Study on the Impact of Sugar Mill Effluent on Germination Behaviour and Some Biochemical Parameters of a Crop Plant, *Cajanas cajan* (L.) Millsp. (Red Gram)

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ABSTRACT

Effect of sugar mill effluent on *Cajanas cajan* (L.) Millsp. was studied. The effluent significantly inhibited germination and growth of root and shoot length. A negative correlation has been found in the concentration of the effluent and biochemical parameters such as chlorophyll, protein, amino acids, nucleic and carbohydrates. In the seedlings, shoots were found to be resistant when compared with roots. The dilution of effluent stimulated the seedling growth and also all other observed biochemical parameters.

Key words: Red gram, effluent, germination, seedlings.

INTRODUCTION

The harmful effect of sugar mill effluent has been well documented. Most of the studies have been carried out to find out the effect of effluent on the crop plants (Kadioglu and Algur, 1990; Tiwari *et al.,* 1993 and Subramani *et al.,* 1995). The studies of Pragasam and Kannabiran (2001) and Kannabiran and Pragasam (1993) on the effect of distillery effluent on leading characteristics of *Vigna mungo* reported that low concentration of distillery effluent may have a beneficial effect on the seedlings. Studies by other authors (Indira and Mohanty, 2006 and Mahapatro and Mohanty, 2006) revealed that the distillery effluent stimulated the plant growth at lower concentration but inhibited the same at higher concentration. Most of the studies were concerned with the seed germination and seedling growth only. The present work brings out the effect of sugar mill effluent on germination behaviour, growth of seedlings and all the important biochemical parameters to correlate the events.

MATERIAL AND METHODS

Aska Sugar Co-operative mill is situated in the town of Aska (Orissa) and discharges the factory effluent into land or nearby water bodies which ultimately reaches the River

Rushikulya. This River is a perennial source of water for domestic use and irrigation of crop land in the villages along the river course.

For our experimentation, the sugar mill effluent was collected from the source at the point of discharge. The collected effluent was stored at 10 ºC and was brought to room temperature before it was used in experimentation. The collected effluent was considered as stock (100% concentration) and diluted to 5, 10, 15, 20, 25, 50, 75, 100% (volume/volume) with distilled water for experimentation. A set with distilled water were also maintained and taken as control.

Healthy seeds of Red gram *(Cajanas cajan* (L.) Millsp.) were obtained from local Orissa University of Agriculture and Technology (O.U.A.T.) extension centre situated at Ratnapur near Berhampur (Ganjam) and were preserved in an incubator at 19°C. The seeds were graded and surface sterilized with 1 % $HgCl_2$ before use.

Germination beds were prepared by placing sterilized cotton and blotting paper in sterilized Petri plate (6″ dia). 30 dry Red gram seeds were placed in each plate containing the germination bed and were soaked with effluent of desired concentration. Emergence of radical was considered as the index of germination.

Roots and shoots of 7-day-old seedlings were taken for estimation of various biochemical parameters. Chlorophyll content (Arnon, 1949), protein (Lowry *et al.,* 1951), Amino Acid (Moore and Stein, 1948), DNA and RNA (Schiemeider, 1957) and soluble sugar (Yoshida *et al.,* 1972) were estimated in both treated and control samples.

All the data were expressed in terms of mean ± SD of 10 replicates. The con-elation analysis was carried out between percentage of effluent concentration and parameter studied and correlation coefficient values are expressed in terms of (r) value at various levels of significance (P) (Mishra and Mishra, 1989).

RESULTS

1. Germination Percentage

The germination of Red gram seeds was recorded after 72 hours of experiment setting both in control and effluent treated categories. In general, there was decrease in germination with an increase in the concentration of effluent percentage. Germination of seeds was reduced and was found to be 27.7 % in case of 100% effluent concentration i.e. undiluted effluent (Table 1).

Table 1: Effect of different concentrations of effluent on Germination percentage of Red gram seeds (Germination was noted after 72 hours of soaking).

S No.	Conc. of Effluent (%)V/V	% of Germination ± SD
1	0	97.22 ± 4.81
2	5	91.66 ± 8.33
3	10	74.99 ± 5.32
4	15	69.44 ± 9.62
5	20	66.60 ± 8.33
6	25	61.60 ± 4.80
7	50	49.90 ± 2.20
8	75	41.60 ± 1.90
9	100	27.70 ± 3.40

2. Vegetative Growth of Seedlings

Red gram seeds took about 72 hours for total germination and another 72 hours were allowed for gradual growth of radicle and plumule. Thus, root and shoot lengths were measured at the end of 7^{th} day. Vegetative growth of the seedling was measured and ratio was calculated between the shoot and root length under varied concentrations of effluent. It shows that in general root and shoot length decreased with increase in effluent concentration and in both the cases it was negatively correlated with higher significance level. In the same manner the shoot and root ratio also decreased from control with increase in the effluent concentration (Table 2).

Table 2: Effect of different concentrations of sugar mill effluent on root and shoot and shoot length of 7 day old Red gram seedlings. Each datum is mean value ± SD

S. No.	% of effluent Concentration	Mean ± SD Root length (cm)	Mean ± SD Shoot length (cm)	S/R
1	0	13.50 ± 2.99	7.83 ± 1.06	0.58
2	5	14.70 ± 2.88	7.85 ± 1.59	0.53
3	10	11.66 ± 2.819	6.75 ± 1.20	0.57
4	15	8.80 ± 1.109	7.50 ± 1.10	0.85
5	20	8.58 ± 1.22	6.76 ± 1.62	0.71
6	25	11.40 ± 1.264	6.31 ± 0.62	0.55
7	50	6.45 ± 0.74	8.15 ± 0.73	1.26
8	75	6.81 ± 0.17	6.21 ± 0.29	0.91
9	100	4.2 ± 0.7	4.58 ± 0.9	1.09

3. Biochemical Parameters

Table 3 depicts the results observed in relation to various biochemical parameters like chlorophyll, amino acids, protein, DNA, RNA and carbohydrates in roots and shoots of 7 day old seedlings with treatment of sugar mill effluent of different concentrations (5% to 100%). The biochemical parameters increased slightly with increase in concentration of effluent treatment. When the effluent concentration was increased (50. 75 and 100%), there was a decrease in biochemical parameters studied, showing a negative correlation. The shoots were more affected than roots in case of DNA and RNA studies.

DISCUSSION

Seed germination and growth are of vital importance for continuation of plant life. For germination and growth of seedlings entrance of water into seeds is a basic requirement to initiate and trigger the intricate sequence of metabolism. At this stage the seed and seedlings are susceptible to environmental stress like the presence of pollutants (effluents) in the environment, which results in deleterious effect on germinating seeds and seedling growth.

A number of studies have been carried out to find out the effect of the effluents of distillery on crop plants by Kadioglu and Algur (1990); Tiwari *et al.* (1993); Muthukumar and Arockiasamy (1994) and Subramani *et al*. (1995) and the effect of distillery effluent on various aspects of green gram and black gram has also been reported by Kannabiran and Pragasam (1993) and Pragasam and Kannabiran (2001). Attempts were also made in the past to assess the effect of distillery effluents on rice seedlings by Sahai *et al.* (1983) and on *Helianthus annus* by Rajendran (1990) who found that it is invariably beneficial up to 5 % of the effluent concentration.

Retardation in seedling growth can be attributed to the impact of sugar mill effluent (Pragasam and Kannabiran, 2001; Sharma *et al.,* 2002 and Kannan, 2001). The retardation in the root growth in the present case inferred in terms of root length in control and effluent treated seedlings suggested that there is high oxygen demand in case of the sugar mill effluent. This is in agreement with the reports of Swaminathan and Vaidheeswaran (1991) on groundnut seeds in oxygen depleted environment. The inhibition of germination and reduction in root and shoot length in *Eleusine coracana* reported by Mishra *et al.* (1989) was attributed to the mito-depressive activity of sugar mill effluents.

It is now commonly believed that effect of pollutants like effluents occurs at biochemical level, which finally results in delay in germination and retardation in seedling growth. However, biochemical injury in seedling does not appear spontaneously.

When the concentration of pollutant effluents exceeds the detoxifying capacity of the tissue through their normal metabolism there is decrease in the biochemical parameters (like chlorophyll, protein, amino acid sugar and nucleic acids) which is again directly proportional to the concentration of effluents.

A decrease in chlorophyll content in effluent treated seedlings suggests pollution injury (Pragasam and Kannabiran, 2001). Changes in pigment concentration by sugar mill effluent treatment (Sharma *et al*., 2002) is also likely to affect carbohydrate metabolism, hence, decrease in chlorophyll content supports the above view.

The increase or decrease in the amount of metabolites either may be due to decreased synthesis or increased metabolism and chemical destruction of metabolite itself (Panigrahy, 1996). The reductions in biochemical parameters in effluent treated seedling of Red gram in the presence of effluent indicate impairment of metabolic machinery.

In all biochemical studies (chlorophyll, amino acid carbohydrate, DNA and RNA) the shoot of seedling was comparatively resistant whereas root was susceptible to the effluent treatment.

The biotoxic effect of the effluent of ACSI, Aska on red gram seedlings was evident from the fact that there is an overall decrease in germination percentage, root shoot length and its ratio and biochemical parameters. In all cases a negative correlation was obtained between concentration of the effluent and the biochemical parameters (chlorophyll, protein, amino acid, nucleic acid and carbohydrates).

The present study also demonstrated that the treatment of sugar mill effluent to red gram seedling produced persistent morphological retardation. Moreover, decline in biochemical parameters may be attributed to the manifestations and decreases in root and shoot length of the Red gram seedling. Therefore, it is extremely necessary to treat the effluent properly before release to a river system for irrigation in agriculture to minimize harmful impact (Sharma *et al*., 2002; Rajesh, 1995; Pandey and Neraliya, 2002; Mishra and Pandey, 2002 and Jothimani and Bhaskaran, 2002).

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REFERENCES

Arnon, D. I. 1994. Copper enzyme is isolated chloroplast polyphenol oxydase in *Beta Vulgaris. Plant Physiol.* **24:** 1-15.

Indira, Subhadarsini and Mohanty, B. K. 2006. Effect of sugar mill effluent on ragi *(Eleusine coracana.* L) and growth behaviour of its seedlings, *Ad Plant Sci* .**19**(II): 463-467.

Jothimani, P. and Bhaskaran, A. 2002. Effects of dilution and dynamics of physical factors during factory effluent irrigation. *J. Ecotoxicol. Environ. Monit.* **12**(40): 255-261.

Kadioglu, A. and Algur, O. F. 1990. The effect of vinasse on the growth of *Helianthus annus* and *Pisum sativum,* part 1. The effects on some enzymes and chlorophyll and protein contents *Environ Pollut.* **67:**1223-1232.

Kannabiran, B. and Pragasam, A.1993. Effects of distillery effluents on seed germination, seedling growth and pigment content of *Vigna mungo* (L). Heper (CVT 9), *Geobios* **20**: 108-112.

Kannan, J. 2001. Effects of distillery effluents on crop plants. *Adv. Plant Sci.* **14**(1):127-132.

Lowry, O. H. Rose-Brough, N. J., Fan, A. L. and Randal, R. J. 1951. Protein measurement with folin-phenol regent *J. Biol chem.* **193:**265-275.

Mahapatra, M and Mohanty, B. K. 2006. Effect of sugar mill effluents on germination of green gram *(Phaseolus mungo)* and growth behavior of its seedling *J. Indus. Poulln. Contl.,* **26**(1) (in press).

Mishra, B. N. and Mishra, M. K. 1989. *Introductory Practical Biostatistics* Naya Prakash, Calcutta.

Mishra, R.N., Panigrahy, G. N. and Patra, K.S. 1989. Studies on physiotoxicity of paper mill effluent on *Eleusine coracana* Var. AKP-2. *Bullen. Pure and Appl. Sci.* **8B**(1-2):6-10.

Misra, V. and Pandey, S.D. 2002. Effect of distellary effluent and leachates of industrial sludge on the germination of black gram *(Cieer arietinum) Pollution Research,* **21**(4):461-467.

Moore, S. and Stein, W. W. 1948. Photometric ninhydrin methods for use in the chromatograph of amino acids *J. Biochem.* **176**:367-388.

Muthukumar, B and Arockosamy, D. I. 1994. Effects of distellary waste on the nitrate reduction system in *Vigna mungo* (Black gram) *J. Ecotoxcol. Environ. Monitoring* **4**:23-27.

Pandey, G.C. and Neraliya, S. 2002. Distellary effluent induced alterations on the seed germination, seedling growth, chlorophyll and protein contents of Bengal gram, *Cicer arietinum* Linn. *Himalayan J. Env. Zool.* **16**(1):77-81.

Panigrahy, N.C. 1996. *Effect of Sulphur Dioxide Gas on Two Crop Plants*. Ph.D. Thesis, Berhampur University, Berhampur, Orissa, lndia.

Pragasam, A and Kannabiran, B. 2001. Effects of distillery effluent on the photosynthetic pigments and nitrogenous compounds in *Vigna mungo* (L) Heper, *J. Samy. Bot. Cl.* **18**: 21-23.

Rajendran, K. 1990. Effect of distillery effluent on the seed germination, seeding growth chlorophyll content and mitosis on *Helianthus annus. Indian Bot contracter,* **7:**139-143.

Rajesh, K. 1995. Effect of sugar mill effluent on the seed germination and seedling growth of *Cicer arietinum* CV. NP 58. *Adv. Plant Sci.* **8**(1):52-56.

Sahai, R., Jabben, S. and Saxsena, P.K. 1983. Effects of distillery waste on seed germination, seeding growth and pigment content of rice *Indian J. Ecol,* **10**:17-20.

Schiemeider, W.C. 1957. Determination of nucleic acids in tissue by pentose analysis in methods. p.680-684. In: *Enzymology* Vol. 3 (S.D. Colowick and N.O.K. Plan, eds.). Academic Press, New York.

Subramani, A., Sundaramonorthy, P. and Lakshmanachry, A.S. 1995. Effect of distillery effluent on growth yield and productivity on *Vigna radiata, Pollun. Res.* **14**:477-481.

Sharma, V., Sharma, R. and Sharma, K.D. 2002. Distellary effluent effect on seed germination, early seedling growth and pigment content of sugar beet (*Beta vulgaris* Linn. Var. Mezzanan Poly). *J. Environ. Biol.* **23**(1):77-80.

Swaminathan, K. and Vaidheeswaran, K. 1991. Effect of dyeing factory effluent on seed germination and seedling development of groundnut (*Arachis hypogea). J. Environ. Biol.* **15(**3):209-212.

Tiwari, P., Elcey, D.D. and Bhatia, L. 1993. Response of two cultivars of Indian mustard (*Brassica Juncea,* L.) (Zern and Cross) to distillery effluents. *Geobios.* **20**:27-32.

Yoshida, S., Forno, D.A., Cock, J.H. and Gomez, K.A. 1972. *Laboratory Manual for Physiological Studies on Rice.* Int. Rice, Res, Inst, Loss Bane, Phillippines, pp.38-41.