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Combinatorial effects of distillery and sugar factory effluents in crop plants

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Abstract: *Under the reutilization and recycling strategy of industrial effluents, treated distillery and sugar factory mixed effluent was used in petridish culture experiments to investigate its effect on seed germination and seedling growth in wheat, garden pea, black gram and mustard. The seed germination and seedling growth were significantly reduced with increase in concentration of the effluent. The fresh matter was found significantly increased in barley (1.16 g per seedling in 25% dilution level of effluents in comparison to 0.93 in control), while other higher dilution levels reduce it. Wheat, garden pea, black gram, mustard invariably showed inhibition in fresh weight. Dry weight was found consistently reduced or unchanged in different treatments. Total chlorophyll contents in barley were significantly increased in different treatments (2.351 and 2.721 mg/g fresh weight of tissue at 25, 50 % dilution levels in comparison to 1.781 of control) while in other crop it was reduced allover the treatments. Amylase activity in wheat, garden pea, black gram and mustard was reduced in all the treatments. Only in barley its level was enhanced from 0.76 to 0.85, 0.96, 0.81 in 25, 50, 75% dilution levels of the effluent mixture respectively. Based on the data of different crops barley was found to be highly tolerant as the 25 and 50% dilution levels of combined effluents. It showed no change in germination %, while seedling growth was increased in lower dilution levels of combined effluent as compared to control. Barley>garden pea>wheat>black gram>mustard gradually showed increased level of sensitivity respectively. Most detrimental effects were seen in mustard. This toxicity might be due to excess of nutrients, beyond the limits of tolerance. Therefore, the higher concentration of mixed effluent was not advisable for irrigation purpose, however, it could be used for irrigation purpose after proper treatment and dilution (one part treated effluent and five parts of available irrigation water), as this dilution level was found growth and yield promotory.*

Key words: *Distillery and sugar factory effluent, Chlorophyll, Amylase, Toxicity, Nutrients*
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Introduction

The problem of environmental pollution on account of essential industrial growth is, due to the problem of disposal of industrial waste as well, whether solid, liquid or gaseous. Polluted water, in addition to other effects, directly affects soil not only in industrial areas but also in agricultural fields and river beds, there by creating secondary source of pollution (Kisku *et al*., 2000; Barman *et al*., 2000). Various industries have been continuously adding lot of waste water containing high level of nutrients, heavy metals and hazardous substances to the cultivable land (Srivastava *et al.*, 2000; Chandra *et al*., 2004; Math *et al.,* 2005; Malaviya and Rathore, 2007). These effluents not only increase the nutrient level, but also excess tolerance limits and cause toxicity (Mishra *et al*., 1999). Avasn and Rao (2000) studied the physicochemical characteristics of sugar mill and distillery effluent respectively. The high values of COD revealed the presence of high concentration of biodegradable organic matter in the effluent Kumar and Gopal (2001).

The various metallic and nonmetallic elements act as nutrients but at the higher concentration they show toxic effects on seed germination and seedling growth, ultimately adversely affecting plant growth and yield. Om *et al.* (1994), while studying the combined effect of different concentrations of wastes of distillery and sugar mill, observed inhibition of seed germination, seedling growth and biomass in okra (*Abelmoschus esculentus* L.). In the distillery effluent, various metals/nonmetals individually may not be toxic to the plant but in combination they may be toxic. On the other hand Zalawadia *et al*. (1996) studied the inhibitory effect of distillery effluent in combination with fertilizer on plants as well as on soil properties. Experiments conducted by Dutta and Boissya (1999) for studying the effect of low concentration of paper mill effluent on growth and field NPK contents in rice showed increase in growth and yield of crop. Kaushik *et al.* (1996) reported that low concentrations of sugar factory effluent had no effect on seed germination of *Triticum aestivum* L. Nath and Sharma (2002), also reported that the lower concentration of sugar factory effluent increases the seedling growth, chlorophyll and amylase contents in green gram (*Phaseolus radiatus* L.) seedlings in a 10 days experiment.

In the past physico-chemical characteristics of industrial effluents as well as their impacts on plants and animals have been worked out extensively (Mishra *et al.*, 1999; Avasn and Rao, 2000; Kumar and Gopal, 2001) but very little is known about their reutilization and combined effects. The mixed treated effluent of sugar and distillery industry, was used for the experiments and the present experiment was undertaken to study the combined effect of this mixed effluent to find out the impact of various dilution levels of the effluent on seed germination and seedling growth, using certain morphological and biochemical parameters.

Materials and Methods

The sugar and distillery treated effluent mixture (Bajaj Hindusthan Limited, Gola Gokaran Nath, Lakhimpur Kheri) was collected in glass bottles from the common discharge channel and properly sealed. Being a single owner of distillery and sugar factory, the mixed treated effluent of both units, is discharged through a single outlet. This effluent was analyzed for various physico-chemical properties as per methods described by APHA (1995). Different dilution levels of this effluent mixture were used for the experiment propose.

Wheat (*Triticum aestivum*, var. – PBW -343), barely (*Hordeum vulgare*, var. – Jyoti), garden pea (*Pisum sativum*, var. - Arcil), black gram (*Phaseolus mungo*, var. – Type 9), mustard (*Brassica campestris*, var. – PD 303) seeds were used for the petridish experiments. The seeds were treated with 0.1% $HgCl_2$ for prevention from surface fungal/bacterial contamination. All petridishes and glasswares were autoclaved before use. The various concentrations of treated effluents (25%, 50%, 75% and 100%) and control (glass distilled water) were taken for the study in triplicate. Twenty seeds were placed on filter paper in each petridish and treated with 10 ml of each concentration. The germinating seeds and seedling were washed with distilled water every alternate day for the prevention of contaminants and fresh solutions were applied for the maintenance of effluent concentration. The growth parameters like percent germination was recorded in first week while plumule and radicle length, total chlorophyll contents and amylase activity were measured in each sample at the end of second week. Fresh weight of five seedlings was measured with the help of digital balance (Shimadzu AY-220), then they were placed at 80±1°C in a hot air oven for 24 hr and dry weight was measured until constant weight was obtained. The chlorophyll was estimated by using the method of Arnon (1949), amended by Lichtenthaler (1987). Amylase concentration was estimated by using the method of Katsuni and Fekuhara (1969). Physico-chemical characeteristics of the distillery and sugar factory mixed effluent was estimated by APHA, 1995.

The data observed in the experiment were statistically analyzed for the calculation of standard error (S.E.) and student 't' test was administered for testing the hypothesis. The data shown in the table are the averages of three replicates ± S.E.

Results and Discussion

The physicochemical properties of effluent mixture shown in Table 1, indicate that effluent was slightly acidic and highly rich in COD, BOD, sulphate, sulphide, chloride, TDS, TSS, oil and grease.

The findings of the experiment are shown in Table 2-4 and Fig. 1. The results in Fig. 1 show % reduction in seed germination in various concentrations of distillery and sugar factory treated combined effluent in different cases. Wheat, barely, garden pea and black gram showed no change in lower concentration of effluent (25%) in comparison to control. Mustard showed highly significant reduction in germination even at lowest concentration of the effluent mixture. The inhibition in 75% and 100% treated effluent concentration was highly significant in all the seeds of different plant species. Fig. 1 clearly emphasizes the sensitivity of different plants to varying dilution levels of combined sugar and distillery treated effluent (wheat, barley, garden pea, black gram and mustard). Barley showed highest tolerance in different levels of effluents regarding seed germination.

Table - 1: Physicochemical characteristics of the distillery and sugar factory mixed treated effluent

S. No.	Property	Value
1	Colour	Brown
2	Odour	Alcoholic
3	Temperature	27°C
4	pH	6.7
5	Dissolved oxygen	Nil
6	BOD	356 mgl^{-1}
7	COD	632 mgl^{-1}
8	Total dissolved solid	247 mgl^{-1}
9	Total suspended solid	567 mgl^{-1}
10	Total hardness	239 mgl^{-1}
11	Total nitrogen	32.5 mgl^{-1}
12	Chloride	55 mgl^{-1}
13	Sulphate	63 mgl^{-1}
14	Sulphide	32 mgl^{-1}
14	Phosphate	10.2 mgl^{-1}
15	Potassium	22.8 mgl^{-1}
16	Iron	12.25 mgl^{-1}
17	Oil and grease	47 mgl^{-1}

There was slight and gradual reduction in plumule and radicle length, and number of lateral roots from lower to higher concentration of effluent in seedlings of different plant species. In barley, the plumule length and number of lateral roots significantly increased in 25% and 50% concentrations of effluent mixture while decreased in 75% and 100% concentrations of the effluent mixture in comparison to control. The inhibition in higher concentration (75% and 100%) of effluent mixture and promotion in lower concentration (25% and 50%) in barley in plumule length, radicle length and number of lateral roots was significant in most of the treatments as shown in Table 2. Growth inhibitory effect in wheat, garden pea, black gram and mustard were invariably observed in all the concentration levels of effluent mixture when compared with the control.

The mean value of five seedlings is shown in Table 3 for data interpretation of fresh and dry weights. The fresh weight in wheat significantly inhibited in different treatments of combined effluent (25, 50, 75 and 100%) as shown in the table. It was 0.70, 0.50, 0.52, 0.32 g/seedling in different treatments in comparison to 0.99 g/ seedling of control, likewise garden pea, black gram and mustard also showed reduction in fresh weight in comparison to their respective controls. In case of barley 25% dilution of the effluent mixture showed increase in fresh weight significantly, as it was 1.16 g in comparison to 0.93 g of control, while all the other dilution level were found inhibitory. Even 50% concentration of effluent mixture showed significant inhibition in barley and black gram. A significant growth

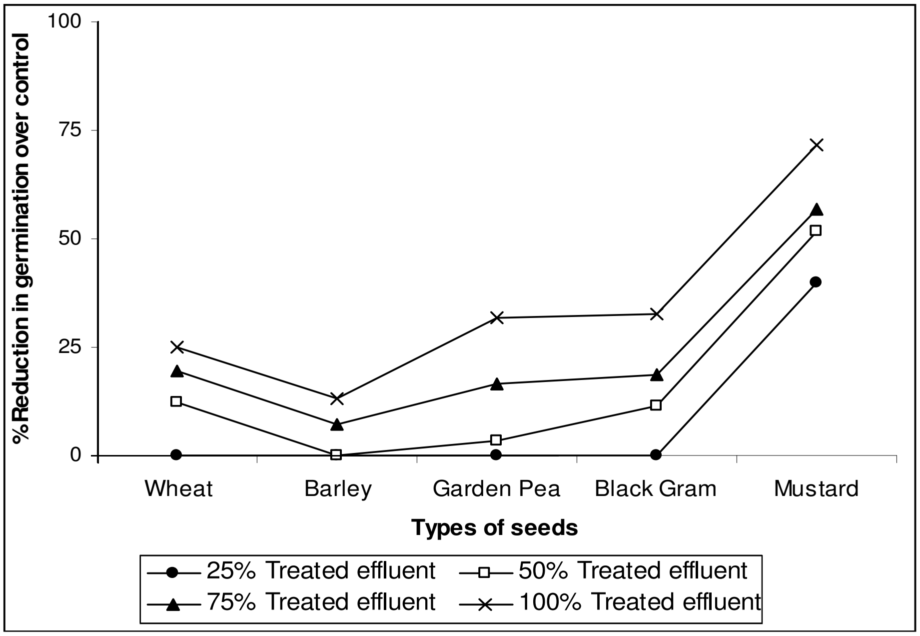

Fig. 1: Effect of distillery and sugar factory mixed effluent on seed germination in crop plant

promotory effect was seen in barley in 25% concentration of combined effluents. The dry weight was found to be reduced with increase in concentration of combined effluent except barley. In barley the dry weight increased at 25% concentration level while other higher concentrations (75% and 100%) reduced the dry weight. The moisture content gradually reduced in most of the cases due to poor water uptake in higher concentrations of effluents, but in case of barley the concentration up to 75% level showed increase in moisture content showing the tolerance potential of the crop.

The total chlorophyll contents and amylase activity were found to decrease from lower to higher concentration of treated combined effluent in wheat, barley, garden pea, and black gram (Table 4). In case of barley both chlorophyll contents and amylase activity increased in 25% and 50% concentrations of combined effluent treatments. They showed decrease in 75% and 100% concentration of effluent mixture in comparison to control, but the reduction was higher than control in 75% concentration of effluent mixture. The total chlorophyll contents and amylase activity were significantly altered in barley in all the treatments.

The investigations carried out during the present experiment clearly showed that barley is a tolerant crop for the irrigation of distillery and sugar factory combined effluent in low concentration level (25% combined effluent + 75% distilled water). While other plants species such as wheat, garden pea, black gram and mustard screened were found sensitive to the effluents mixture used, showing their increase level of sensitivity in ascending manner. (barley> garden pea> wheat>black gram> mustard).

The results of the effect of treated distillery and sugar factory combined effluent on seed germination and seedling growth are conclusive that various metallic and nonmetallic elements act as nutrients but at the higher concentration they show toxic effects on seed germination and seedling growth. Previously, people tried to explore the impact of industrial effluent on plants. Om *et al.* (1994), while studying the combined effect of wastes of distillery and sugar mill, observed inhibited seed germination, seedling growth and biomass in okra. Under the same series of studies Zalawadia *et al.* (1996) reported the inhibitory effects of distillery effluents on seed germination, seedling growth, fresh weight and dry weight *etc.* in onion. Similar results were reported by Subramani *et al.* (1995) while studying the effect of distillery effluent on *Vigna radiata*. Recently Ramana *et al.* (2001) also observed similar kind of inhibitory effects in mustard, cauliflower and radish.

The amylase activity in seedlings under the influence of treated distillery and sugar factory combined effluent was found decreased in comparison to control except lower levels of concentration in barley, which showed increase in amylase activity. The poor germination rate and seedling growth in effluent can be correlated to this reduced amylase activity. Amylase and its important role during seed germination through hydrolysis of reserve starch and release of the energy have been worked out by Thevenot *et al.* (1992). Studies performed by others (Dunn, 1974; Chang, 1982) on role of amylase with the increased seed germination were correlated with more hydrolysis of starch and release of energy. Recently, Chandra *et al.* (2004) studied the effect of distillery effluent on *Phaseolus aureus* L. and found that distillery effluent (more than 10% in concentration) decreased the chlorophyll content. The reduction in chlorophyll contents in our experiment is in agreement to these studies. This reduction in amylase activity and chlorophyll contents under the influence of different levels of treated distillery and sugar factory combined effluent, ultimately resulted into

Table - 2: Effect of different dilution levels of distillery and sugar factory mixed treated effluent on plumule and radicle length (cm) and number of lateral roots in 2 week old seedlings

Type of seed	Control			25%			50%			75%			100%		
	Plumule	Radicle	Lateral roots	Plumule	Radicle	Lateral roots	Plumule	Radicle	Leteral roots	Plumule	Radicle	Lateral roots	Plumule	Radicle	Lateral roots
Wheat	7.03 ± 0.08	4.93 ± 0.08	5.83 ±0.60	6.76* ± 0.03	4.26* ± 0.18	4.70 ± 0.32	4.36* ± 0.14	2.56* ± 0.14	3.76* ± 0.29	1.90* ± 0.05	1.46* ± 0.17	2.30* ± 0.15	1.23* ± 0.08	0.90* ± 0.11	1.66* ± 0.33
Barely	5.76 ± 0.17	5.50 ± 0.15	5.70 ± 0.17	8.33* ± 0.17	5.43 ± 0.12	6.63* ± 0.14	9.53* ± 0.17	5.53 ± 0.13	7.26* ± 0.17	5.86 ± 0.20	4.46 ± 0.41	6.00 ± 0.28	4.73* ± 0.14	3.60* ± 0.11	4.86* ± 0.29
Garden pea	4.63 ± 0.08	4.10 ± 0.05	3.43 ± 0.08	4.40 ± 0.11	3.93 ± 0.03	3.20 ± 0.15	3.83* ± 0.18	3.23* ± 0.12	2.73* ± 0.14	2.53* ± 0.08	2.16* ± 0.06	1.60* ± 0.17	1.20* 0.10	1.56* ± 0.28	1.16* ± 0.17
Black gram	2.16 ± 0.12	1.56 ± 0.06	5.90 ± 0.15	1.83 ± 0.06	1.33* ± 0.03	5.36 ± 0.12	1.63* ± 0.08	0.83* ± 0.03	3.90* ± 0.11	0.83* ± 0.03	0.50* ± 0.05	2.06* ± 0.12	0.36* 0.03	0.40* ± 0.05	1.50* ± 0.25
Mustard	2.53 ± 0.17	1.36 ± 0.03	3.20 ± 0.15	1.93* ± 0.03	1.03* ± 0.03	2.30* ± 0.11	1.13* ± 0.06	0.76* ± 0.03	1.80* ± 0.11	0.63* ± 0.08	0.46* ± 0.03	1.26* ± 0.12	0.43* 0.03	0.33* ± 0.03	0.60* ± 0.15

Values are mean of three replicates ± S.E.
* Statistically significant at p<0.05 level

Table - 3: Effect of different dilution levels of distillery and sugar factory mixed treated effluent on fresh and dry weights (g) and moisture contents (%) in 2 week old seedlings

Type of seed	Control			25%			50%			75%			100%		
	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content	Fresh weight	Dry weight	Moisture content
Wheat	0.99 ± 0.020	0.21 ± 0.006	78.58 ± 1.35	0.70* ± 0.015	0.18* ± 0.005	74.28* ± 0.55	0.52* ± 0.011	0.15* ± 0.003	70.48* ± 0.89	0.52* ± 0.015	0.17* ± 0.005	67.18* ± 2.08	0.32* ± 0.011	0.11* ± 0.012	64.69* ± 3.06
Barely	0.93 ± 0.008	0.20 ± 0.010	78.58 ± 0.93	1.16* ± 0.026	0.18 ± 0.012	84.79* ± 1.03	0.93 ± 0.015	0.20 ± 0.012	81.48 ± 2.89	0.85* ± 0.018	0.10* ± 0.006	88.59* ± 0.64	0.63* ± 0.017	0.16 ± 0.012	74.86 ± 2.57
Garden pea	3.36 ± 0.038	0.90 ± 0.017	73.33 ± 0.63	3.28 ± 0.005	0.98* ± 0.023	69.81* ± 0.73	3.22* ± 0.032	0.91 ± 0.012	71.80 ± 0.63	3.20* ± 0.012	1.04* ± 0.026	67.47* ± 0.70	2.59* ± 0.020	0.82* ± 0.015	68.28* ± 0.81
Black gram	1.20 ± 0.011	0.36 ± 0.020	70.00 ± 1.68	1.14 ± 0.017	0.34 ± 0.023	70.00 ± 1.73	1.06 ± 0.049	0.30 ± 0.015	71.80 ± 0.13	0.90* ± 0.051	0.28* ± 0.017	68.64 ± 0.51	0.43* ± 0.033	0.15* ± 0.006	64.33 ± 2.33
Mustard	0.18 ± 0.005	0.03 ± 0.003	85.26 ± 1.50	0.15 ± 0.012	0.03 ± 0.003	81.66 ± 2.56	0.11* ± 0.008	0.02 ± 0.006	84.42 ± 5.90	0.08* ± 0.005	0.02 ± 0.006	71.62 ± 7.14	0.05* ± 0.012	0.01* ± 0.000	78.57 ± 5.99

Values are mean of three replicates ± S.E.
* Statistically significant at p< 0.05 level

Table - 4: Effect of different dilution levels of distillery and sugar factory mixed treated effluent on total chlorophyll contents and total amylase activity in 2 week old seedlings

Type	Control		25%		50%		75%		100%	
of seed	Chlorophyll	Amylase	Chlorophyll	Amylase	Chlorophyll	Amylase	Chlorophyll	Amylase	Chlorophyll	Amylase
Wheat	2.153 ± 0.090	0.67 ± 0.020	1.700* ± 0.116	0.64 ± 0.017	1.233* ± 0.069	0.51* ± 0.012	0.734* ± 0.026	0.43* ± 0.025	0.587* ± 0.034	0.25* ± 0.025
Barely	1.781 ± 0.077	0.76 ± 0.023	2.351* ± 0.124	0.85 ± 0.036	2.721* ± 0.126	0.96* ± 0.024	1.897 ± 0.058	0.81 ± 0.014	1.123* ± 0.104	0.59* ± 0.018
Garden pea	1.207 ± 0.058	0.54 ± 0.012	1.008 ± 0.068	0.52 ± 0.011	0.814* ± 0.059	0.47* ± 0.008	0.612* ± 0.031	0.33* ± 0.021	0.308* ± 0.038	0.23* ± 0.032
Black gram	1.100 ± 0.052	0.48 ± 0.013	0.901* ± 0.016	0.42* ± 0.013	0.801* ± 0.016	0.32* ± 0.021	0.475* ± 0.025	0.23* ± 0.015	0.250* ± 0.030	0.20* ± 0.021
Mustard	0.814 ± 0.020	0.42 ± 0.020	0.652* ± 0.016	0.34* ± 0.005	0.439* ± 0.031	0.29* ± 0.018	0.252* ± 0.024	0.18* ± 0.017	0.105* ± 0.011	0.12* ± 0.017

Chlorophyll contents measured in mgg^{-1} fresh weight of tissue
Amylase activity measured in terms of starch hydrolyzed in mgg^{-1} fresh weight of tissue
Values are mean of three replicates ± S.E.
* Statistically significant at $p < 0.05$ level

decreased seed germination and seedling growth (Malla and Mohanty, 2005; Singh *et al.*, 2006).

The dilution level (more than 5%) of distillery effluents has been reported as toxic by Sharma *et al.* (2002), and they also recommended the use of distillery effluent for irrigation purpose after proper dilution. Our results clearly show that the different lower dilution levels were growth inhibitory and toxic while higher dilution levels were within tolerance limits. Hence the results obtained in our experiment are also in resemblance to the findings of Sharma *et al.* (2002). These results are also in resemblance to the findings of Rajannan *et at.* (1998), Kumar (1999) and Kumar and Prasad (1999), who showed the carbonaceous sugar mill effluent as a liquid fertilizer and noticed its inhibitory effects on chlorophyll contents, if used in higher concentration. They also studied the alteration in physico-chemical properties of soil irrigated with water having sugar factory effluent.

The treated sugar factory effluent as well as untreated sugar factory effluent as such have been shown as toxic (Goel and Kulkarni *et al.*, 1994; Nighat *et al.*, 1991; Om *et al.*, 1994; Patil *et al.*, 2001). Likewise, in our results they were found toxic in the experiment, when used in lower dilution levels, but the different higher dilution levels of treated distillery and sugar factory combined effluent can be used for irrigation at different stages in different plants.

This way the present findings provide a suitable strategy under reutilization and recycling of industrial effluents for irrigation purpose in agriculture with reference to different plant species.

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