010	0.018	0.55
PE_{50}	3.2	1.7	4.9	0.194	0.072	0.003	0.006	0.53
PE_{75}	1.4	2.5	4.03	0.111	0.055	0.003	0.005	0.56
PE_{100}	-	-	-	-	-	-	-	-
				Secondary Treated Effluent (SE)[3]				
SE_{25}	2.6	5.5	8.1	0.257	0.062	0.004	0.006	0.67
SE_{50}	2.5	4.5	6.8	0.188	0.059	0.003	0.004	0.8
SE_{75}	2.5	4	6.5	0.182	0.054	0.004	0.006	0.74
SE_{100}	1.1	2.16	3	0.159	0.055	0.001	0.002	0.66

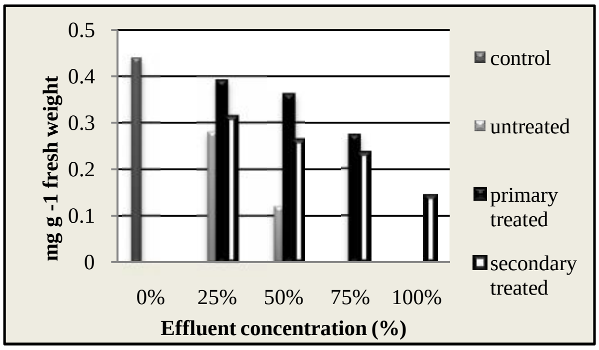
[1] $UE_{25,}$ $UE_{50,}$ UE_{75},$UE_{\ 100}$: 25, 50, 75 and 100% untreated CBDE, respectively
[2] $PE_{25,}$ PE_{50}, PE_{75}, $PE_{\ 100}$: 25, 50, 75 and 100% primary treated CBDE, respectively
[3] $SE_{25,}$ $SE_{50,}$ $SE_{75,}$ $SE_{\ 100}$: 25, 50, 75 and 100% secondary treated CBDE, respectively

The minimum values for all the positive germination parameters were observed at 100% effluent. Similarly, in primary treated and secondary treated CBDE the maximum values for all these parameters were found in 25% effluent concentration which were gradually declined with increase in effluent concentration up to 100%. The results also showed that minimum values for negative germination parameters like delay index (3) and percent inhibition (0) were found in 25% untreated effluent which was found to increase with increase in effluent concentration i.e. the maximum values for these parameters were seen in 100% concentrations of all the effluent treatments.

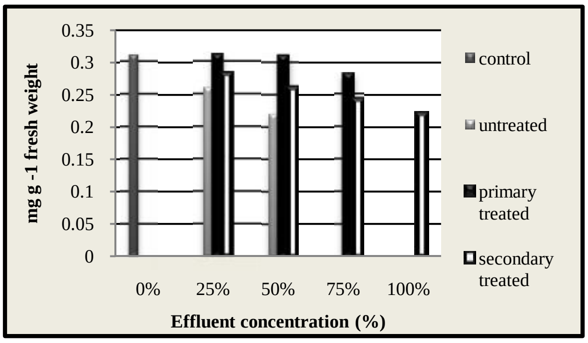
The effect of CBDE on various growth parameters of *Cicer arietinum* L. variety GNG26054 are shown in Table-2 which depicts a decreasing trend with increase in effluent concentration for all parameters (root length and shoot length, fresh and dry seedling weight, dry root and shoot weight and root-shoot ratio).

The maximum values were observed at 25% untreated effluent whereas minimum values were shown at 50% untreated effluent as there was no germination in 75% and 100% untreated effluent concentrations. The maximum values of all these parameters like root length (8.2 cm), shoot length (12.83 cm), fresh seedling weight (0.472 g) and dry seedling weight (0.134 g) were found in 25% primary treated effluent which were higher than the values shown by 25% secondary treated effluent. The minimum values of all these parameters were shown by seedlings irrigated with 75% primary treated effluent as there was no germination and seedling growth in 100% primary treated effluent concentration.

The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations are shown in the Figure 1 (a and b) and Figure 2 (a and b). The maximum values in untreated effluent were seen in 25% concentration and minimum in 50% concentration.



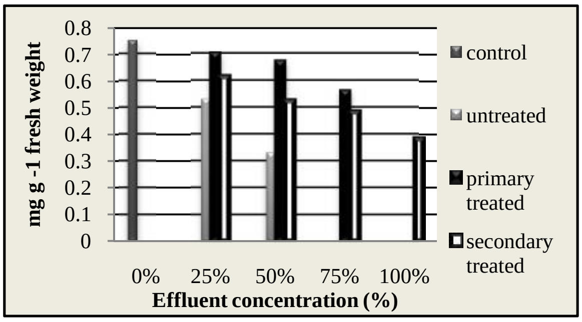
a) Chlorophyll a



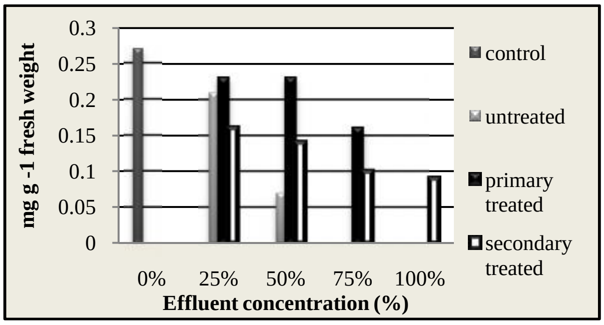
b) Chlorophyll b

Fig 1 Effect of different concentrations of untreated, primary treated an secondary treated CBDE on Chlorophyll a and chlorophyll b of *Cicer arietin* var. GNG26054.

In primary treated effluent, the maximum values of the pigments like chlorophyll a (0.39 mg g^{-1} fw), chlorophyll b (0.31 mg g^{-1} fw), total chlorophyll (0.70 mg g^{-1} fw) and carotenoid (0.23 mg g^{-1} fw) were found in 25% concentration which decreased gradually with increase in concentration showing lowest value at 75% effluent. In secondary treated effluent also, the maximum chlorophyll a and b, total chlorophyll and carotenoid concentrations were measured at 25% effluent which decreased gradually with minimum values observed at 100% effluent concentration.



a) Total chlorophyll



b) Carotenoids

Fig 2 Effect of different concentrations of untreated, primary treated and secondary treated CBDE on Total chlorophyll and carotenoid content of *Cicer arietinum* L. var. GNG26054**.**

DISCUSSION

Sufficient water absorption is essential for proper seed germination, without which seedling growth and development is severely affected (Kelly *et al*., 1992). Reduction in seed germination at higher effluent concentration may be due to higher amount of solids present in the effluent which causes changes in the osmotic relationship of the seed and water. Thus, reduction in the amount of water absorption takes place, which results into retardation of seed germination (Adriano *et al*., 1973). Present findings are corroborated well with the Narain *et al*. (2012), who studied the impact of distillery effluent on seedling growth and pigment concentration of *Cicer arietinum* L. and found that the percent germination, vigour index and germination index showed a gradual decline with increase in effluent concentration. They observed that the distillery effluent did not show any inhibitory effect on seed germination at low concentration (25%). Seeds germinated in 100% effluent but did not survive for longer period.

The better growth at 25% effluent concentration may be due to the growth promoting effect of nitrogen and other mineral elements present in the effluent. Kathirvel (2012) also reported that at lower concentrations the germination and other parameters of *Cicer arietinum* L. were high and subsequently higher concentrations showed minimal growth and germination rate.

Our study is also consistent with the findings of Malaviya and Sharma (2011a) who found that the untreated distillery effluent at lower concentration (20%) favored the germination of *Brassica napus* L. Our findings are also consistent with the study of Malaviya *et al*. (2012) who found that the low effluent concentration (20%) of dyeing industry caused a positive impact on germination and growth of *Pisum sativum*. They also observed that all the growth parameters increased at 25% effluent and decreased at 100% effluent concentration. At 100% effluent, nutrients were raised too high to become toxic resulting in retarded root and shoot length (Dutta and Boissya, 1996). Kohli and Malaviya (2013) in their study on *Triticum aestivum* L. var. PBW-373 found that very low concentration (4.5%) of tannery effluent showed positive impact on overall growth of seedlings as compared to the relatively high effluent concentrations.

Pigment content pattern is also similar with the findings of Narain *et al*. (2012), who reported that the chlorophyll a and chlorophyll b contents in *Cicer arietinum* were increased upto 25% effluent concentration. Similarly, Kohli and Malaviya (2013) reported that chlorophyll a, chlorophyll b and total chlorophyll in *Triticum aestivum* L. var. PBW-373 have been increased upto 4.5% effluent concentration and then gradually decreased with an increase in effluent concentration.

CONCLUSION

The present study showed that Combined Brewery Distillery Effluent (CBDE) has toxic effect on germination, seedling growth and pigment content of *Cicer arietinum* L. var. GNG26054 when applied at higher concentrations whereas, it showed beneficial effect at lower effluent concentration (25%) on the above mentioned parameters. The CBDE if applied in lower concentrations can not only prevent it from being environmental hazard but also serve as a potential source of liquid fertilizer for agricultural crops. It will also reduce the quantity of water required for irrigation and help in water conservation and provide nutrients to the plants and agricultural field. Thus it can be suggested that after post-field trials the suitably diluted effluent can be used for cultivation of *Cicer arietinum* L. var. GNG26054.

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