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Irrigational impact of untreated and treated brewery-distillery effluent on seed germination of marigold (*Tagetes erecta* L.)

Anuradha Sharma and Piyush Malaviya*
Department of Environmental Sciences, University of Jammu, Jammu-180 006, India
*Corresponding Author E-mail : **piyushmalaviya@rediffmail.com**

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Abstract

Current study presents the effect of irrigation with different concentrations (20, 40, 60, 80 and 100%) of untreated and treated brewery-distillery effluent on germination behaviour of marigold (*Tagetes erecta* L. var. Pusa Basanti). The 100% untreated effluent showed acidic pH (4.80) and higher values of BOD (1500.00 mg l^{-1}), COD (4000.00 mg l^{-1}), chloride (1742.20 mg l^{-1}), TSS (900.00 mg l^{-1}) as compared to that of treated effluent. *Tagetes* seeds were exposed to different concentrations of effluent and the results revealed maximum values of germination parameters viz., percent germination, peak value, germination value, germination index, speed of germination and vigour index at 20% untreated and 60% treated effluent concentrations, whereas the values for negative germination parameters viz., delay index, germination period and percent inhibition were minimum at 20% untreated and 60% treated effluent concentrations.

Key words

Effluent, Germination, Irrigation, Plant, Pollution

Introduction

Disposal of wastewater is a worldwide problem faced by industries (Malaviya and Rathore, 2001; Kohli and Malaviya, 2013). Among various industries, molasses based industries (distilleries) are recognised as one of the most polluting agro-based industries generating huge quantities of distillery spentwash (SW) loaded with high BOD, COD values, low pH, dark brown colour with a foul smell, phenolic compounds, sulphate and heavy metal load (Chandra *et al*., 2004). The liquid residues produced during the industrial phase of alcohol production are liquor, sugarcane washing water, water from condensers and cleaning of equipments (Pant and Adholeya, 2007).

Effluent, when discharged as such (untreated or partially treated) into the water bodies, adversely affects aquatic ecosystem by reducing the penetration power of sunlight and ultimately reduces the photosynthetic activities and dissolved oxygen content, whereas in agricultural soil it causes inhibition of seed germination by reducing soil alkalinity and manganese availability (Kumar and Chandra, 2006). Hence, wastewater requires pre-treatment before its safe disposal into the environment (Kumar and Chandra, 2006; Mohana *et al*., 2009).

Utilization of industrial waste as soil amendment has generated interest in recent times (Kaushik *et al*., 2005). Although, distillery effluents contain many constituents which are phytotoxic at higher concentration but some of the constituents at lower concentrations are beneficial for growth and development of plants. In view of this, many workers have shown deleterious effects of distillery effluent on plant parameters at higher concentrations (Bhargava *et al*., 2008; Pandey *et al*., 2009; Malaviya and Sharma, 2011a; Malaviya and Sharma, 2011b). However, the effect of distillery effluent is crop-specific and due care should be taken before using the effluent for pre-sowing irrigation purposes (Ramana *et al*., 2002). Therefore, in the present study, an attempt was made to study the impact of untreated (raw) and treated brewery-distillery effluent on germination parameters of marigold (*Tagetes erecta*).

Materials and Methods

The untreated and treated effluent samples used for the present study, collected in pre-cleaned containers at regular intervals (on weekly basis) from a Brewer and

Distiller located in Jammu (India), were analysed for their physico-chemical characteristics using standard methods of APHA (1998). Tested seeds (of above 90% viability) of *Tagetes erecta* L. var. Pusa Basanti were procured from Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu. The garden soil, collected from the Department of Arboriculture, University of Jammu, Jammu, was sun-dried and mixed with sand and farmyard manure in 3:1:1 ratio (Soil: Sand: F.Y.M). Soil treatment was also done by drenching the soil with Bavistine (fungicide) @1.5 g l^{-1} of water.

The experimental set-up was designed in the form of eleven treatment sets; each consisted of three replicates; plastic trays (length 450 mm; width 350 mm and height 75 mm) filled with equal quantities (5 kg) of the prepared soil. Out of the eleven treatment sets, first set was taken as control (E_0) in which tap water was used for irrigation and for the next five treatments (designated as UE_{20}, UE_{40}, UE_{60}, UE_{80}, UE_{100}); different concentrations (20, 40, 60, 80 and 100%) of untreated effluent were used. In remaining five treatments, different concentrations of treated effluent (20, 40, 60, 80, and 100%) were used for irrigation and designated as TE_{20}, TE_{40}, TE_{60}, TE_{80}, TE_{100}, respectively. In each replicate, hundred seeds were sown (about 2-3 cm deep) giving proper spacing among them. Seeds were irrigated with equal quantity (400 ml $tray^{-1}$ d^{-1}) of various concentrations of untreated and treated effluent.

The number of seeds germinated was recorded on alternate days from the 3rd day after sowing (DAS) upto the 30th DAS when the number of seeds germinated became constant and percent germination was obtained. The emergence of radicle of 2 mm length was taken as a criterion for germination (Malaviya *et al*., 2012). All the experiments were carried out in triplicates and the results were averaged. Number of seedlings emerging in each replicate was noted and various indices were calculated. Germination index was calculated by the formula of Zucconi *et al*. (1981). Delay index (delay in germination time over control) was calculated to compare the performance of crop under different effluent concentrations (Modified after Kaushik *et al*., 2005). Vigour index was estimated following the method of Abdul Baki and Anderson (1973). Similarly, some other parameters like percent inhibition (percent germination of treatment over control), peak value (cumulative percent germination divided by the number of days since initial imbibition), germination value (multiplication of peak value and % germination) and speed of germination (rate of seed germination over time) were estimated by formula of Rao *et al*. (1979) and Czabator (1962).

Statistical analysis : All the experiments were conducted in triplicate. The data obtained were statistically analysed using SPSS Inc. (v.16.0) software for mean and standard error. Multivariate analysis of variance was performed to observe the effect of effluent concentrations and plant age on germination parameters of *Tagetes erecta*. The quantitative changes due to effluent application were evaluated at 5% using Duncan's Multiple range Test.

Results and Discussion

The untreated effluent used in the present study was dark brown in colour with acidic pH (4.8), while treated effluent

Table 1 : Effect of different concentrations of untreated and treated Brewery-Distillery effluent on various germination parameters of *Tagetes erecta*

Treatments	Parameters								
	Cumulative % germination	Peak value	Germination value	Germination Index (%)	Speed of germination	Vigour index	Delay index	Germination period (days)	Percent inhibition
Control (E_0)	74.33	4.96	368.68	-	28.51	1728.17	-	12	-
Untreated effluent (UE)[1]									
UE_{20}	74.67	4.98	371.86	101.57	28.57	17.53.99	-	12	-0.34
UE_{40}	63.00	3.71	233.73	82.07	23.34	1432.62	0.22	14	11.33
UE_{60}	56.67	3.33	188.71	72.97	19.75	1281.31	0.44	14	17.66
UE_{80}	53.00	3.12	165.36	67.18	19.36	1191.44	0.44	14	21.33
UE_{100}	51.00	2.68	136.68	63.24	19.28	1138.83	0.89	16	23.33
Treated effluent (TE)[2]									
TE_{20}	75.00	5.00	375.00	103.53	28.59	1787.25	-	12	-0.67
TE_{40}	77.00	5.13	395.01	107.45	28.71	1867.25	-	12	-2.67
TE_{60}	79.00	5.27	416.33	115.58	29.01	1949.72	-	12	-4.67
TE_{80}	74.00	4.35	321.90	98.62	27.28	1704.96	-	14	0.33
Te_{100}	64.67	3.80	245.75	85.21	22.60	1484.18	0.22	14	9.66

[1]UE_{20}, UE_{40}, UE_{60}, UE_{80}, UE_{100} : 20, 40, 60, 80 and 100% untreated effluent; [2]TE_{20}, TE_{40}, TE_{60}, TE_{80}, TE_{100} : 20, 40, 60, 80 and 100% treated effluent, respectively

Table 1.: Effect of different concentrations of untreated and treated Brewery-Distillery effluent on germination[1] (%) *Tagetes erecta*

	Days after sowing (DAS)								
Treatments	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**
Control (E_0)	$1.67^{a}\pm0.33$	$5.33^{a}\pm0.33$	$34.67^{ab}\pm4.06$	$60.67^{ab}\pm1.76$	$67.33^{ab}\pm0.88$	$73.33^{ab}\pm1.67$	$74.33^{a}\pm1.20$	$74.33^{abc}\pm1.20$	$74.33^{ab}\pm1.20$
Untreated Effluent (UE)[2]									
UE_{20}	$0.67^{ab}\pm0.33$	$3.67^{abc}\pm0.33$	$36.00^{ab}\pm1.15$	$67.00^{a}\pm1.15$	$72.00^{a}\pm1.15$	$72.67^{ab}\pm0.67$	$74.67^{a}\pm0.33$	$74.67^{abc}\pm0.33$	$74.67^{ab}\pm0.33$
UE_{40}	$1.00^{ab}\pm0.00$	$3.00^{bc}\pm0.58$	$28.67^{abc}\pm2.91$	$48.67^{cd}\pm1.20$	$57.00^{c}\pm0.58$	$58.67^{cd}\pm0.67$	$61.00^{cd}\pm0.58$	$63.00^{cde}\pm1.15$	$63.00^{bcd}\pm1.15$
UE_{60}	$0.67^{ab}\pm0.33$	$3.00^{bc}\pm0.58$	$15.00^{d}\pm0.58$	$43.67^{d}\pm3.18$	$48.67^{cd}\pm4.06$	$52.00^{de}\pm2.31$	$53.67^{cde}\pm1.20$	$56.67^{def}\pm0.33$	$56.67^{cde}\pm0.33$
UE_{80}	$1.00^{ab}\pm0.58$	$2.00^{c}\pm0.58$	$18.00^{cd}\pm2.31$	$44.00^{d}\pm1.73$	$49.00^{cd}\pm0.58$	$52.00^{de}\pm0.00$	$52.00^{de}\pm0.00$	$53.00^{ef}\pm0.00$	$53.00^{de}\pm0.00$
UE_{100}	$1.67^{a}\pm0.33$	$2.00^{c}\pm0.00$	$25.33^{bcd}\pm4.81$	$40.00^{d}\pm5.77$	$43.33^{d}\pm7.22$	$47.67^{e}\pm8.37$	$49.67^{e}\pm8.67$	$50.00^{f}\pm8.96$	$51.00^{e}\pm9.24$
Treated Effluent (TE)[3]									
TE_{20}	$0.00^{b}\pm0.00$	$3.00^{bc}\pm0.58$	$39.00^{a}\pm2.31$	$67.33^{a}\pm1.76$	$71.67^{a}\pm2.60$	$74.00^{a}\pm3.46$	$75.00^{a}\pm3.79$	$75.00^{ab}\pm3.79$	$75.00^{ab}\pm3.79$
TE_{40}	$1.67^{a}\pm0.33$	$3.67^{abc}\pm0.67$	$35.33^{ab}\pm4.84$	$54.00^{bc}\pm4.04$	$73.33^{a}\pm1.76$	$75.00^{a}\pm1.15$	$77.00^{a}\pm2.31$	$77.00^{a}\pm2.31$	$77.0^{a}\pm2.31$
TE_{60}	$0.67^{ab}\pm0.33$	$3.67^{abc}\pm1.20$	$35.67^{ab}\pm8.29$	$58.00^{b}\pm1.15$	$71.00^{a}\pm3.46$	$78.67^{a}\pm2.33$	$79.00^{a}\pm2.31$	$79.00^{a}\pm2.31$	$79.00^{a}\pm2.31$
TE_{80}	$1.67^{a}\pm0.33$	$4.67^{ab}\pm0.67$	$23.67^{bcd}\pm0.33$	$60.67^{ab}\pm2.91$	$69.67^{a}\pm1.20$	$72.67^{ab}\pm1.20$	$72.67^{ab}\pm1.20$	$74.00^{abc}\pm2.08$	$74.00^{ab}\pm2.08$
Te_{100}	$0.67^{ab}\pm0.33$	$2.67^{bc}\pm0.33$	$19.0^{cd}\pm0.58$	$45.67^{cd}\pm1.76$	$58.67^{bc}\pm5.21$	$63.00^{bc}\pm4.62$	$63.00^{bc}\pm4.62$	$64.67^{bcd}\pm5.55$	$64.67^{bc}\pm5.54$

Within each column, values not followed by the same letter are significantly different at $p < 0.05$; [1]values are mean of three replicates ±SE; [2]UE_{20}, UE_{40}, UE_{60}, UE_{80}, UE_{100} : 20, 40, 60, 80 and 100% untreated effluent; [3]TE_{20}, TE_{40}, TE_{60}, TE_{80}, TE_{100} : 20, 40, 60, 80 and 100% treated effluent

was light brown in colour and slightly alkaline in nature having 8.3 pH. Physico-chemical analysis of untreated and treated effluent revealed that 100% untreated effluent exhibited high BOD (1500.00 mg l^{-1}), COD (4000.00 mg l^{-1}), chloride (1742.20 mg l^{-1}) and TSS (900.00 mg l^{-1}) which exceeded CPCB discharge standards (1998), while TDS (1909.50 mg l^{-1}) was found within the permissible limits. The values of the above mentioned parameters of treated effluent was found within the permissible limits. Magnitude of the analyzed parameters was lower for treated effluent than untreated effluent. Values of various parameters for different effluent concentrations (both untreated and treated) showed an increasing trend of EC, Op, turbidity, chloride, TDS, TSS, and TS with increasing concentration of both the effluents. Minimum values were observed in 20% treated effluent, while maximum values were noted in 100% untreated effluent. However, there was a decreasing trend in pH with increasing concentration of untreated effluent, with highest pH (6.13) noted in 20% effluent and lowest (4.80) in 100% effluent concentration, respectively.

Table 1 shows the values of various germination parameters (cumulative % germination, peak value, germination value, germination index (%), speed of germination, vigour index) which were maximum at 20% untreated and 60% treated effluent concentrations as compared to control and then decreased gradually with increase in effluent concentrations (both untreated and treated). Similarly, for negative germination parameters (delay index, germination period, percent inhibition) the values were minimum at 20% untreated and 60% treated effluent and increased with increase in effluent concentrations (highest at 100% of both untreated and treated effluent). The results revealed that there existed a specific correlation between different effluent concentrations and various germination parameters.

The value of germination index was found to be highest in UE_{20} (101.57) and lowest in UE_{100} (63.24) when treated with 20% and 100% untreated effluents. However, germination index was highest in TE_{60} (115.58) and lowest in TE_{100} (85.21) when, treated with 60% and 100% treated effluent, respectively. This may be attributed to the fact that high salt concentration in the effluent induces high osmotic pressure and anaerobic condition which affects various biochemical processes such as movement of solute, respiration process of seeds and enzymatic steps of seed germination (Bharagava and Chandra, 2010). Increasing effluent concentration showed deleterious effect on the vigour index. The values of vigour index were relatively higher in UE_{20} (1753.99) and TE_{60} (1949.72) than control, but gradual decrease in vigour index with increase in effluent concentration (both untreated and treated) *i.e.* in UE_{100} (1138.83) and TE_{100} (1484.18) was observed. Similar trends were observed by Pandey *et al.* (2009) while studying the effect of treated distillery effluent on pea and wheat. They found that the vigour index was maximum at 50% treated distillery effluent which further decreased with increase in effluent concentration. Indra and Mycin (2009) found that low concentration (10%) of tannery effluent, promoted vigour index in five varieties of *Vigna mungo* L.

It was observed that germination value was highest in UE_{20} (371.86) followed by E_0 (368.68) and E_{40} (233.73) and lowest in UE_{100} (136.68) in case of untreated effluent, while for treated effluent germination value was highest in TE_{60} (416.33) and lowest in TE_{100} (245.75) as compared to control. This may be attributed to the toxicity caused due to increasing amount of various organic and inorganic compounds present at higher concentrations of distillery effluent (Malaviya and Sharma, 2011a). It was also observed that peak value was maximum in UE_{20} (4.98) and TE_{60} (5.27), while minimum in UE_{100} (2.68) and TE_{100} (3.80). Speed of germination also followed similar trend, and was found to be maximum in UE_{20} (28.57) and TE_{60} (29.01) and minimum in UE_{100} (19.28) and TE_{100} (22.60), respectively. On the other hand, percent inhibition was found to be maximum in UE_{100} (23.33) and TE_{100} (9.66) and minimum in UE_{20} (-0.34) and TE_{60} (-4.67), which can be attributed to increase in salt content with increasing effluent concentrations.

The present findings are corroborated well with that of Pandey *et al*. (2007) who studied the impact of treated distillery effluent on seeds. *Triticum aestivum*, *Pisum sativum* and *Abelmoschus esculentus* and found that germination speed, peak value and germination values increased gradually from control upto 50% effluent concentration and then decreased furthers. Ramana *et al*. (2002) found that higher concentrations (75% and 100% raw spentwash) of distillery effluent caused inhibitory effects on seed germination (%), germination speed peak value and germination value of some vegetable crops (*Lycopersicum esculentum*, *Capsicum annum*, *Lagenaria siceraria*, *Cucumis sativus*, *Allium cepa*). Garg and Kaushik (2008) found that low concentration (6.25%) of textile effluent did not show any inhibitory effect on seed germination and delay index of two cultivars of *Sorghum vulgare.*

The percent germination on exposure of different concentrations (both untreated and treated) and duration upto 30 DAS is presented in Table 2. Maximum germination was recorded at 20% untreated (74.67) and 60% treated (79.00) effluent concentrations, whereas at 100% concentration of untreated and treated effluent, minimum values of percent germination (51.00 and 64.67), were recorded. Overall, irrigation with untreated effluent showed the following trend for percent germination as compared to control: $UE_{20}>E_0>UE_{40}>UE_{60}>UE_{80}> UE_{100}$. On the other hand, the germination pattern following treatment with treated effluent showed the following trend: $TE_{60}>TE_{40}>TE_{20}>E_0> TE_{80}>TE_{100}$. It is also evident that the germination became constant in Control (E_0), UE_{20}, TE_{20}, TE_{40} and TE_{60} on 15 DAS whereas in UE_{40}, UE_{60}, UE_{80}, TE_{80} and TE_{100} on 17 DAS and in UE_{100}, it became constant on 19 DAS.

The results of the present study are consistent with the findings of Wins and Murugan (2010) who observed that germination of *Vigna mungo* L. Hepper was maximum at lower concentration (25%) of textile mill effluent which decreased further with increase in effluent concentration. Similar results were obtained by Nagajyothi *et al*. (2009) while observing the effect of industrial effluent on green gram (*Phaseolus aureus* Roxb). Maximum germination was found at lower concentration (25% effluent) which further decreased with increase in effluent concentration, as compared to control. Inhibition of seed germination at higher effluent concentration is attributed to high level of dissolved solids, which enhances salinity and conductivity of the effluent (Singh *et al*., 2006). Further, Rehman *et al*. (2009) found reduction in germination rate in *Brassica napus* at higher effluent concentration (100% untreated effluent) of textile industry which might be due to the presence of high concentration of Ni^{2+}, Cd^{2+}, Pb^{2+} and other toxic organic compounds in the textile effluent that cause a range of cellular toxicities. The present resent findings are also supported by Bharagava *et al*. (2008) who observed that 50% (v/v) post-methanated distillery effluent increased seed germination in *Brassica nigra* L. (mustard), which decreased with increase in effluent concentration (75 and 100% v/v). It can be concluded from the present study that appropriate dilutions of the effluent *i.e.,* 20% untreated and 60% treated brewery-distillery effluent could be used for irrigation to enhance germination of *Tagetes erecta*.

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