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EFFICACY OF DISTILLERY EFFLUENT ON SEED GERMINATION AND BIOCHEMICAL PARAMETERS OF CHICKPEA (*Cicer arietinum* L.)

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Abstract: In the present investigation an attempt has been made to assess the effect of distillery effluent on germination and biochemical parameters of Chickpea. Maximum percentage of germination was observed in 25% effluent concentration and it was found to be reduced as the concentration of the effluent increased. The root and shoot length, fresh and dry weight, vigour index and tolerance index, was maximum in 25% effluent concentration and it was significantly reduced in higher effluent concentrations (50%, 75% and 100%), when compare to control. Phytotoxicity was absent in 25% effluent concentration but was increased in 100% effluent concentrations. The chlorophyll, protein and carbohydrate content were found to be decreased as the concentration of the effluent increased except in 25% concentration. Reduction in seed germination and seedling growth parameters in chickpea at higher effluent concentration indicate that it is toxic to the growth of crop plant. If the effluent is diluted to optimum level (25%) it can be used to irrigate agricultural fields.
Keywords: Distillery effluent, seed germination, seedling growth, chickpea.

Introduction

Distillery industry is one of the major Agro-based industries that play a major role in pollution of Environment. Pollution prevention focuses on preventing the generation of waste, while waste minimization refers to reducing the volume or toxicity of hazardous wastes by water recycling and re use and process modification and the byproduct recovery as a distillery effluent can be used for beneficial purpose. Distillery effluent is acidic and reddish dark brown coloured and contains high value of BOD, COD suspended, dissolved solids and total dissolved solids SO_4, K, Na, Ca^+, Cl^- and N. One of the important ecological benefits of waste disposal is the utilization of distillery industrial effluent for irrigation purposes (Raja and Vijayakumari, 1989 a,b; Singh and Mishra, 1987, and Sindu *et al.,* 2007). The highly colored components of the spent wash reduce sunlight penetration in river, lakes or lagoons which in turn decrease both photosynthetic activity and dissolved oxygen concentration (Kumar *et al.,* 1995). Application of distillery effluent to soil without proper

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monitoring perilously affects the ground water quality by altering its physico chemical properties such as colour , pH, electrical conductivity etc. due to leaching down of organic and inorganic ions (Mohan *et al.,* 2009). Hence before discharge of the effluent to the ecosystem the effluent or spent wash must be treated. Patak *et al.* (1999) have shown that distillery effluent contains large quantities of plant nutrient in addition to high organic load and dissolved organic matter. However during production of alcohol no harmful chemicals are added to the spent wash at any stage. It has been estimated that every cubic meter of spent wash contains 1kg of nitrogen, 0.24 kg of phosphorus, 10kg of potassium oxide, and contain large quantities of calcium, magnesium and sulphate and appreciable quantities of micro nutrients (Srinivasamurthy *et al*., 2006). Most of these nutrients are in soluble form and are easily available to the plants. Distillery effluent serves as an additional potential source of fertilizer for agricultural use and prevents the waste water from being an environment hazard (Day, 1973). In water scarce situation, effluent is single permanent water source for irrigation, consequently causes positive effect at lower concentrations. In this regard studies have been carried out by Rani and Srivastava, (1998), Subramani (1995) Pandey *et al*. (2008), Sukanya and Meli (2005, a, b), and Tharakeshwari & Jagannath (2006).

The present study has been undertaken to study the effect of of distillery effluent collected from Chamundi Distilleries Pvt. Ltd, Maliyur, on seed germination and biochemical parameters chickpea.

MATERIALS AND METHODS

The effluent sample was collected from the Chamundi Distilleries located at Maliyur, T. Narasipura Taluk, Mysore district. Karnataka. The seed samples of chickpea variety KAK-2 was procured from G.K.V.K Bangalore. Healthy seeds of chickpea were surface sterilized with 0.2% Mercuric chloride for 2 minutes and washed thoroughly using distilled water to remove excess of chloride. Ten seeds of chickpea were placed per petriplate and 10 ml of various concentrations of effluent (25%, 50%, 75% and 100%) was added. One set of seeds treated with distilled water served as control. Germination percentage, root and shoot length, fresh and weight was calculated according to ISTA (1985) standard. Vigour index (Abdul Baki and Anderson, 1973). Tolerance index (Turner and Marshal, 1972). Percentage of phytotoxicity (Chion and Muller, 1972). Biochemical studies *viz,* total chlorophyll (Arnon, 1949), carotenoids (Kirk and 1965), estimation of total protein (Lowry's *et al.* 1951) and total carbohydrate (Hedge and Hofreiter 1962) was conducted. The obtained data were subjected to statistical analysis using SPSS package version 14.0 with Tukey's significant test at 5% (Tukey s, 1949).

RESULT AND DISSCUSSION

Seed germination, root and shoot length of chick pea showed significant difference in different effluent concentrations. Seed germination percent was increased in lower concentration of effluent (25%), while at higher effluent concentration (50%,75% and 100%) germination percentage , root and shoot length decreased (table 1). Increased germination percentage, root and shoot length at lower concentration effluent treatment have been reported by Behera and Mishra (1982), Elcey and Tiwari (1991), Tarakeshwari and Jagannath (2006). The reduction in the germination percentage and root and shoot length was due to high amount of total dissolved solids that disturbed the osmotic relation and other metabolic constituent in the crop plants (Sahai *et al* 1983). The fresh and dry weight of chickpea decreased with increase in effluent concentration. At higher effluent concentration there was a significant reduction in fresh and dry weight. The lower concentration favorably increased the fresh and dry weight of seedling. Similar result have been reported by Sahai *et al.* (1983) and Sahai and Neelam (1987). The tolerance index also was increased in 25% effluent concentration and decreased in higher effluent concentration

In the present study the vigour index in the seedling showed a gradual decrease with increase in effluent concentration (table 2). This is similar to the earlier findings of Kannan (2001) in *Pennisetum typhoides.* Elcey and Tiwari (1991). Phytotoxicity in the present study was gradually increased with increase in effluent concentration. 25% effluent showed least phytotoxicity (table 2). Similar observation was reported in *Vigna radiata* by Subramani *et al.* (1995), and Tharakeshwari and Jagannath in (2006, a and b) .Higher concentration of the distillery effluent was found to be toxic to germination due to fungal attack (Pandy and Soni, 1994, and Ramana *et al.,* 2001).

The mean values of protein content under different effluent concentrations are present in Table 3. The protein content showed significant increase at T_1 effluent concentration (027.333mg/g F. Wt.) over control plant (026.333 mg/g F. Wt.). With increasing concentration (T_2, T_3 and T_4) the protein content decreased (024.666, 011.666 and 06.666 mg/g F. Wt. respectively). Increase in protein content over control plants at lower concentration of effluent have been reported by Jabeen and Saxena (1990) in *Pisum sativum*, Chandra *et al.* (2004) in *Phaseolus aureus* in *Vigna radiata* and *V. unguiculata*

(Tharakeshwari and Jagannath, 2006). Increase in protein content at lower concentration of effluent was due to transportation of nitrogen absorbed by plants to last stages (Kodioglu and Algur 1990).In the present study protein content was gradually decreased with increase in effluent concentration. Similar results have been reported by Kumar *et al.* (1995) in *V. radiata* and *V. mungo.* The protein content reduction at higher concentration may be attributed to reduction in absorption and metabolism of nitrogen leading to decrease in total physiological activities (Kodioglu and Algur, 1990). The mean values of carbohydrates content under different concentrations of effluent treatment are present in Table 4. The carbohydrate content observed at T_1 effluent concentration was maximum (340.000 mg/g F. Wt.) over control (283.333 mg/g F. Wt.) and significantly decreased with increasing concentration of effluent-T_2, T_3 and T_4 (300.000, 266.666, and 226.666 mg/g F. Wt. respectively). Minimum values were recorded at T_4 effluent concentration. T_1 effluent concentration exhibited significant increase when compared to other effluent concentrations. The present study showed an increase in the carbohydrates content at T_1 effluent concentration. There was a gradual decrease in carbohydrate content at higher concentration of the effluent. Similar results decrease in carbohydrate content has been reported by Suresh *et al.* (2003).

Effects of distillery effluent concentrations on pigment content of chick pea are presented in Table 4. The chlorophyll-a content showed maximum values at T_1effluent concentration (0.950 mg/g F. Wt.). Chlorophyll a content decreased with increasing concentration of T_2, T_3, and T_4 effluent concentrations (0.109, 0.09 and 0.074 mg/g F. Wt.) respectively. Similar results have been reported by Sahai *et al*. (1983) in paddy, Sahai and Srivastava (1986) in *Cajanus cajan* , Jabeen and Saxena, (1990) in *Pisum sativum*, Rajendra (1990) in *Helianthus annus*, Kannabiran and Pragasam (2001) in *Vigna mungo*, Kumar *et al.* (1997) in *V.radiata*, and Chandra *et al.* (2004) in *Phaseolus aureus*.

The mean values of chlorophyll b also showed similar trend as that of chlorophyll a. There was maximum chlorophyll b content at T_1effluent concentration (0.366mg/g F. Wt.), while at increasing concentration of T_2, T_3 and T_4 the chlorophyll b content was minimum (0.104, 0.082, 0.068 mg/g F. Wt.) respectively. T_1 effluent concentration exhibited highly significant difference when compared to other treatment (T_2, T_3, and T_4). A large number of physical and chemical agents are known to cause reduction in pigment content. Chlorophyll content reduction may be regarded as one of criteria to determine the pollution level of the effluent.

Reduction of chlorophyll content at increasing concentration of effluent may be due to increase in TDS and TSS which destabilize the chlorophyll pigment. (Sahai *et al*., 1983).

Mean values of total chlorophyll content (table 4) at T_1 effluent concentration was maximum (1.155 mg/g F. Wt.),) and subsequently decreased with increasing concentrations. At T_4 effluent treatment total chlorophyll content showed minimum values (0.143 mg/g F. Wt.). There was significant difference observed under different effluent treatment in mean values of carotenoid content .The carotenoids content was maximum in T_1(25%) effluent concentration over control (T_0) and decreased with increasing concentration of T_2 , T_3 and T_4. Increase in chlorohpyll a, b and total chlorophyll content at lower concentration might be due to presence of optimum quantities of nutrient element such as Mg and K required for pigment biosythesis (Sahai and Srivastava 1986; Jabeen and Saxena, 1990; Kodioglu and Algur, 1996. The present study reveals that higher concentration of distillery effluent had deleterious effect on the seed germination and seedling growth of chick pea. Hence distillery effluent can be used to irrigate agricultural field after proper treatment and further dilution up to 25% for cultivation of chickpea.

Table 1: Effect of distillery effluent on germination, root, and shoot length, fresh & dry weight of chickpea**.**

Conc.	Germination (%)	Root length(cm)	Shoot length (cm)	Fresh weight (mg/g)	Dry weight mg/g
T0	100.000^{b}±0.000	002.019^{a}±0.701	005.300^{c}±1.449	007.715^{a}±`0.318	003.255^{a}±0.035
T1	100.00^{b}±0.000	002.309^{c}±0.812	005.275^{a}±1.411	008.070^{b}±1.400	003.290^{b}±0.509
T2	073.330^{c} ±5.77	000.833^{b}±0.983	001.830^{b}±1.709	004.325^{c}±0.007	002.165^{c}±0.077
T3	056.66^{d} ±5.77	000.609^{a}±0.704	000.990^{a}±1.182	003.980^{d}±1.286	001.910^{d}±0.551
T4	043.33^{a}±11.54	000.047^{d}±0.174	000.375^{d}±0.539	002.875^{e}±0.134	001.780^{e}±0.028

Means with different superscriptions are significantly different from each other as indicated by Tukey's HSD.

Table 2: Effect of distillery effluent on vigour index, tolerance index and phytotoxicity of chickpea.

Conc.	Vigour index	Tolerance index	Phytotoxicity (%)
T0	733.000^{a}±35.35	100.000^{a}±0.000	000.000^{a}±0.000
T1	770.00^{a}±57.982	102.50^{b}±3.535	-001.369^{a} ±1.689
T2	324.800^{b}±39.59	065.430^{c}±32.201	040.770^{c}±10.832
T3	217.500^{c}±44.547	041.665^{d}±11.787	054.798^{d}±14.648
T4	054.375^{d}±32.703	017.00^{d}±0.070	081.200^{e}±11.186

Means with different superscriptions are significantly different from each other as indicated by Tukey's HSD

Table 3: Effect of distillery effluent on Protein and carbohydrate content (mg/g) in chick pea

Conc.	Protein (mg/g F. Wt.)	Carbohydrate (mg/g F. Wt.)
T0	026.333^{a}±2.886	283.333^{d}±41.633
T1	027.333^{b}±6.350	340.000^{e}±0.00
T2	024.666^{c}±10.785	300.000^{a}±0.000
T3	011.666^{c}±1.527	266.666^{b}±30.550
T4	006.666^{d}±1.154	226.666^{c}±41.633

Means with different superscriptions are significantly different from each other as indicated by Tukey's HSD.

Table 4: Effect of distillery effluent on pigment content (mg/g) in chick pea

Conc.	Chlorophyll a(mg/g)	Chlorophyll b(mg/g)	Total chlorophyll (mg/g)	Carotenoid(mg/g)
T0	000.791^{a}±0.000	000.356^{a}±0.000	001.148^{a}±0.000	000.635^{a}±0.000
T1	000.950^{b}±0.000	000.366^{b}±0.000	001.155^{b}±0.000	000.636^{b}±0.000
T2	000.109^{c}±0.059	000.104^{c}±0.012	000.160^{c}±0.135	000.080^{a}±0.051
T3	000.09^{d}±0.000	000.082^{d}±0.000	000.173^{d}±0.000	000.069^{c}±0.00
T4	000.074^{e}±0.000	000.068^{e}±0.000	000.143^{e}±0.000	000.056^{d}±0.00

Means with different superscriptions are significantly different from each other as indicated by Tukey's HSD.

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