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Toxicity of distillery effluent on seed germination, seedling growth and metabolism in *Pisum sativum*

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Abstract: Different concentrations of distillery factory treated effluent were used in petridish culture experiments to investigate its effect on seed germination and seedling growth in *Pisum sativum*. The higher concentration of the different elements (already present in effluent), BOD and COD affected the seed germination, seedling growth and ultimately plant growth and yield. The seed germination and seedling growth were significantly reduced with increase in concentration of the distillery effluent. It adversely affected seedling growth (radicle and plumule size), number of lateral roots, total chlorophyll, total amylase, fresh weight, dry weight and moisture content while catalase and peroxidase increased due to stress caused by distillery effluent.

Key words: Distillery effluent, Toxicity, Seed germination, Chlorophyll, Amylase, Catalase, Peroxidase

Introduction

Industrial waste has been a major cause in reducing soil fertility and causing great damage because effluent are being added to the neighbouring soil and water (frequently used for irrigation) continuously (Hosetti, 1995). The effluent treatment plants in industrial setup do reduce the toxic and hazardous levels of various wastes releasing them within permissible limits (Metcalf and Eddy, 1995) but in actual practice, besides damaging human health and crop productivity, these effluents exert deleterious effects on soil, water and air (Misra, 1981).

The harmful nature of Industrial effluents in relation to plant growth and development is well recognized owing to the presence of toxic chemicals present in it. Agricultural production in many countries is being severely affected by the reckless discharge of these effluents to the water bodies near the industrial establishments which are the main source of irrigation. In addition to providing large quantities of water, some effluents contain considerable amount of essential nutrients, which may prove beneficial for plants (Veer and Lata, 1987; Gautam and Bishnoi, 1992; Swaminathan *et al.,* 1989).

Polluted water directly affects soil not only in industrial areas but also in agricultural fields and river beds, creating secondary sources of pollution (Kisku *et al.*, 2000; Barman *et al.*, 2000). Untreated distillery effluent is very rich in organic carbon, potassium, sulphate, phosphate, chloride, magnesium and moderate levels of nitrogen but decrease 10-15 times after proper treatment and dilution (Om *et al.*, 1994; Ramana *et al.,* 2001; Gupta *et al.*, 2001). Some macro- and micro-nutrients, which are important for plant growth and yield, become toxic, beyond tolerance limit, and cause adverse effect on plant growth and yield.

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 to others 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 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.

These effluents cause toxicity to different crops being grown in that area (Srivastava *et al*., 2000; Chandra *et al*., 2004; Nath and Sharma, 2002). There are 325 distilleries in India producing 2.7 billion liters of alcohol and generating 65 billion liters of wastewater annually. Potassium plays an important role in soil plant relationship along with Ca, Mg and Na. Their presence in the soil in adequate amount and in suitable proportion to one another and to other exchangeable anion such as Al^{3+}, H^{+} and NH^{4} is necessary as the soil is a suitable medium for plant-root development. Because of the detrimental effects of industrial solid waste and water on crop growth and yield, the people started exploring the chief causes of damage and its remedies.

Materials and Methods

Pea (*Pisum sativum* L.) seeds were used for the petridish experiment. The treated distillery effluent samples were taken from the distillery factory. All seeds were surface sterilized with 0.1% $HgCl_2$ for the prevention of fungal / bacterial contamination. The 10, 25, 50, 75 and 100% concentrations of treated effluents were prepared while distilled water was taken as control. In the petridishes, 25 seeds were sown (in each petridish 15 ml quantity of each solution was used as described above) upon the filter paper. The experiment was under observation for 21 days. The fresh and dry weights were taken with the help of digital balance and dry weight measured by placing different treatments' seedlings at $80^0 \pm 1^0C$ in a oven, weighing at 24 hours.

The germination % and different growth parameters were observed (*i.e.* plumule, radicle length, and number of lateral roots) in each petridish. The chlorophyll was estimated by using the method of Arnon (1949). The estimation of enzymes *i.e.* amylase, catalase and peroxidase, was done by using the method of Katsuni and Fekuhara (1969), Euller and Josephson (1927) and Luck (1963) respectively. The effluent was analyzed for various physico-chemical properties as per methods described by APHA (2005). The data observed in the experiment were statistically analyzed for the calculation of standard error (SE) and student 't' test for testing the hypothesis.

Table - 1: Physico-chemical characteristics of the treated distillery factory effluent

Property	Value
Colour	Brown
Odour	Alcoholic
Temperature	28°C
pH	6.2
Dissolved oxygen	Nil
BOD	357 mgL^{-1}
COD	712 mgL^{-1}
Total dissolved solid	350 mgL^{-1}
Total suspended solid	654 mgL^{-1}
Total hardness	289 mgL^{-1}
Total nitrogen	41.0 mgL^{-1}
Chloride	50 mgL^{-1}
Sulphate	58 mgL^{-1}
Sulphide	45 mgL^{-1}
Phosphate	11.0 mgL^{-1}
Potassium	25.3 mgL^{-1}
Iron	10.25 mgL^{-1}
Oil and grease	35mgL^{-1}

BOD= Biological oxygen demand, COD=Chemical oxygen demand

Results and Discussion

The physicochemical properties of distillery factory effluent shown in Table 1, indicate that it was slightly acidic and the values of COD, BOD, sulphate, sulphide, chloride, TDS, TSS, oil and grease were found higher than tolerable limits.

There was significant reduction in germination % in different dilution levels of distillery effluent and complete inhibition of germination in 100% concentration. Plumule and radicle length decreased significantly with increase in different dilution levels of treated effluent. Number of lateral roots, fresh weight, dry weight and moisture % also decreased significantly with increasing concentration of treated distillery effluent (Table 2).

The total chlorophyll contents were decreased in all the treatments with increase in distillery effluent concentration which was quite apparent in phenotypic condition of seedlings. The total amylase activity was declined by the effluent in the treatments indicating poor starch breakdown followed by poor growth of seedlings. The catalase and peroxidase activity increased with increasing concentration of treated distillery effluent if compared to control which indicates the stress of distillery effluent on seedlings. Overall the data emphasize the inhibitory and toxic effect of distillery effluent (Table 3).

The results obtained in the experiment regarding the inhibitory effects of distillery factory effluents on seed germination and seedling growth are conclusive and emphasize 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 have tried to explore the impact of various industrial effluents on plants. Om *et al.* (1994), while studying the combined effect of wastes of distillery and sugar mill, observed inhibition of seed germination, seedling growth and biomass in okra. Zalawadia *et al.* (1996) reported the inhibitory effects of distillery effluents on seed germination, seedling growth, fresh weight and dry weight in onion. Raman *et al.* (2001) observed similar kind of inhibitory effects in mustard, cauliflower and radish. Results, obtained by us regarding the inhibition of seed germination and seedling growth in distillery effluent treatments can be correlated to the decreased amylase activity. Important role of this enzyme during seed germination through hydrolysis of reserve starch and release of energy has been worked out by Thevenot *et al*. (1992). Studies of Dunn (1974), Chang (1982) on amylase correlated increased seed germination with more hydrolysis of starch and release of energy. The reduced % of germination can be correlated with the inhibition of total amylase activity under the influence of distillery effluents. Kannan (2001) studied the effect of distillery effluent on commonly cultivated crop plants (*Phaseolus aureus* and *Pennisetum typhoides*) and found that in the 100% effluent irrigation, the germination percentage was zero. The dilution level (more than 5%) of distillery effluents has also been reported as toxic (Sharma *et al.*, 2002) who have also recommended the use of distillery effluent for irrigation purpose after proper dilution.

Several cellular roles have been described for plant catalases. It neutralizes H_2O_2, which is produced during photorespiration (Zelitch and Ochoa, 1953) and acyl CoA oxidation of fatty acids (Yamaguchi *et al*., 1984). Catalase has also been suggested to play a role in the protection against environmental stress (Taiz and Zeiger, 2002). Catalase activity was increased in all the treatments. Our findings clearly show that the different dilution

Table - 2: Effect of different concentrations of treated distillery effluents on seed germination and seedling growth in pea (*Pisum sativum* L.) at 21st day

Treatments	Germination (%)	Plumule length (cm)	Radicle length (cm)	No. of lateral root	Fresh wt. (gm)	Dry wt. (gm)	Moisture (%)
Control	93.33 ± 4.80	9.26 ± 0.85	11.45 ± 0.20	12.32 ± 0.85	5.021 ± 0.65	0.526 ± 0.027	89.52 ± 0.162
10% TE	78.66 ± 1.33*	8.82 ± 0.65	10.62 ± 0.35	10.35 ± 0.56	4.245 ± 0.24*	0.511 ± 0.045	87.96 ± 0.25
25% TE	62.66 ± 1.33*	5.32 ± 0.82*	7.16 ± 0.44*	6.55 ± 0.64*	2.456 ± 0.16*	0.458 ± 0.015*	81.35 ± 0.87*
50% TE	36.00 ± 2.309*	3.92 ± 0.26*	4.10 ± 0.67*	3.44 ± 0.034*	1.112 ± 0.08*	0.254 ± 0.010*	77.15 ± 0.85*
75% TE	14.66 ± 1.33*	1.28 ± 0.37*	1.53 ± 0.304*	0.00 ± 0.00*	0.505 ± 0.11*	0.186 ± 0.005*	63.16 ± 0.35*
100% TE	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*

Values are mean of three replicates ± SE, *Statistically significant at 0.05 level, TE= Treated effluents

Table - 3: Effect of different concentrations of treated distillery effluents total chlorophyll content (mg g^{-1} fresh weight of tissue), total amylase (starch hydrolyzed mg g^{-1} freshweight of tissue), catalase (ml H_2O_2 degraded g^{-1} fresh weight of tissue) and peroxidase activity (Δ OD g^{-1} fresh weight of tissue) in pea (*Pisum sativum* L.) at 21st day

Treatments	Total chlorophyll	Total amylase	Catalase	Peroxidase
Control	3.0526 ± 0.204	2.11 ± 0.032	114.50 ± 10.25	18.66 ± 1.12
10% TE	2.1452 ± 0.152*	1.23 ± 0.065*	154.65 ± 12.55*	32.66 ± 0.96*
25% TE	1.0021 ± 0.025*	0.86 ± 0.022*	256.25 ± 18.33*	45.45 ± 1.25*
50% TE	0.5752 ± 0.035*	0.38 ± 0.062*	214.00 ± 14.00*	42.00 ± 1.11*
75% TE	0.2242 ± 0.011*	0.11 ± 0.030*	156.50 ± 10.12*	10.57 ± 0.55*
100% TE	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*	0.00 ± 0.00*

Values are mean of three replicates ± SE, *Statistically significant at 0.05 level, TE= Treated effluents

levels of distillery effluent increased the catalase activity. It seems that synthesis of catalase is initiated in early stage of seed germination and increases towards the later stage of seedling growth. In distillery effluent treated plants the activity of another H_2O_2 scavenging enzyme, *i.e.* peroxidase, increased pronouncedly as has been observed earlier by Tewari *et al*. (2002). This might be attributed to rapid diffusion of H_2O_2 produced in the cytosol or might be due to accumulation of high phenols and low protein formation in such conditions.

The water potential of solutions may be dissected into individual components, usually written as:

$$\psi_w = \psi_s + \psi_p + \psi_g$$

The terms ψ_s, ψ_p and ψ_g denote the effect of solutes, pressure, and gravity, respectively, on the free energy of water. The gravitational component is ignored because it is negligible when vertical distances are small (less than 5 m). The reference state used to define water potential is liquid water at ambient pressure and temperature. This means that ψ_w is proportional to the work required to move 1 mol of pure water at ambient pressure and temperature. In most of cases ψ_w inside plant cells is negative, because pure water has a higher potential than the water inside the cell. Solutes reduce the free energy of water by diluting water than the rate of water intake inside the cell is reduced (Taiz and Zeiger, 2002). Low ψ in distillery effluent treated plants suggests reduced availability of water under effluent stress, and this is also one of the region to reduce amylase activity. Recently Chandra *et al.* (2004), Kumar and Gopal (2001), Rajannan *et al*. (1998), Subramani *et al.* (1995), Nath *et al.* (2007 a, b, c) studied the effect of distillery effluent on various plants and found that distillery effluent (more than 10% in concentration) decreased the morphological parameters, chlorophyll contents and amylase while they increase the antioxidative enzymes like catalase and peroxidase. The changes in verious morphological and biochemical parameters in our experiment is in agreement to these studies.

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