

Utilization of Distillery Industry Wastewater as Liquid Biofertilizer: Seed Bioassay Test for Feasibility and Toxicity Measurement

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ABSTRACT

The industrial effluents have been recommended as potential source of irrigation water and nutrient sources for commercial cropping system. However, the high nutrient loads and presence of some growth retarding substances in industrial effluent may cause severe impact on plant germination and gross productivity of a commercial crop. Prior to field application the industrial effluent should be tested for its ecotoxicity using seed bioassay test. The of this study was to investigate the toxicity of distillery effluent (DE) using seeds of moong (*Vigna radiata*), guar (*Cyamopsis tetragonoloba*), Makai (*Zea mays*) and gehu (*Triticum aestivum*). For lab trial, a total of a total of five different concentrations of distillery effluents (20, 40, 60, 80 and 100%) were prepared by adding required quantity of distilled water. Seed germination, root length, shoot length, root weight, shoot weight and chlorophyll level etc. were measured in experimental set-ups. Result suggested 40% DE strength suitable for plants: *C. Tetragonoloba* (67.7%), *T. aestivum* (64%) and *Z. Mays* (92.3%) except in *V. radiata* (98% germination with 60% DE). The high DE indicates a toxic impact on seed germinations. The seedling growth and biomass also showed a close relationship with strength of DE in treatment set-up. In majority of set-ups, set-up with 20 – 60 % DE strength showed the better results of plant groths. The biochemical stress of high DE strength on seedling was also observed in this study. The study clearly support the utility of DE for plant production but after proper dilutions of the effluent.

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I. Introduction

The wastewater discharged from distillery industries poses serious threat to the environment. The safe disposal of wastewater is the prime and most concern option for wastewater treatment planners (Pandey et al., 2008). The wastewater composition, on the other hand, advocated its role as a resource which further utilized for productive uses, since wastewater contains nutrients that can be used for the cultivation of agricultural crops (Kumar and Chopra, 2012). The application of distillery discharge as a soil amendment and irrigation of agricultural crops has been increased in past decade (Biswas et al., 2009). This industrial discharge is rich in organic and inorganic matter and serves as an excellent source of plant nutrients (N, P, K, S etc) (Bharagava et al., 2008). Besides, it may contain some toxic compounds as well, that may suppress the soil properties and plant productivity. After proper primary and secondary treatment processes, the wastewater can be essentially acts as a soil fertilizer and utilized for crop irrigation purpose. There present several studies that advocated the adequacy of distillery effluent in enhancing the productivity and agronomic value of various crops (Khan and Srivastava, 1996; Banerjee et al., 2004; Kannan and Upreti, 2008; Bharagava et al., 2008; Pandey et al., 2008; Biswas et al., 2009). However, a proper compositional characteristic of distillery effluent may play crucial role in deciding the productivity of the agricultural system. Therefore, there is an urgent need to study usage

But prior to recommendation of such industrial effluents for land irrigation and crop production the toxicological impact of industrial effluent of commercial plant should be assessed.

The seed bioassay technology has been suggested as lab scale and precise method to assess the toxicity of any substance on commercial crops. The seedling germination and seedling growth patterns under different concentrations of industrial effluent can give some idea about the suppressing or toxicological impact of industrial effluents on plants. Keeping in view the above facts, a lab-scale bioassay of distillery effluent was conducted using few commercial cereal crops in order to seed the feasibility of utilizing distillery effluent for crop irrigation purposes.

II. Methodology

The distillery effluent was collected from lagoon located near to discharge point of a sugar mill. The effluent water was collected in pre-sterilized dark-coloured bottles and kept in ice boxes in field. In lab the effluent was stored in freezer at as per standard protocol described in APHA-AWWA-WPFC (1994). Before preparing different dilutions of distillery wastewater, different physico-chemical characteristic of wastewater were determined. For seed bioassay experimentation, a total of five different concentrations of distillery effluents (20, 40, 60, 80 and 100%) were prepared by adding required quantity of distilled water. The seeds of moong (*Vigna radiata*), guar (*Cyamopsis tetragonoloba*), Makai (*Zea mays*) and gehu (*Triticum aestivum*) were procured from a local Govt. Seed Centre, Hanumangarh.

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The experiment was carried out in plastic containers (21 cm dia. × 18.5 cm height) filled with garden soil. The seeds were treated with 0.1% mercuric chloride (MgCl₂) for 3 minutes and washed in distilled water to remove the surface fungi and microorganisms. Twenty healthy and uniform sized seeds were sown in the respective plastic containers. The distillery effluents with different concentrations were used as irrigation medium for all crops. However, in case of control tap water was used. Seed germination was studied after 5 days of sowing and germination index was calculated accordingly. At the end of the experiment four plants were randomly exhorted from each container and root length, shoot length, root weight, shoot weight and chlorophyll were measured.

2.2 Analytical methods

The different physico-chemical characteristic of wastewater was studied using standard methodology as described in APHA-AWWA-WPFC (1994). In order to obtain final root and shoot biomass weight (DW in g), three plants with similar fresh weight were taken from each treatment set-ups at the end of experiment. The weight was measured gravimetrically using weighing machine. The root and shoot length was determined using simple measuring scale. The chlorophyll pigments were measured spectrophotometrically by using standard method of Martin et al. (2003). Germination percentage was calculated using equation 1

$$\text{Germination (\%)} = \frac{\text{Seed germinate 3d each day}}{\text{Total number of seed taken}} \times 100$$

(equation 1)

The seed germination index was calculated on the basis of relative seed germination (RSG) and relative root growth (RRG). The percentages of relative seed germination (RSG), and relative root growth (RRG) and germination index (GI) after treatment duration were calculated as follows:

$$\text{RSG (\%)} = \frac{\text{number of seeds germinated using distillery effluent}}{\text{number of seeds germinated in control}} \times 100$$

$$\text{RRG (\%)} = \frac{\text{Mean root Length of plant grown in distillery effluent}}{\text{Mean root Length of plant grown in control}} \times 100$$

$$\text{GI (\%)} = \frac{\text{RSG} \times \text{RRG}}{100}$$

2.3 Statistical analysis

Experiments were carried out in triplicates and data were averaged with standard deviation (SD). SPSS® statistical package (Window Version 13.0) was used for data analysis. All statements reported in this study are at the $p < 0.05$ levels.

III. Experimental Results

The physico-chemical characteristics of undiluted distillery wastewater were found to be pH (7.22), EC (4.22), BOD (567.66 mg/L), COD (2156.33 mg/L), Cl⁻ (349.66 mg/L), NO₃⁻ (256.33 mg/L), SO₄²⁻ (150.0 mg/L), totP (85.72 mg/L), totN (92.31 mg/L), totK (195.51 mg/L), totNa (138.45 mg/L), totCa (568.48 mg/L), TDS (4476 mg/L), TSS (2912 mg/L),

Fe (6.84 mg/L), Mg (122.72 mg/L) and Zn (11.75 mg/L). Statistically (ANOVA) there exist a significant difference among germination rate (%) of different plant species grown in different concentrations of effluent treated soil: *Vigna radiata* ($F = 483654.5$, $p > 0.05$), *Cyamopsis tetragonoloba* ($F = 37322.8$, $p > 0.05$), *Zea mays* ($F = 292642.6$, $p > 0.05$) and *Triticum aestivum* ($F = 202177$, $p > 0.05$). The result on seed germination rate (%) and germination index (GI) validates that distillery effluent concentration markedly influences the germination process in all the studied plant (Table 1). It has been found that among all plants, germination rate was the maximum in *Vigna radiata* (98.0%) at the effluent strength of 60% and minimum was observed in *Vigna radiata* (26.0%) at the effluent strength of 100%. In case of *Vigna radiata*, germination rate range between 26-98%, with maximum at 60% strength (98.0%), followed by 40% (94.0%) and 20% (76.3%) and was markedly higher than those reported by control (65.6). For *Cyamopsis tetragonoloba*, germination rate lies between 49.3-67.6%. The maximum was reported at the effluent concentration of 40% (67.6%), followed by 20% (63.0%) and 60% (57.3%). The minimum was observed at the effluent strength of 100% (49.3). *Zea mays* showcases peculiar germination trend lies in the range of 60.6-92.3%, with maximum reported at 40%, followed by 100% (62.6%) and 80% (60.6%). All effluent strengths offers higher germination rate when compared to that of control. In case of *Triticum aestivum*, germination rate falls in the range of 36.5-64.0%. The maximum was recorded at the effluent strength of 40%, followed by 20% (46.6%) and 80% (37.3%). Overall results suggested that *Zea mays* germination rate for all distillery effluent strengths were comparatively higher than other crop species (Fig 1). The germination index (GI) was calculated by combining relative shoot growth and relative root growth of respective crops (Zucconi et al., 1981). There exists a statistical significant difference in the germination index (GI) of different plants grown such as *Vigna radiata* ($F = 25.168$, $p > 0.05$), *Cyamopsis tetragonoloba* ($F = 69.53$, $p > 0.05$), *Zea mays* ($F = 75.86$, $p > 0.05$) and *Triticum aestivum* ($F = 59.16$, $p > 0.05$). In *Vigna radiata*, GI followed the order as 60% (146.4) > 80% (66.1) > 40% (59.3) > 20% (52.5) > 100% (44.6). For *Cyamopsis tetragonoloba*, maximum GI was recorded in 40% treatment (184.1) followed by 80% (170.4), 60% (114.6), 20% (95.1) and the least was observed in 100% (58.08). For *Zea mays* the reduction in GI was in order of 20% (260.40) > 80% (201.1) > 100% (196.6) > 60% (102.2) > 40% (95.1) and for *Triticum aestivum*, the GI tend to decrease in the order of 100% (373.7) > 20% (248.4) > 40% (159.9) > 80% (127.63) > 60% (125.3). Among the entire experimental plants maximum germination index was observed in *Triticum aestivum* (373.7 at 100% effluent strength) followed by *Zea mays* (260.4 at 20% effluent strength) and *Triticum aestivum* (248.4 at 20% effluent strength). Overall, the minimum germination index was found to be occurring in *Vigna radiata* and best results was observed in *Zea mays* (Table 1).

Table 1. Impact of different concentrations of distillery effluent on seed germination (%) and germination index of some commercial crops (mean ± SD; n=3).

DE strength	<i>Vigna radiata</i>		<i>Cyamopsis tetragonoloba</i>		<i>Zea mays</i>		<i>Triticum aestivum</i>	
	SG (%)	GI(%)	SG (%)	GI(%)		SG (%)	GI(%)	SG (%)
20%	76.3±2.6	52.5±4.6	63.0±2.8	95.1±4.1	61.0±2.5	260.4±22.5	64.0±1.0	248.4±9.3
40%	94.0±3.1	59.3±1.9	67.6±1.2	184.1±6.9	92.3±2.0	95.1±5.1	46.6±2.4	159.9±17.8
60%	98.0±2.6	146.4±16.3	57.3±1.4	114.6±9.8	58.3±1.4	102.2±3.1	36.6±2.4	125.3±12.7
80%	45.6±2.6	66.1±4.9	55.0±1.7	170.4±6.8	60.6±1.2	201.1±11.0	37.3±2.3	127.6±16.9
100%	26.0±2.6	44.7±3.7	49.3±2.0	58.1±1.0	62.6±1.4	196.6±0.4	36.5±1.4	373.7±17.4
Control*	65.6± 2.6	59.1±1.2	59.0±1.7	65.4±2.2	57.6±3.5	78.5±2.1	61.0±1.5	97.6±2.4

*Control represents plant irrigated with tap water

The growth characteristics of the plant were predicted on the basis of root and shoot length, root and shoot weight and pigment concentration. All studied plants shown peculiar growth characteristic based on the strength of distillery effluent concentration used. There exists a statistically significant difference among all plants for studied growth parameter. The ANOVA value for root length was presented as follows: *Vigna radiata* ($F=128433.0$, $p>0.05$), *Cyamopsis tetragonoloba* ($F = 238328.4$, $p>0.05$), *Zea mays* ($F = 80190.9$, $p>0.05$) and *Triticum aestivum* ($F = 4385.1$, $p>0.05$). In case of shoot length, *Vigna radiata* ($F = 70848.1$, $p>0.05$), *Cyamopsis tetragonoloba* ($F = 3.73$, $p>0.05$), *Zea mays* ($F = 11869.5$, $p>0.05$) and *Triticum aestivum* ($F = 97124.1$, $p>0.05$). For root biomass, the ANOVA values were as follows: *Vigna radiata* ($F = 1166.3$, $p>0.05$), *Cyamopsis tetragonoloba* ($F = 30.36$, $p>0.05$), *Zea mays* ($F = 140.1$, $p>0.05$) and *Triticum aestivum* ($F = 63.4$, $p>0.05$) and for shoot biomass, the value were *Vigna radiata* ($F = 15871.1$, $p>0.05$), *Cyamopsis tetragonoloba* ($F = 13092.4$, $p>0.05$), *Zea mays* ($F = 1468.7$, $p>0.05$) and *Triticum aestivum* ($F = 10036.1$, $p>0.05$).

In *Vigna radiata* the root length (cm) and shoot length (cm) lies between 2.8-7.9 and 6.1-16.1, respectively (Table 2). Maximum root length (7.9 cm) has been reported at 60% effluent strength followed by 20% (5.3 cm) > 80% (3.46cm) > 40% (3.4 cm). The trend for shoot length was as follows: 20% (16.1 cm) > 60% (15.7 cm) > 40% (12.0 cm) > 80% (8.5 cm) > 100% (6.1 cm). The root and shoot weight (g) was in the ranges of 3.8-44.6 and 70.7-280.4, respectively. The maximum root weight was reported at effluent strength of 80% (44.6 g) followed by 40% (13.3 g) > 20% (11.2 g) > 80% (3.8 g). Likewise, shoot weight (g) increases from 280.4 (20%) > 266.5 (60%) > 200.1 (40%) > 177.8 (80%) > 70.7 (100%). The chlorophyll a, chlorophyll b and total chlorophyll content varies between 95-198, 66-180 and 162-293 mg/g, respectively. It has been noticed that with increasing distillery effluent strength in soil, the pigment concentration in plant also goes on increasing. The effluent strength of 100% markedly diminished the growth characteristics and chlorophyll content in plant, which can be further related to the normal plant growth and development.

In *Cyamopsis tetragonoloba*, root and shoot length were in the ranges of 3.4-10.6 and 6.0-8.1 cm, respectively (Table 3). The increasing root length (cm) reported a trend of 10.6 (100%) > 6.1 (20%) > 4.7 (60%) > 4.4 (80%) > 3.4 (40%). Similarly, shoot length (cm) increases in the order of 40% (8.1) > 60% (7.5) > 100% (7.4) > 80% (7.3) > 20% (6.0). The root and shoot weight (g) falls between 2.6-6.1 and 20.5-142.4, respectively. The maximum root weight of 6.1 g was found at effluent strength of 20%, followed by 4.8 g (at 100% effluent strength) and 4.5 g (at 60% effluent strength). The minimum was at effluent strength 80%. Likewise, shoot weight was maximum (142.4 g) at 100% effluent strength, followed by 101 g (at 20% effluent strength) and 60 g (at 40% effluent strength). The root length and root weight keeps on decreasing with increasing effluent strength, but the effluent strength of 100% showcases sudden increase in both length and weight. The pigment content of plant lies in the ranges of 0.8-1.9 (mg/g) for total chlorophyll, 0.4-1.2 (mg/g) for chlorophyll a and 0.2-0.8 (mg/g) for chlorophyll b. It has been noticed that increasing effluent strength reduces the pigment content in *Cyamopsis tetragonoloba*.

In case of *Zea mays*, root length (cm) and biomass (g) lies in the range of 1.2-5.1 and 7.1-34.6, respectively (Table 4). The maximum root length was witnessed at effluent strength of 40% (5.1), later follows a trend of 60% (3.0) > 80% (1.6) > 100% (1.5) > 20% (1.2). For root biomass (g) the maximum was observed at strength of 80% (34.6), followed by 60% (22.6) and 40% (18.8). Similarly, shoot length (cm) and biomass (g) falls in the ranges of 3.3-4.9 and 106.6- 273.1, respectively. The maximum shoot length (cm) observed were 4.9 (at 40%), followed by 4.2 (at 100%), 4.0 (at 20%), 3.5 (at 80%) and 3.3 (at 60%). The shoot biomass follows a trend with 273.1 (60%) > 264.9 (at 20%) > 192.3 (40%) > 181.4 (80%) > 106.6 (at 100%). It has been observed that with increasing effluent strength in the soil, the root and shoots length increases till strength of 40%, then after their occur a massive decrease in both. However, in case of root and shoot biomass their seen a progressive trend till 80% effluent strength. For the chlorophyll content *Zea mays* also followed the similar pattern as the earlier discussed plants.

Table 2. Impact of different concentrations of distillery effluent on growth characteristics of *Vigna radiata* (mean \pm SD; n=3).

DE strength	Root (cm)	Shoot (cm)	Root (g)	Shoot (g)	Chl. a (mg/g)	Chl. b (mg/g)	Total Chl (mg/g)
20%	5.4 \pm 0.2	16.1 \pm 0.1	11.2 \pm 0.1	280.4 \pm 0.5	198 \pm 32.2	180 \pm 14.8	293 \pm 12.1
40%	7.9 \pm 0.0	12.0 \pm 0.2	13.3 \pm 0.2	200.1 \pm 0.2	176 \pm 11.7	168 \pm 8.9	246 \pm 11.9
60%	3.5 \pm 0.4	15.7 \pm 0.1	6.9 \pm 0.0	266.5 \pm 0.4	154 \pm 20.1	139 \pm 11.9	221 \pm 10.9
80%	3.5 \pm 0.1	8.5 \pm 0.1	44.6 \pm 1.1	177.8 \pm 1.0	130 \pm 10.6	119 \pm 13.1	192 \pm 10.5
100%	2.9 \pm 0.1	6.1 \pm 0.4	3.8 \pm 0.1	70.7 \pm 0.7	95 \pm 9.8	66 \pm 12.7	162 \pm 13.8
Control*	3.3 \pm 0.2	8.2 \pm 0.0	21.5 \pm 0.3	151.7 \pm 0.7	185 \pm 12.1	164 \pm 8.7	256 \pm 11.2

*Control represents plant irrigated with tap water

Table 3. Impact of different concentrations of distillery effluent on growth characteristics of *Cyamopsis tetragonoloba* (mean \pm SD; n=3).

DE strength	Root (cm)	Shoot (cm)	Root (g)	Shoot (g)	Chl. a (mg/g)	Chl. b (mg/g)	Total Chl (mg/g)
20%	6.1 \pm 0.1	6.0 \pm 0.1	6.1 \pm 0.2	101.0 \pm 0.4	1.2 \pm 0.2	0.8 \pm 0.6	1.9 \pm 0.8
40%	3.4 \pm 0.1	8.1 \pm 0.1	3.5 \pm 0.2	60.0 \pm 0.4	1.1 \pm 0.3	0.6 \pm 0.0	1.7 \pm 0.6
60%	4.7 \pm 0.3	7.5 \pm 0.2	4.5 \pm 0.3	20.5 \pm 0.4	0.8 \pm 0.4	0.4 \pm 0.2	1.4 \pm 0.8
80%	4.4 \pm 0.2	7.3 \pm 0.1	2.6 \pm 0.2	40.3 \pm 0.4	0.6 \pm 0.1	0.3 \pm 0.3	1.1 \pm 0.3
100%	10.6 \pm 0.3	7.4 \pm 0.2	4.8 \pm 0.1	142.4 \pm 0.3	0.4 \pm 0.5	0.2 \pm 0.1	0.8 \pm 0.1
Control*	5.4 \pm 0.3	7.6 \pm 0.1	3.1 \pm 0.1	55.9 \pm 1.3	1.6 \pm 0.4	1.0 \pm 0.0	2.2 \pm 0.6

*Control represents plant irrigated with tap water

Table 4: Impact of different concentrations of distillery effluent on growth characteristics of *Zea mays* (mean \pm SD; n=3).

DE strength	Root (cm)	Shoot (cm)	Root (g)	Shoot (g)	Chl. a (mg/g)	Chl. b (mg/g)	Total Chl (mg/g)
20%	1.2 \pm 0.1	4.0 \pm 0.0	7.0 \pm 0.1	264.9 \pm 1.9	2.4 \pm 0.7	1.9 \pm 0.1	4.8 \pm 1.1
40%	5.1 \pm 0.1	4.9 \pm 0.1	18.8 \pm 0.7	192.3 \pm 0.2	1.9 \pm 0.7	1.7 \pm 0.2	3.1 \pm 1.9
60%	3.0 \pm 0.0	3.3 \pm 0.1	22.6 \pm 0.5	273.1 \pm 3.2	1.6 \pm 0.4	1.4 \pm 0.1	2.1 \pm 1.0
80%	1.6 \pm 0.1	3.5 \pm 0.2	34.6 \pm 1.6	181.4 \pm 0.4	1.5 \pm 0.1	1.3 \pm 0.1	1.8 \pm 2.5
100%	1.5 \pm 0.2	4.2 \pm 0.1	14.9 \pm 0.0	106.6 \pm 0.9	1.2 \pm 0.0	1.1 \pm 0.2	1.6 \pm 1.0
Control*	3.1 \pm 0.1	3.5 \pm 0.1	20.3 \pm 0.3	165.0 \pm 1.4	3.2 \pm 0.8	2.2 \pm 0.1	5.3 \pm 1.2

*Control represents plant irrigated with tap water

Table 5: Impact of different concentrations of distillery effluent on growth characteristics of *Triticum aestivum* (mean \pm SD; n=3).

DE strength	Root (cm)	Shoot (cm)	Root (g)	Shoot (g)	Chl. a (mg/g)	Chl. b (mg/g)	Total Chl (mg/g)
20%	3.1 \pm 0.1	9.1 \pm 0.0	5.7 \pm 0.1	324.4 \pm 2.1	0.02 \pm 6.8	0.02 \pm 6.2	0.03 \pm 3.0
40%	3.5 \pm 0.2	11.5 \pm 0.5	7.9 \pm 0.2	260.7 \pm 0.5	0.01 \pm 2.9	0.01 \pm 4.9	0.02 \pm 4.7
60%	3.5 \pm 0.3	7.4 \pm 0.2	7.8 \pm 0.1	439.8 \pm 0.4	0.004 \pm 7.5	0.007 \pm 5.1	0.01 \pm 5.1
80%	3.5 \pm 0.3	7.1 \pm 0.0	11.2 \pm 0.4	150.9 \pm 0.9	0.002 \pm 4.6	0.005 \pm 7.1	0.01 \pm 2.0
100%	2.6 \pm 0.1	9.5 \pm 0.3	13.8 \pm 0.6	184.6 \pm 0.9	0.001 \pm 3.9	0.002 \pm 2.9	0.01 \pm 1.0
Control*	7.2 \pm 0.1	8.6 \pm 0.1	14.3 \pm 0.8	172.0 \pm 0.8	0.03 \pm 4.9	0.04 \pm 5.1	0.05 \pm 3.7

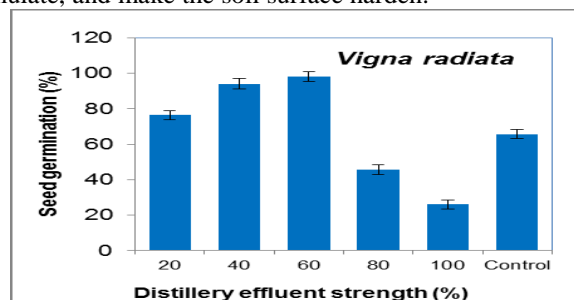
*Control represents plant irrigated with tap water

A decreasing trend in the chlorophyll content with the increasing effluent treatment strength was observed. The total chlorophyll (mg/g) content in the plants was recorded to be 4.8 (at 20%) > 3.1 (at 40%) > 2.1 (at 60%) > 1.8 (at 80%) > 1.6 (at 100%). The chlorophyll a and chlorophyll b level also follows the same trend as total chlorophyll. The *Zea mays* grown on 20% effluent treated soil showed best result in terms of plant growth.

The plants of *Triticum aestivum* showed decreased root and shoot growth and deduction in biomass at higher concentration (Table 5). The effluent application of different concentrations influences the growth characteristics of the *Triticum aestivum*. Contrastingly, at lower concentration improved growth was recorded. Root length and biomass lies in the range of 2.6-3.5 and 5.7-13.8. The maximum root length (3.5 cm) was observed at effluent concentration of 80%, decreasing in the order 3.5 (at 60%) > 3.5 (at 40%) > 3.1 (at 20%) and 2.6 (at 100%). The root biomass showcase a increasing order of 13.8 (at 100%) > 11.2 (at 80%) > 7.9 (at 40%) > 7.8 (at 60%) > 5.7 (at 20%). The plant shoot length and biomass supported that plants were healthier at varying effluent concentrations, when compared to control. The shoot length lies in the range of 7.1-11.5 cm and shoots biomass ranges between 184.6-439.8 g. The maximum shoot length of 11.55 cm was observed at effluent strength of 40%, followed by 9.5 (at 100%), 9.1 (at 20%), 7.4 (at 60%) and 7.1 (at 80%). Likewise, shoot biomass follows a trend of 439.8 (at 60%) > 324.4 (at 20%) > 260.7 (at 40%) > 184.6 (at 100%) > 150.9 (at 80%). As effluent strength in soil get increases, the pigment concentration keeps on decreasing. The maximum concentration of 0.3 (mg/g) total chlorophyll, 0.02 (mg/g) chlorophyll a and 0.02 (mg/g) chlorophyll b was found at 20% effluent strength. The chlorophyll content in the given plant was comparatively higher than those grown on control soil.

It has been noticed that in all studied crop plant (*Vigna radiata*, *Cyamopsis tetragonoloba*, *Zea mays*, *Triticum aestivum*) the shoot biomass contributes more and was comparatively higher than that of root biomass. Also the pigment concentration in the respective plant plays a very crucial role in deciding the normal well being of the plant (Tharakeshwari and Jagannath, 2011). The pigment concentration is directly correlated with the photosynthetic activities of the plant, which later govern the biomass production (Kumar and Chopra, 2013). The results suggested

that effluent with 20% strength when used for irrigation purpose seems to be beneficial for plant germination and growth. This was in accordance to the results of Bajpai and Dua, 1972, Kannan and Upreti (2008), Tharakeshwari and Jagannath (2011) Kumar and Chopra (2012), Kumar and Chopra (2013) and Mohamed and Ebead (2013). The study by Bharagava et al. (2008) also states that post distillery effluent irrigation lead to increases in chlorophyll and protein contents in Indian mustard plants (*Brassica nigra* L.) at the lower concentrations (25% and 50%) of distillery effluent followed by a decrease at higher concentrations (75% and 100%) of distillery effluent as compared to their respective controls. The performance differentiation among different strengths of distillery wastewater was due to the disparity in nutrient load (Kumar and Chopra, 2012). The collaborative interaction among soil pH and wastewater EC plays a significant role in the deciding the response of plant toward the irrigation source (Mohamed and Ebead, 2013). The physico-chemical properties (mainly pH) of soil is govern by multitude of factors such as water holding capacity, soil practical size (textural properties and structure), moisture content, organic matter and salt content (Miller and Turk, 2002). In the acidic or basic nature of soil provide microenvironment for microbial activity and nutrient exchange (Charman and Murphy, 1991). The availability of basic cations (like Ca^{2+} , Mg^{2+} , K^+ and Na^+) decreases as per increase in the acidic character of the soil and it directly affects the germination and growth of the crop plants (Kalaiselvi et al., 2010). Similarly, salts are usually the most damaging to young plants, but not necessarily at the time of germination. It has been found in study by Pandey et al., 2008 that high salt concentration can down regulated and slow down seed germination process. This may because of the percolation of soluble salts readily into water, where they accumulate, and make the soil surface harden.



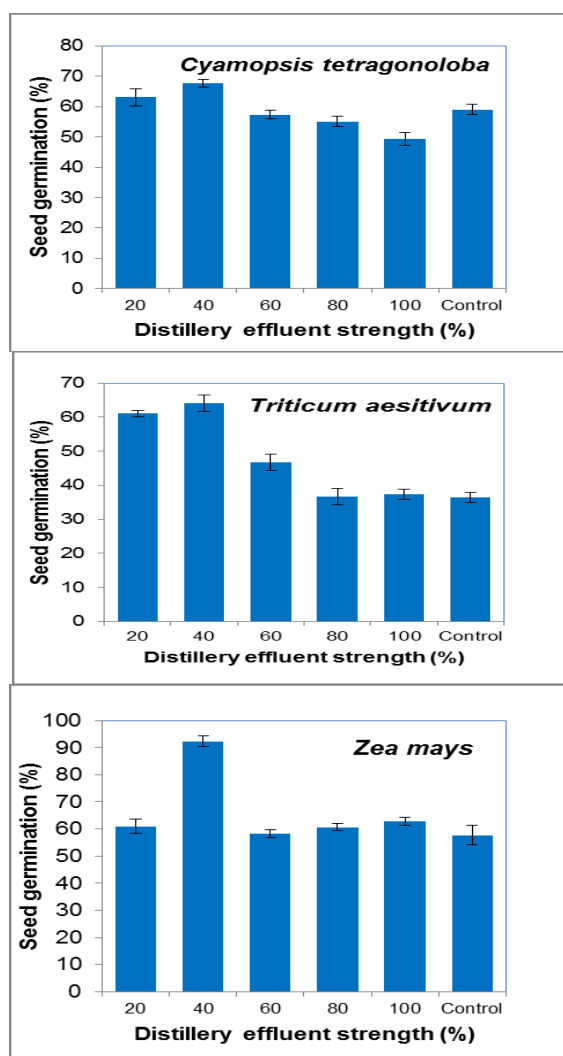


Fig 1. The seed germination rate (%) in different crop plants.

As a result, there might be delay in seed germination (Kumar and Chopra, 2012). The obtained data strongly indicates that long term irrigation with distillery effluent having high nutrient level deliberately diminishes the plant growth and dynamics. However, an appropriate dilution to the effluent may solve the purpose of its utilization for irrigation purpose and remarkably improves the availability of nutrients in the soil. The dilution of effluent upto 25 to 50% makes it an excellent nutrient fertilizer and promotes plant growth (Mohamed and Ebead, 2013).

It is possibly due to excess of nitrogen, phosphate, potassium, sulphate, calcium, and chloride by affecting the water absorption and other metabolic process in plants (Rani and Srivastava, 1990; Subramani et al., 1995). However, the response varies between different plants. A study by Lakshmi and Sundaramoorthy, (2001) reported that high concentrations of the sugar mill effluent reduced the growth of seedlings which may be due to excess amount of minerals present in the effluent. The plant-soil interaction dynamics were the key factors for determining suitability of distillery wastewater as irrigation source (Sahai and Neelam 1987). The increase in the chlorophyll content at low sludge concentration was due to the presence of essential nutrients and metal ions (Suthar et al., 2005). The observation in *Zea mays* was in accordance with a study by Shinde and Trivedi (1981). According to their study improved yield of maize was seen with the use of distillery spent wash and hence, maize was recommended as the tolerant

crop for growing in distillery spent wash. However, the toxicological aspects reveals that, the concentration of nutrient beyond particular level suppose to be lethal for plant survival (Sukanya and Meli, 2004). It was stated that distillery effluent has high oxygen demand and may result in oxygen depleted environment in soil (Suthar et al., 2005). It may further drastically affects seed germination and plant development processes (Baruah and Das, 1998). Therefore, a threshold limit should be estimated before supplying any kind of nutrient rich industrial discharge to the crop irrigation.

IV. Conclusion

The distillery industries produces huge amount of wastewater rich nutrients and heavy metals. The proper dilution of distillery wastewater reduces the strength of nutrient in it and supported its role in land restoration and sustainable plant production. The present study reveals that higher percentage of germination and better seedling growth was witnessed at lower concentration of distillery effluent. The nutrients presents in the diluted effluent might have played a role in promoting the seed growth at lower concentrations, however, at higher concentration of the effluent the soil nutrients are raised to level which probably become toxic resulting in inhibition of root and shoot growth. The dilution of wastewater (25-50%) showed positive effect on plant growth and production and supposed to be beneficial for crops. The result available after seed bioassay test advocated the use of pure distillery effluent without dilution may adversely affect the plant productivity.

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