IMPACT OF DISTILLERY EFFLUENT ON SEEDLING GROWTH AND PIGMENT CONCENTRATION OF *Cicer arietinum* L.

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ABSTRACT

The impact of different concentrations (10%, 25%, 50%, 75% and 100%) of distillery effluent on seed germination (%), germination index, plumule and radicle length, fresh and dry weight of plumule and radicle, chlorophyll and carotenoid content of *Cicer arietinum* was investigated. The germination percentage of seed, seedling growth and chlorophyll content showed a gradual decline with increase in effluent concentration. 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 was at 25% effluent concentration, may be due to the growth promoting effect of nitrogen and other mineral elements present in the effluent.

Key Words : Chlorophyll, *Cicer arietinum*, Distillery effluent, Germination index, Concentration

INTRODUCTION

Land and water are precious natural resources on which rely the sustainability of agriculture and the civilization of mankind. Unfortunately, these have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities. Pollution includes point sources such as emission, effluents and solid discharge from industries, vehicle exhaust and metals from smelting and mining, and nonpoint sources such as soluble salts (natural and artificial), insecticides/ pesticides, industrial and municipal wastes disposed in agricultural fields and fertilizers[1].

India has emerged during recent years as the largest alcohol producer in the world, which bears immense significance as a basic chemical for rapidly advancing chemical industry and as a readily available source of energy. Therefore, in the present scenario as well as for future, demand for alcohol will increase in the country and so also the number of distilleries producing alcohol. This is clearly evident from the two-fold increase in number of alcohol industries in India during the last decade. Distilleries are one of the 17 most polluting industries listed by the Central Pollution Control Board (CPCB). Presently there are 285 distilleries in India producing 2.7 billion litres of alcohol and generating 40 billion litres of wastewater annually.

Although the benefits of wastewater use in irrigation are numerous but precautions should be taken to avoid short and long-term environmental related risks. Earlier studies have shown that the effect of an industrial effluent vary from crop to crop[2]. So it is essential to study the effect of industrial effluents on individual crop prior to use the industrial waste in agricultural fields.

In the present investigation the impact of distillery effluent in various concentrations (0%, 10%, 25%, 50%, 75% and 100%) on seed germination

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(%), germination index, vigour index, plumule and radicle length, dry and fresh weight and chlorophyll and carotenoid content of *Cicer arietinum* has been explored.

MATERIAL AND METHODS

Study site

Mohan Meakins Brewery Ltd. (MMBL) Lucknow Distillery, Uttar Pradesh, India was selected as the study site. It is situated at Dali Ganj, Lucknow on the bank of Gomti River. Mohan Meakin Brewery is Asia's first commercial brewery. It produces high quality beer and other alcoholic products.

Physico-chemical analysis of distillery effluent

Distillery wastewater was collected from the Mohan Meakins Brewery Limited Distillery, Lucknow in containers and brought to laboratory. Various parameters of the treated effluent viz, colour, temperature, pH, EC, TS, TDS, TSS, BOD, COD, DO, alkalinity, total hardness, chloride and sulphide were determined[3].

Seed germination and seedling growth

The present study was conducted to assess the impact of distillery effluent on germination per cent, germination index and length of radicle and plumule of gram seeds. For this seeds of gram (*Cicer arietinum* L.) obtained from CSA University, Kanpur, India, were surface sterilized with 0.1% aqueous solution of mercuric chloride, followed with repeated washings by using sterilized double distilled water. An experimental design with 3 replicates and 15 seeds per petri dish was used. In particular, these experiments were performed both on undiluted and variably diluted (10%, 25%, 50%, and 75% in water) effluents and distilled water was used as control. These pre-treated grains were then germinated in sterilized petri plates lined with absorbent cotton moistened with double distilled water or appropriate effluent with desired dilution and incubated at 25±2°C. The seed germination bioassay was evaluated by computing germination index, a factor of relative seed germination and relative root elongation[4]. The length of radicle and plumule was measured with the help of scale.

Pigment concentration

Arnon's method[5] was used to estimate the pigment concentration. For this leaf sample was crushed in 80% chilled acetone and then centrifuged at 10,000 rpm for 10 min at 4°C. The absorbance was recorded at 663, 645, 470 and 540 nm on UV-Vis spectrophotometer.

RESULTS AND DISCUSSION

Effluent sample collected from MMBL Distillery Lucknow was analyzed for different physico-chemical parameters. The effluent was dark brown in colour with unpleasant odour. The values obtained for Electrical Conductivity (EC), total hardness, alkalinity, dissolved oxygen and pH are tabulated in **Table 1**. The concentration of iron was found maximum in the effluent.

It is evident that an increase in the effluent concentration resulted in a gradual decline in germination percentage so much that no germination took place in the pure effluent **(Table 2)**. Distillery effluent is purely of industrial origin and contains large quantities of soluble organic matter and plant nutrients. The brown colour could be ascribed to the presence of melanoidin, the reaction product of sugar amine condensation. The unpleasant odour due to the presence of skatole, indole and other sulphur compounds which are not effectively decomposed by yeast or methanogenic bacteria during distillation is also an issue of public concern[6-7]. The pH i.e., the hydrogen ion concentration is an important parameter and the pH value determines the quality of water sample. The pH of treated effluent is 8.0 that can be considered as normal range for irrigation water. Industrial effluents generally change the natural pH level of the receiving water body to some extent. Such changes can tip the ecological balance of the aquatic system, excessive acidity in particular can result in the release of Hydrogen sulphide (H_2S) in air. The presence of salts in the wastewater increases the Electrical Conductivity (EC) of the effluent and is an alternative indicator of Total Dissolve Solids (TDS).

The higher EC alter the chelating properties of receiving water systems, which create conditions for free metal availability to flora and fauna[8].

Table 1 : Physico-chemical characteristics of distillery effluent

Parameters (Unit)	Mean ± SD
Colour	Dark Brown
Odour	Pungent
pH	8.25 ± 0.05
EC (dSm^{-1})	7.15 ± 0.08
TS ($mg\ l^{-1}$)	3263.29 ± 1.82
TDS ($mg\ l^{-1}$)	3029.55 ± 1.68
TSS ($mg\ l^{-1}$)	233.74 ± 2.21
DO ($mg\ l^{-1}$)	Nil
BOD ($mg\ l^{-1}$)	5980.83 ± 1.97
COD ($mg\ l^{-1}$)	21999.47 ± 1.99
Alkalinity ($mg\ l^{-1}$)	1441.40 ± 1.91
Total Hardness ($mg\ l^{-1}$)	475.77 ± 1.74
Chloride ($mg\ l^{-1}$)	748.07 ± 2.28
Sulphide ($mg\ l^{-1}$)	16.89 ± 0.07
Residual Chlorine ($mg\ l^{-1}$)	25.72 ± 0.35
Free CO_2 ($mg\ l^{-1}$)	10.40 ± 0.23
Metals ($\mu g\ ml^{-1}$)	
Iron	1017.37 ± 0.61
Manganese	414.88 ± 1.53
Copper	153.29 ± 2.52
Zinc	101.85 ± 3.05

The potassium salts were mainly responsible for increasing the EC of the effluent. Total Dissolved Solids (TDS) refers to all dissolved materials present in the water. Discharge of water with a high TDS level would have adverse impact on aquatic life, render the receiving water unfit for drinking, and reduce crop yields if used for irrigation[9]. High levels of total solids (3263 mgl^{-1}) in the distillery effluent, attributed to high concentrations of carbonates, chlorides, sulfates and nitrates of Ca, Mg and Na contributing to high salinity in water and eventually in soils into which it is leached out[10]. However, exceptionally high organic loading is likely to diminish the nutrient potential of the spent wash. The contamination of surface and ground waters, destruction of aquatic life and excessive accumulation of salts in soils pose serious threat to sustenance of soil and environmental health. A perusal of the data showed the effluent to be devoid of dissolved oxygen and high in BOD (5980 mgl^{-1}) and COD (21999 mgl^{-1}). Such high levels of BOD, which is indicative of relatively high proportion of the organic matter, causes depletion of dissolved oxygen in water, is known to be a threat to aquatic life. The high COD of the effluent might be due to the presence of large quantity of chemicals[11]. Hardness of water is usually due to the dissolved chlorides, sulphates, carbonates and bicarbonates of calcium and magnesium ions. Chlorides are highly soluble and do not precipitate or sediment and so cannot be removed easily by biological treatment. The chlorides and sulphides cause considerable difference in taste to the ground water. The presence of a very high amount of chlorides and sulphides is responsible for high degree of hardness and further it increases the degree of eutrophication. The discharge of effluent to a larger water body could lead to the dilution of different chemicals including metals but continuous discharge of effluent can cause harmful effect on the living system.

The germination percent of *C. arietinum* was 77.88% (10%), 84.45% (25%), 53.33% (50%), 13.33% (75%) and nil (100%) than 62.22% in control **(Fig. 1a)**. Increased significant result was observed in 25% concentration of the effluent at $p<0.05$ level while higher significant reduction ($p<0.01$) was observed at 75% concentration of effluent. The vigour index of C. arietinum was 839.03 (10%), 987.25 (25%), 357.64 (50%), 52.58 (75%) and nil (100%) over control (410.18). Statically significant ($p<0.01$) increase was observed upto 25% concentration which ranged from 104-140% while a decrease of 12.8-87% was noticed at higher concentration. Seed germination index of *C. arietinum* in different concentrations (10%, 25%, 50%, 75% and 100%) of distillery effluent was 210.66, 277.16, 119.99, 14.19 and nil respectively **(Table 2 and Fig. 1)**. There was a significant increase ($p<0.01$) of 110-177% at 25% concentration while a significant decrease ($p<0.01$) of 85.8% was noticed at 75% effluent concentration in comparison to respective control.

The data for radicle and plumule lengths of both *C. arietinum* indicate that the effluent, at upto 25% concentration, had a marked promoting effect on the overall growth of the seedling. The radicle and plumule length of *C. arietinum* increased significantly ($p<0.01$) as

83.5% and 69.6% respectively at 25% effluent concentration in comparison to control. However, the decrease was non-significant (p>0.05) at 50% and 75% for radicle and at 50% concentration for plumule length. The decrease in both radicle and plumule length

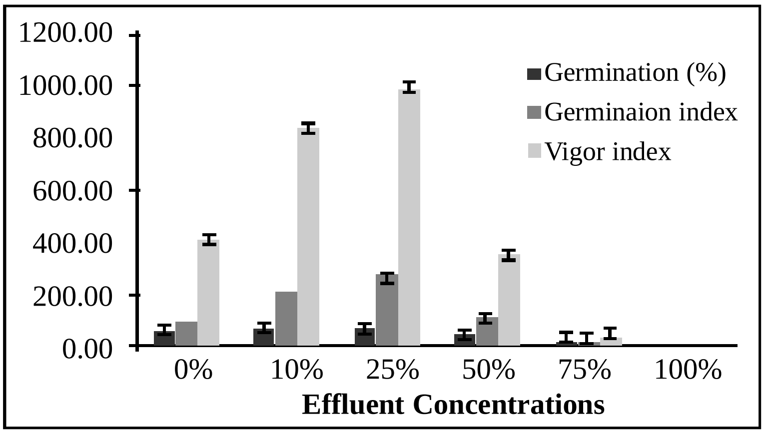

Fig 1 : Effect of different concentrations of distillery effluent on seed percent germination, germination index and vigour index

was 31% and 42% respectively, compared to their respective control **(Fig 2).**

In *C. arietinum* fresh and dry weight of radicle and plumule increased significantly (p<0.05 or p<0.01) upto 25% concentration of distillery effluent, thereafter reported decrease at higher concentrations. The increase in fresh and dry weight of radicle was 163% and 449% respectively at 25% concentration, whereas a decline of 19-58% and 12-47% in fresh and dry weight of radicle was noticed at higher

Table 2 : Effect of different concentrations of distillery effluent on seed germination and seedling growth of *Cicer arietinum*

Parameters	Effluent Concentrations (%)					
	0	10	25	50	75	100
% Germination	62.22 ± 7.70	ns77.78 ± 7.70	*84.45 ± 3.85	ns53.33 ± 6.67	**13.33 ± 6.67	--
Radicle Length (cm)	2.29 ± 0.25	*3.49 ± 0.69	**4.19 ± 0.66	ns2.89 ± 0.49	ns1.57 ± 0.66	--
Plumule Length (cm)	4.44 ± 0.45	**7.36 ± 0.32	**7.52 ± 0.33	ns3.87 ± 0.45	**2.55 ± 0.35	--
Germination Index	100.00 ± 0.00	**210.66 ± 2.10	**277.16± 2.45	**119.99 ± 2.15	**14.19 ± 2.66	--
Vigour Index	410.18 ± 23.50	**839.03 ± 14.01	**987.25 ± 26.82	*357.64 ± 8.76	**52.58 ± 20.22	--
Fresh Weight of Radicle (g)	0.103 ± 0.013	**0.264 ± 0.032	**0.270 ± 0.015	ns0.083 ± 0.010	*0.043 ± 0.013	--
Fresh Weight of Plumule (g)	0.217 ± 0.032	**0.371 ± 0.033	**0.462 ± 0.027	ns0.160 ± 0.021	**0.111 ± 0.031	--
Dry Weight of Radicle (g)	0.037 ± 0.013	*0.096 ± 0.026	**0.205 ± 0.020	ns0.033 ± 0.011	ns0.020 ± 0.010	--
Dry Weight of Plumule (g)	0.096 ± 0.012	**0.190 ± 0.010	**0.248 ± 0.018	ns0.074 ± 0.016	**0.043 ± 0.011	--

Data in replicate (n=3) are summarized as Mean ± SD; ns- *p*>0.05, *- *p*<0.05, **- *p*<0.01.

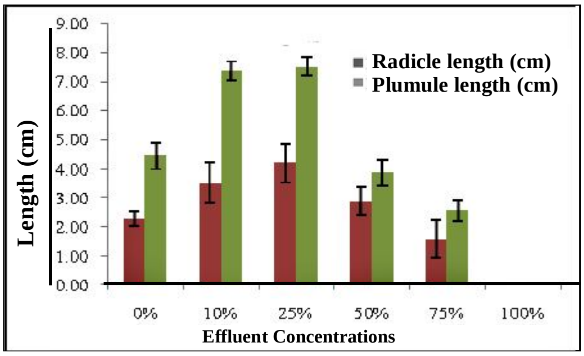

Fig. 2 : Effect of different concentrations of distillery effluent on radicle and plumule length (cm)

concentration in comparison to their respective controls. Plumule fresh and dry weight was decreased 26-48% and 23-55% respectively, compared to their respective controls **(Fig. 3)**.

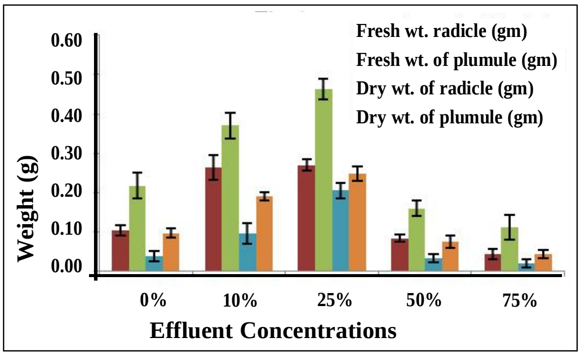

Fig. 3 : Effect of different concentrations of distillery effluent on fresh and dry weight (gm) of radicle and plumule

Lower concentrations of effluent showed promontory effect on seed germination, germination index, vigour index and effluent tolerance index but higher concentrations suppressed these parameters so much that there was no germination in 100% effluent. The percentage germination and germination index increased upto 25% concentration of the effluent and at higher concentrations germination percentage and percent germination index decreased, so much that no germination took place in the pure effluent. The inhibition of seed germination at higher concentration of the distillery effluent may be due to high salt content[12] and presence of toxic heavy metals[13]. Inhibition of seed germination, may be due to high level of dissolved solids[14]. Another reason of reduced seed germination at higher concentrations might be due to impermeability and high level of total dissolved solids which enrich the salinity and conductivity of solute absorbed by seeds before germination. Seed germination is reduced at higher concentrations of the distillery effluent due to high osmotic pressure caused by high salt concentrations in effluent[15-16]. The maximum vigour index was at 25% for *C. arietinum* (964.44). The root and shoot length of the seedlings indicate that effluent at upto 25% concentration had a marked promoting effect on the overall growth of the seedlings. At 25% of effluent concentration, increase in root and shoot length as compared to control was observed, whereas at 50% and 75% of effluent concentration decrease in length of root and shoot was recorded. The fresh and dry weight of the plumule and radicle was also found maximum at 25% concentration and at higher concentration, fresh and dry weight decreased. The visible effects produced in the roots and aerial parts of the seedlings show great resemblance to the nickel-toxicity effects[17-18]. The distillery effluent under investigation is toxic to growth of plants irrigated with it. The toxicity of the different species of crops to distillery effluent indicates that the toxicity of a variety of species depends upon chemical nature of the effluent, type of crop species and interaction of effluent with seed coat. The seed germination and seedling growth of *Phaseolous radiatus* and *Cajanus cajan* were significantly reduced at higher concentration of distillery effluent[19-20]. Inhibition in root growth of Pisum sativum at higher concentration, which may be due to high BOD and excess soluble salts present in distillery effluent[21]. Experiment shows that dilution of distillery effluent from tap water reduces the phytotoxicity in crop plants. Ramana et al. conducted a laboratory experiment to see the impact of different concentrations of distillery effluent on the seed germination, speed of germination, peak value and germination value in some crops and found that irrespective of crop species, at 75% and 100% concentration of the effluent complete failure of germination was observed[22]. Tomeulescu et al. studied the toxicity of Cu, Zn, individually and in combinations at germination stage[23]. They observed that

Cu significantly inhibited the germination, which increased with increasing concentration, Zn also inhibited the germination. The toxic effect was more pronounced when Cu and Zn were applied together. The results indicate that root growth was the most sensitive end point measurement than the shoot growth. Phytotoxicity assessment plays an important role in environmental monitoring and risk assessment especially in metal contaminated soil. The effect of industrial effluent on seed germination as well as radicle growth depends on the nature of toxic substances, which alter the seed water interaction necessary for triggering activity[24-25].

The chlorophyll a, chlorophyll b and total chlorophyll contents were increased upto 25% concentration and decreased significantly at higher concentrations of the effluent **(Fig. 4)**. The chlorophyll a and chlorophyll b contents were increased upto 25% concentration and decreased significantly at higher concentrations of the effluent. Similar observation has been reported by other co-workers[26] in *Phaselous radiatus* treated with distillery effluent. Decline in the total chlorophyll content has been reported in many other plants also[27]. Decrease in chlorophyll content could also be due to disturbance in the Hill reaction. Carotenoid is a non-enzymatic antioxidant, playing vital role in the protection of plant from the adverse impact of reactive oxygen species[28]. Carotenoid also protects chlorophyll from photo-oxidative destruction within the chloroplast[29]. The carotenoid content also increased up to 25% effluent concentration and decreased at higher concentrations. Many workers have reported the loss of carotenoid content caused by various pollutants[30]. Oxidation of carotenoids takes place through a light catalyzed reaction resulting in the formation of epoxide, which is further reduced in dark by an enzyme-catalyzed reaction.

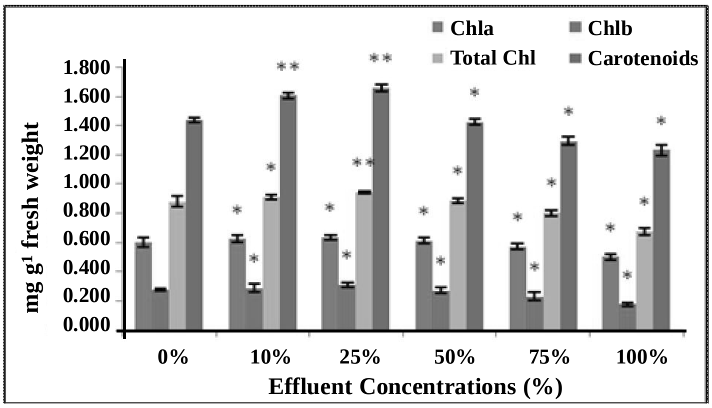

Fig.4 : Effect of different concentrations of distillery effluent on pigment concentration (mg g^{-1} fresh weight) of *Cicer arietinum* seedling

CONCLUSION

The observation made in the study showed that the high concentration of the effluent is toxic whereas it has beneficial effect at lower concentrations. The use of the distillery effluent in low concentration 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 field and plants. Thus the study suggested that the effluent could be used safely for *Cicer arietinum* cultivation, after proper treatment and dilution.

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