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IMPACT OF DISTILLERY EFFLUENT ON GERMINATION, MORPHO-LOGICAL AND BIOCHEMICAL PARAMETERS OF *LYCOPERSICUM ESCULANTUM* MILL. AND *CAPSICUM ANNUM*. L

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ABSTRACT

This study was undertaken to evaluate the effect of different concentrations of distillery effluent on various parameters of seed germination and growth, morphological and biochemical parameters of tomato (*Lycopersicum esculantum* Mill.) and capsicum (*capsicum annum*. L).The 100% effluent showed no growth. The study showed the maximum values of positive germination viz. speed of germination (1.86) and (2.2), peak value (3) and (3.3), percentage germination (90) and (100) at 25% effluent concentration in case of tomato and capsicum respectively. The maximum values of morphological parameters viz. shoot length (22.41cm) and (14.86cm), root length (10.4cm) and (5.83cm) were observed at 25% effluent concentration in tomato and capsicum respectively. In case of biochemical parameters, the chlorophyll and protein content showed a gradual decline with increase in effluent concentration. Among the two plants studied, *C. annum was* found to be more resistant to effluent effect than *L. esculentum*.

Keywords: *Capsicum annum*, Distillery effluent, *Lycopersicum esculentum*, Physico chemical parameters

INTRODUCTION

Human evolution has led to gigantic scientific and technological progress. Global development, on the other hand, raises new challenges, particularly in the field of environmental protection and conservation [1]. Almost every government around the world wants surroundings free from harmful contamination for their people. Yet, the demand for a country's economic, agricultural and industrial development outweighs the demand for a safe, clean, and natural environment. Ironically, it is the economic, agricultural and industrial developments that are often linked to polluting the environment [2]. Environmental pollution constitutes a great health hazard to human being, flora and fauna [3]. Among the various kinds of pollution, the water pollution is the serious problem in India. Water is one of the 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 thus, its careful utilization is important. The problem of environmental pollution due industrial growth has led to the problem of disposal of industrial waste, whether solid, liquid or gaseous. All three types of wastes probably pollute the water. Polluted water, in addition to other directly affects soil not only in industrial areas but also in agricultural fields, as well as the beds of rivers, creating secondary sources of pollution [4, 5]. In India, there are about 7500 medium to large industrial units, which produce lot of wastewater, which is difficult and costly to treat. Wastewater quality 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). Currently, there are 319 distilleries in India with an installed capacity of 3.29 billion litre of alcohol [6]. In the distillery industry, about 15 litres of spent wash is released as wastewater, for every litre of alcohol produced. The spent wash is acidic, dark brown liquid with high BOD and COD and emits unbearable odour [7]. 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 [8, 9, 10, 11]. The present investigation was carried out to examine the impact of distillery effluent on the various germination, morphological and biochemical parameters of *Lycopersicum esculantum* Mill and *capsicum annum*.

MATERIAL AND METHODS

The distillery effluent was collected and various physico-chemical parameters like colour,

odour, pH, EC (Electrical conductivity), TSS (Total suspended solids), TDS (Total dissolved solids), BOD (Biological oxygen demand) and COD (Chemical oxygen demand) were analysed as per the method of [12]. Different concentrations of effluent (5%, 25%, 50%.75% and 100%) were prepared by mixing stock effluent and distilled water (v/v). The healthy and viable seeds of *Lycopersicum esculentum and capsicum annum* were surface sterilized with 0.1% mercuric chloride for one minute and washed with distilled water. Then the seeds were soaked in distilled water for about 2 hours. The 10 seeds of Tomato and capsicum were sown in different earthen pots containing uniformly mixed garden soil (sand, red soil and Black soil 1:1:1 ratio) and allowed to germinate in controlled light. Initially the pots were irrigated with tap water for 7 days. There after the pots were supplied with respective concentration of the effluent for a week. Control sets were maintained with tap water. After 30 days, the soils and plants of respective doses and control were collected and measured the physiochemical characteristic and biochemical parameters.

Growth parameters

The growth parameters such as root length, shoot length, leaf area and number of leaves/plant were measured. Germination Index (GI) was calculated by the formula given by [13], Delay Index (DI) by following the methodology modified after [14] and Vigour Index by [15]. Similarly, some other parameters like percent inhibition, germination value, peak value, speed of germination time were also estimated using formulae adopted from [16, 17].

Biochemical characters

Chlorophyll a, chlorophyll b and total chlorophyll, fresh leaf tissues were extracted with 100% acetone and were quantified using the formula of [18]. Leaf soluble protein [19] and Total phenol [20] were analysed both for control and experimental seedlings of *L. esculentum*, Mill. and *C. annum.*

Statistical analysis

Means, standard deviation and 95% confidence intervals were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA). Data was subjected to a single way analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Physico-chemical analysis of distillery effluent

The distillery effluent used for the present study was a light brown coloured odorous waste released from the industry. Analysis of the effluent for its various physico-chemical characteristics showed that it had high values of COD (2489.6 mg l^{-1}), TDS (1434.6 mg l^{-1}) and BOD (790.1mg l^{-1}) (Table1).

Physiochemical characteristics of soil irrigated with different concentrations of distillery effluent

Physiochemical characteristic of soil with different concentrations of distillery effluent were measured after 30 days of treatment and tabulated in table-2. pH is an important parameter for soil which determine the availability of nutrients in soil. It was found that at lower concentration the soil pH was increased over control further increase of effluent concentration in soil resulted a decreased value of pH. Dilution factor effect the alkalinity of soil pH, above 75% dilution the soil shifted towards acidity. The previous studies reported that at a particular pH range the nutrient availability enhanced which increased plant growth and availability of nutrients in soil is inhibited with change in pH range from a particular limit that effected the growth and development of plant [21]. EC of soil increased over control and a negative correlation found between doses of effluent concentration in soil and its EC values. At higher effluent concentration, the EC values increased which might be due to the increased different salt concentration in soil. The present result showed a positive correlation with moisture content and effluent concentration. It may be suggested that the effluent load with higher doses in soil increase the soil particle size, it may negatively impact on soil moisture content. Water holding capacity is related to the number, size and distribution of soil pore and quality of organic matter in soil medium. The present result observed that WHC decrease when effluent dose concentrations were increased in soil. It might be suggested the effluent salt load and organic matter increased in soil with higher doses which decreased number, size and quality of soil pore and it leads to determine the WHC. It was reported that WHC is related to soil moisture content and organic matter [22]. Organic carbon content of soil increased considerably with the application of different doses to soil starting from 5 % to 100 %. Increased organic content with higher doses of effluent with soil resulted in soil sickness due to poor aeration and higher BOD. These findings were supported by the results of [23, 24, 25]. Total phosphate, Nitrogen and Potassium in treated soil were increased in different doses of effluent i.e. 5%, 25%, 50%, 75% and 100% over control soil.

Effect on germination parameters

The effect of distillery effluent on various germination parameters of (*Lycopersicum esculantum* Mill.) and capsicum (*capsicum annum*. L) are shown in Table 3. It was observed that values for positive germination parameters i.e. germination percentage, mean

germination time, germination value, speed of germination, mean daily germination and peak value were found to be highest in 25% effluent concetration in case of tomato and capsicum respectively and lowest in 75% effluent concentration.

It has been observed that the germination value in case of tomato was highest in 25% effluent concentration (0.9) followed by 5% (0.69) and control (0.55) and in case of capsicum highest value was observed in 25% effluent concentration (1.09) followed by 5% (0.9) and control (0.67) and lowest in 75% effluent concentration. The decreasing trend of germination value of the plants with increasing effluent concentrations may be attributed to the lesser uptake of nutrients and increase in osmotic potential of the soils irrigated with higher effluent concentrations [26]. while studying the effect of distillery effluent on some vegetable crops observed the similar results. Therefore, plants irrigated with higher concentrations of effluents have lower germination rates. It was also observed that peak value of germination was found to be maximum in 25% of effluent concentration (3) and (3.33) in tomato and capsicum respectively and speed of germination also followed the same trend whereas percent inhibition was found to be maximum in 75% effluent concentration (57.2) and (37.5) in tomato and capsicum respectively, which can be attributed to the increase in salt content with increasing concentrations of the effluent.

Effect on morphological parameters

The effects of distillery effluent on various morphological parameters of (*Lycopersicum esculantum* Mill.) and capsicum (*capsicum annum*. L) are shown in Table-4 which depicts a decreasing trend with increase in effluent concentration for all the parameters (root length and shoot length, leaf surface area, leaves/plant and length of leaves).

The maximum values were observed at 25% of effluent concentration in tomato and capsicum respectively whereas minimum values were revealed at 75% effluent concentration and there was no germination in 100% effluent concentrations. Similar study was observed that distillery effluent showed positive impact on all the growth parameters at lower effluent concentration and then decreased with increase in effluent concentration [27]. The better growth at 25% effluent concentration may be attributed to the growth promoting effect of nitrogen and other mineral elements present in the effluent.

Effect on biochemical parameters

The effects of distillery effluent on various biochemical parameters of (*Lycopersicum*

esculantum Mill.) and capsicum (*capsicum annum*. L) are shown in Table-5 the pigment content showed a declining trend with increasing concentrations of distillery effluent. [28] studied the impact of dye industrial effluent on *Phaseolus mungo*, L., and reported a declining trend in pigment content. The same was reported in Vigna mungo, by distillery effluent [29]. Similar reduction in pigment level was observed in many plants by various industrial effluent irrigation by [30]. One of the major molecules severely affected by stress is protein [31]. Hence, the total soluble protein in leaf was analyzed both for tomato and capsicum. The results revealed that the reduction in protein content was very obvious with an increase in the concentrations of effluent, when compared to the control plants. Phenol is one of the major chemical constituents, providing good protection against stress injury in plants [32]. It has also been illustrated that, the synthesis of phenol triggered only under stressful environments [33]. Hence, the impact of distillery effluent on the total phenol content was analyzed. It was found that there was more prominent increase in the phenol content of the distillery effluent treated *capsicum annum*. L than that of the effluent treated *Lycopersicum esculantum.*

Table 1: Physico-chemical characteristics of distillery effluent

Parameters	Values*
Colour	Light brown
Odour	Pungent
pH	5.56 ± 0.05
Ec (dSm^{-1})	4.23 ±0.15
TSS ($mg\ l^{-1}$)	1715.3 ±6.09
TDS ($mg\ l^{-1}$)	1434.6 ±5.81
BOD ($mg\ l^{-1}$)	790.1 ±9.06
COD ($mg\ l^{-1}$)	2489.6 ±2.31

*** Data are presented as mean ± sd (n = 3)**

Table 2: Physiochemical characteristics of soil after treatment with different concentration of distillery effluent

Parameters	Effluent concentration (%)					
	Control	5%	25%	50%	75%	100%
pH	7.17 ± 0.08^{cd}	7.52 ± 0.11^{e}	7.29 ± 0.09^{d}	7.12 ± 0.06^{c}	6.48 ± 0.16^{b}	6.10 ± 0.10^{a}
EC (dSm^{-1})	1.93 ± 0.13^{a}	2.20 ± 0.10^{b}	2.43 ± 0.07^{c}	2.62 ± 0.17^{d}	2.75 ± 0.11^{d}	3.18 ± 0.11^{e}
WHC (%)	46.09 ± 0.18^{f}	44.86 ± 0.50^{e}	40.94 ± 0.44^{d}	37.79 ± 0.30^{c}	34.00 ± 0.20^{b}	25.60 ± 0.23^{a}
Soil moisture (%)	37.80 ± 1.08^{cd}	40.81 ± 0.73^{e}	38.59 ± 1.10^{d}	37.34 ± 0.53^{c}	34.54 ± 0.43^{b}	30.81 ± 0.69^{a}
OC (%)	1.26 ± 0.05^{a}	1.41 ± 0.11^{b}	1.84 ± 0.14^{c}	2.34 ± 0.10^{d}	2.58 ± 0.08^{e}	3.54 ± 0.21^{f}
Available phosphorus (kg/ha)	6.34 ± 0.44^{a}	6.53 ± 0.27^{a}	7.56 ± 0.18^{b}	8.62 ± 0.15^{c}	9.52 ± 0.21^{d}	10.09 ± 0.08^{e}
Available potassium(kg/ha)	227.2 ± 1.55^{a}	274.7 ± 2.80^{b}	311.4 ± 0.58^{c}	340.6 ± 1.27^{d}	386.5 ± 0.75^{e}	412.9 ± 0.15^{f}
Available nitrogen (kg/ha)	121.3 ± 0.13^{a}	149.1 ± 0.3^{b}	172.3 ± 0.26^{c}	228.5 ± 0.3^{d}	256.8 ± 0.3^{e}	313.1 ± 0.15^{f}

Data are presented as mean ± sd (n = 3). Means with same superscript in row do not vary significantly ($p < 0.05$) from each other

Table 3: Effect of different effluent concentrations on various germination parameters of (*Lycopersicum esculantum* Mill.) and capsicum (*capsicum annum*. L) after 30 days

Parameters	Tomato (Effluent concentration %)					Capsicum (Effluent concentration %)				
	control	5%	25%	50%	75%	control	5%	25%	50%	75%
Germination (%)	70	80	90	50	30	80	90	100	60	50
Speed of germination	1.1	1.46	1.86	0.86	0.6	1.4	1.9	2.2	1.1	0.9
Mean germination time(MGT)	10.33	12.66	14.65	8.16	5.56	13.16	15.17	16.83	10.5	7.5
Mean daily germination(MDG)	0.23	0.26	0.3	0.16	0.1	0.26	0.3	0.33	0.2	0.16
Peak value(PV)	2.33	2.7	3	1.5	1	2.66	3	3.33	2	1.7
Vigour index	1562.4	1992	2383.2	993	510	1546.4	1875.6	2308	1054.2	740
Percentage Inhibition (%)	_	-14.3	-28.6	28.5	57.2	_	-12.5	-25	25	37.5

Table 4: Effect of different effluent concentrations on various morphological parameters of (*Lycperscum esculantum* Mill.) and capsicum (*capsicum annum*. L) after 30 days

Parameters	Tomato (effluent concentration)					Capsicum (effluent concentration)				
	control	5%	25%	50%	75%	control	5%	25%	50%	75%
Shoot length (cm)	17.3 ±0.1^{c}	19.3±0.2^{d}	22.4±0.0^{e}	14 ±0.02^{b}	11.2 ±0.16^{a}	12.1 ±0.15^{c}	13.4 ±0.17^{d}	14.8±0.31^{e}	11.2±0.0^{b}	10.2 ±0.25^{a}
Root length (cm)	8.3±0.19^{c}	9.6 ±0.10^{d}	10.4±0.3^{e}	7.5 ±0.23^{b}	6.7 ±0.19^{a}	4.5 ±0.19^{c}	5.6 ±0.21^{d}	5.8 ±0.20^{e}	4.2 ±0.11^{b}	4.0 ±0.14^{a}
Length of leaves (cm)	13.4±0.0^{c}	14.4±0.0^{d}	15.1±0.0^{e}	11.1±0.1^{b}	9.1 ±0.07^{a}	8.1 ±0.01^{c}	9.8 ±0.02^{d}	10.5 ±0.02^{e}	6.0±0.0^{b}	5.2 ±0.09^{a}
Leaves /plant	19.6±1.5^{c}	23.6±1.5^{d}	28.3±0.5^{e}	16.6±0.5^{b}	11.3 ±1.52^{a}	17 ±0.47^{c}	19.6 ±0.57^{d}	21.3 ±0.45^{e}	15.3±0.1^{b}	13.6 ±0.20^{a}
Leaf surface area (cm^2)	6.4±0.02^{c}	8.0±0.05^{d}	8.6 ±0.15^{e}	6.0 ±0.06^{b}	5.3 ±0.27^{a}	13.3 ±0.20^{c}	15.2 ±0.15^{d}	17.47±0.25^{e}	12 ±0.10^{b}	8.9 ±0.26^{a}

Data are presented as mean ± sd (n = 3). Means with same superscript in row do not vary significantly ($p < 0.05$) from each other

Table 5: Effect of different effluent concentrations on various biochemical parameters of (*Lycopersicum esculantum* Mill.) and capsicum (*capsicum annum*. L) after 30 days

Parameters	Tomato (effluent concentration)					Capsicum (effluent concentration)				
	control	5%	25%	50%	75%	Control	5%	25%	50%	75%
Chlorophyll a (mg g^{-1} F.W)	1.29±0.01^{c}	1.64±0.03^{d}	1.82±0.02^{e}	0.87±0.01^{b}	0.64±0.01^{a}	1.10±0.003^{c}	1.62±0.01^{d}	2.11±0.04^{e}	0.84±0.2^{b}	0.69±0.01^{a}
Chlorophyll b (mg g^{-1} F.W)	0.59±0.02^{c}	0.76±0.01^{d}	0.96±0.03^{e}	0.43±0.02^{b}	0.28±0.05^{a}	0.65±0.005^{d}	0.71±0.02^{c}	0.81±0.01^{d}	0.52±0.08^{b}	0.32±0.1^{a}
Total chlorophyll (mg g^{-1} F.W)	1.88±0.04^{c}	2.40±0.03^{d}	2.79±0.01^{e}	1.31±0.01^{b}	0.92±0.1^{a}	1.76±0.006^{c}	2.34±0.03^{d}	2.92±0.01^{e}	1.36±0.3^{b}	1.01±0.1^{a}
Total proteins (mg g^{-1} F.W)	2.14±0.02^{c}	2.23±0.04^{d}	2.45±0.02^{e}	1.68±0.01^{b}	1.30±0.06^{a}	12.07±0.06^{c}	14.55±0.0^{d}	15.91±0.1^{e}	11.48±0.2^{b}	9.50±0.3^{a}
Total phenols (mg GAE/g F.W)	1.27±0.06^{a}	1.97±0.03^{b}	2.14±0.01^{c}	3.24±0.02^{d}	3.61±0.2^{e}	1.95 ±0.03^{a}	2.67±0.04^{b}	3.31±0.01^{c}	3.97±0.07^{d}	4.12±0.3^{e}

Data are presented as mean ± sd (n = 3). Means with same superscript in row do not vary significantly ($p < 0.05$) from each other

CONCLUSION

It can be concluded that distillery effluent is one of the alternative resource to meet the water demand for agriculture practices. On the basis of the above experiment it can be suggested that distillery effluent should be treated to reduce the concentration of pollutants. The use of wastewater for irrigation may serve as an additional source of water with fertilizing properties after appropriate dilution. Irrigation water quality not only affect the growth of crops, but also have long term effects on soil health, grain quality, fodder quality and health of consumers. So finally, it is suggested that long term experiments should be conducted to explore the effect of distillery effluent on above suggested aspects before its use for irrigation.

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