J. Phytol. Res. **19**(2) : 261-266, 2006

CYTOLOGICAL EFFECT OF INDUSTRIAL EFFLUENT ON ROOT MERISTEM OF *CAPSICUM ANNUM L.*

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The effluent waste water of Amruth Distilleries Ltd., Bangalore was used to assess its cytotoxic effect on mitosis of *Capsicum annuum* root system. Cytotoxicity of effluent was studied by treating roots with different concentrations of effluents for different durations along with control. The high mitotic index and lower percentage of abnormality was very much evident in roots treated with diluted effluent. The mitotic index showed decreasing tendency with the increase in effluent concentration. C-metaphase, binucleate and sticky anaphase and some of the common abnormalities observed in all the treatments. Different durations and concentration of the treatment influenced the cell division. The types and percentage of abnormalities observed confirmed chronic impact of effluent on crop and other biota of agro-ecological system.

Keywords: *Capsicum annuum*; Chromosomal abnormalities; Industrial effluent.

Introduction

Waste water of industrial origin represents a chemical complex and their nature depends on the type of industry. In some cases in the same industry the composition of waste water varies depending on the materials used. Due to rapid urbanization combined with industrialization have lead to the generation and disposal of enormous amount of waste water, which apart from containing toxic materials are also rich in several plant nutrients. Treatment problems combined with water scarcity in several areas of our country have made it obligatory for the farmers living in the low-lying areas of the city to depend on this valuable resource as a source of water for agriculture. However, several studies on the effect of effluent on crop plants have reported their beneficial effects on overall plant growth. Although the effluent support plant growth at lower concentrations, where as higher concentrations, are likely to affect metabolic and cellular activities because of the presence of several toxic components in them. Despite, effluents generated from food processing units, sugar mills, paper mills, distilleries and tanneries have been reported to induce clastogenic and turbagenic effects on crop plant like *Allium cepa*[1-7].

Materials and Methods

Amruth Distillery Private Limited is located 15 km away from Bangalore City, Karnataka, India. It generates nearly 30,000 to 50,000 litres of waste water per day. The effluents run in the form of channel for a distance of about 3 to 4 ½ km and subsequently join one of the main sewage channels of outskirts of the Bangalore City. Waste water effluents are responsible for complete elimination of plant communities through burnt effect. The effluents were collected at monthly intervals for one year at the point of discharge from the treatment plant of factory and analyzed for 30 physico-chemical parameters using standard method[8]. The effluents were then used to carry out cytological study in *C. annuum.*

Healthy *Capsicum* seeds (Chilies) were selected and placed in Petri plates, soaked with water for fresh root primordial. Seeds were germinated and produced the roots of around 1-2 cm in length. Roots were placed in the Petri plates containing different concentrations of effluents viz., 10, 25, 50 and 75% and raw effluent (100%) and treated for 24, 48, 72 and 96 hr. The different concentrations of the effluent were prepared using distilled water.

A control with distilled water was maintained in all the cases. The experiment was conducted at room temperature under diffused light at 27±2°C. After treatment, the root tips were excised and treated with colchicines (0.2%) for 2 ½ hrs and fixed in freshly prepared Carnoy's fluid for 24 hr. The fixed roots were stored in 70% ethanol in refrigerator for further processing. The micro-preparation was made using aceto-orcein and feulgen stain techniques[9].

Toxicity to mitotic apparatus was calculated using the formula:

$$\text{Mitotic index} = \frac{\text{No. of cells in division x 100}}{\text{Total No. of cells}}$$

The Karyotoxicity is the toxicity caused to a specific component of mitotic apparatus, which ultimately lead to the constitutional alteration of the chromosomal component. This was calculated by using the following formula:

$$\text{Percentage of aberrated cells} = \frac{\text{No. of aberrated cells x 100}}{\text{Total No. of cells}}$$

Results and Discussion

Waste water of industrial origin are known to induce deleterious effects among plants and animal cells[10]. Fishbein[11,12] opined that industrial effluents are responsible for causing genetic damage among plants and animals. Cook and Wood[13] have reviewed the earlier work on the genetic effects induced by pollutants. The physico-chemical analysis of the effluents is presented in Table 1. The cytotoxicological effects of Amruth Distilleries effluents on somatic cells were estimated on the basis of changes in the mitotic index and apparatus. Kabarity *et al.*[14] and Soheirel – Khodary *et al.*[15] reported frequent C-metaphase anomalies in *A. cepa* treated with chemicals and insecticides. Dixit and Nerle[1] and Joshi and Singh[2] observed the induction of chromosomal abnormalities in the form of breakage, bridges, laggards, stickiness and pycnotic nuclei in *A. cepa* exposed to different industrial effluent waste water.

The C-mitosis predominated the roots treated with all the concentration of effluents. Tamali *et al.*[16] also observed the inhibition of cell division as well as induction of C-mitosis by galvanizing industrial waste water. Binucleate cells were frequently found at higher concentrations. Chauhan *et al.*[17] with respect to insecticide treatment and Thangapandian *et al.*[6] with respect to industrial effluents also observed binucleate as common anomaly. Onfelt and Klasterska[18] considered that mitotic – abnormalities were insignificant from the industrial effluents also observed binucleate as common anomaly. In this context the effluents in the present study were regarded as mitotic poison. Moretton *et al.*[19] assessed the genotoxic potential of tannery wastewater by induction of gene conversion and point mutation in *Sacharomyces cerevisae* (d7) strain. This study revealed that induction of C-mitosis and binucleate cells can be considered as important parameters for assessing industrial effluent toxicity in *C. annuum*. Despite, the frequency of abnormalities were significantly decreased by diluted effluents. However, sometimes numerical increase in chromosome was also recorded. Shanthamurthy and Rangaswamy[4] and Kaushik *et al.*[3] also made similar point of observations. The inhibitory effects of effluents on mitosis of *C. annuum* are evident from Table 2. A strong dose dependent impact is obvious in terms of decline in the mitotic index with increase in concentration. There is a continuous decrease in mitotic index with the increase in distillery effluent concentration. Although, the percentage of abnormalities showed reverse trend (Fig. 1 and 2).

Further, the percentage of cell division and abnormalities induced also remained dose and duration dependent with 10% producing least impact and 100% inducting maximum effect. Nearly 5, 6 and 7% decrease in mitotic index was observed at 100 and 25% concentration of effluent, respectively. Both the concentration and duration of treatment showed negative correlation with mitotic index. The root tips treated for 24 hr induced low percentage of abnormalities and maximum was recorded at 96 hrs treatment. The effluent in the range of 75 to 100% induced higher percentage of abnormalities and lower mitotic index. The root tips treated with lower concentration of effluent however showed higher mitotic index and lower percentage of abnormalities. Similar suppressing effects of industrial effluents on mitotic activities in *A. cepa* has been reported by Somashekar *et al.*[5] and Chikkaswamy[20].

The various types of chromosomal abnormalities observed were chromosomal breaks, disintegration, bridges fragments, binucleate condition, C-metaphase and sticky anaphase. These abnormalities were probably due to impact of effluent on spindle to the action of higher level of chloride and sodium ions present in the distillery effluent. The effects may be synergistic or individualistic, sometime both.

In general, mitotic index of the effluent treated root tips showed gradual decreasing tendency with increasing effluent concentration. Further, high percentage of abnormalities was recorded in raw effluent treated *Capsicum* roots for longer duration.

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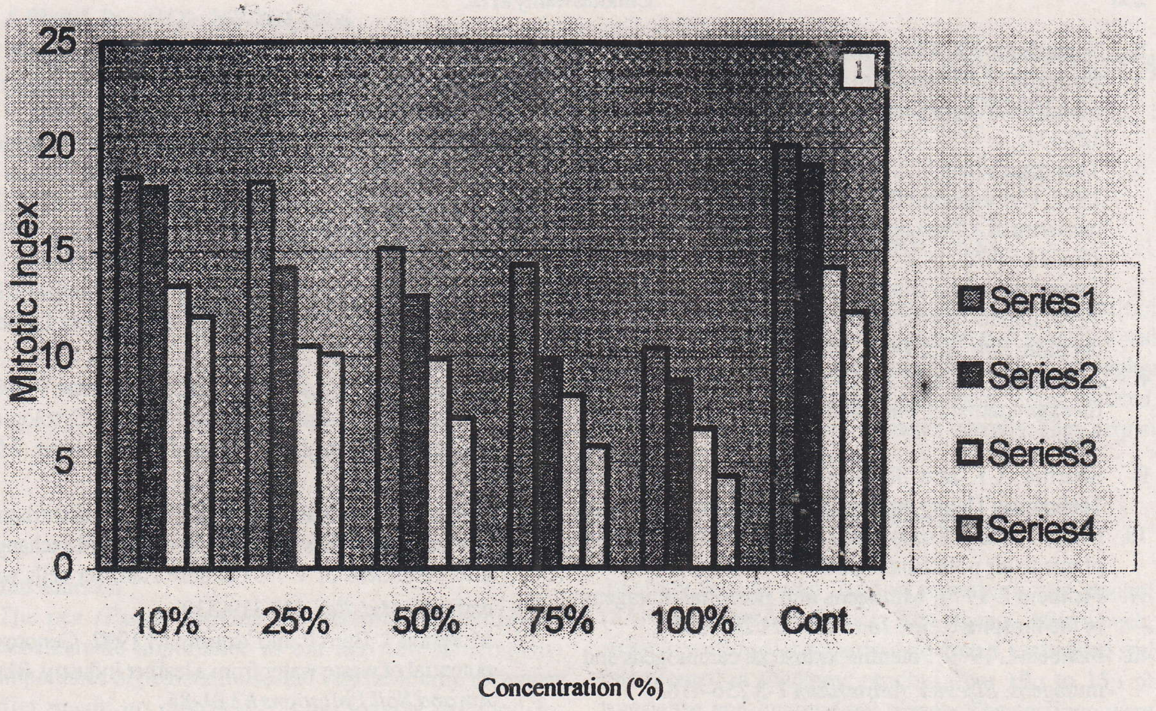
Table 1. Range and mean values of physico-chemical characteristics of the waste water from Amruth Distilleries, chemical characteristics expressed in mg/l (except pH and EC)

1	pH	3.5-4.2-3.85
2	Electrical conductance	14750.40-16252.00-15501.2
3	Total solids	38050.50-39716.60-38883.55
4	Total dissolved solids	35706.12-36713.50-36209.81
5	Total suspended solids	2230.50-3535.20-2882.85
6	Turbidity	2875.50-3130.65-3003.07
7	Total alkalinity	1850.30-2130.70-1990.5
8	Total hardness	4550.40-6841.50-5695.95
9	Residual chlorine	0.00-0.30-0.15
10	Hydrogen sulphide	0.00-1.80-0.9
11	Dissolved oxygen	1.40-3.30-2.35
12	Biological oxygen demand at 20°C for 5 days	18450.00-21450.00-19950.00
13	Chemical oxygen demand	49635.00-54360.00-51997.50
14	Chlorides as Cl	3780.00-4276.20-4028.10
15	Fluorides as F	1.61-2.92-2.26
16	Calcium as Ca	1375.20-1635.10-1505.15
17	Magnesium as Mg	436.20-515.40-475.80
18	Manganese as Mn	0.00-1.50-0.75
19	Sodium as Na	460.12-535.20-497.66
20	Potassium as K	960.21-1051.60-1005.90
21	Iron as Fe	10.00-11.40-10.70
22	Sulphates as SO_4	1890.60-2145.30-2017.95
23	Phosphates as PO_4	0.00-1.20-0.60
24	Nitrates as NO_3	0.00-0.50-0.25
25	Nitrites as NO_2	0.00-0.30-0.15
26	Silicates as SiO_2	0.00-1.40-0.7
27	Ammonical nitrogen	58.60-63.20-60.90
28	Nickel as $Ni^{2}+$	1.32-2.07-1.69
29	Lead as $Pb^{2}+$	0.82-1.92-1.37
30	Zinc as $Zn^{2}+$	19.20-37.42-28.31
31	Copper as $Cu^{2}+$	3.36-8.72-6.04
32	Cobalt as $Co^{3}+$	1.22-1.61-1.41
33	Chromium as $Cr^{3}+$	1.34-3.30-2.32

Table 2. Impact of distillery effluent on different phases of mitosis of *Capsicum annuum L.*

Hrs	Conc. %	Total Cells	No. of Cells In Division	Prop-hase	Meta-phase	Ana-phase	Telo-phase	Abnor-mality%	Mitotic Index	Mito dep. ression
24	10	2000	371	305	24	28	14	Nil	18.55	7.71
	25	2000	366	266	25	29	16	Nil	18.30	8.95
	50	2000	302	253	21	17	11	8	15.10	24.87
	75	2000	286	245	16	23	2	18	14.30	28.85
	100	2000	208	180	14	14	Nil	20	10.40	48.25
	Cont.	2000	402	342	31	15	6	Nil	20.10	Nil
48	10	2000	316	264	13	32	7	Nil	18.05	5.74
	25	2000	283	228	24	22	9	Nil	14.15	26.10
	50	2000	257	210	16	20	11	14	12.85	32.05
	75	2000	197	162	15	20	Nill	29	9.85	48.56
	100	2000	178	145	23	10	Nill	59	8.90	53.52
	Cont.	2000	383	322	31	20	10	Nil	19.15	Nil
72	10	2000	268	232	10	20	6	Nil	13.40	5.63
	25	2000	210	181	8	17	4	Nil	10.50	26.05
	50	2000	198	172	7	15	4	17	9.90	30.28
	75	2000	162	141	6	13	2	41	8.10	42.95
	100	2000	132	120	4	8	Nil	72	6.60	53.53
	Cont.	2000	284	211	36	15	22	Nil	14.20	Nil
96	10	2000	238	210	7	17	4	Nil	11.90	1.65
	25	2000	202	182	9	7	3	9	10.10	16.52
	50	2000	142	132	5	8	Nill	29	7.10	41.32
	75	2000	115	94	6	15	Nill	46	5.75	52.47
	100	2000	88	69	6	13	Nill	76	4.40	63.63
	Cont.	2000	242	197	13	19	13	Nil	12.10	Nil



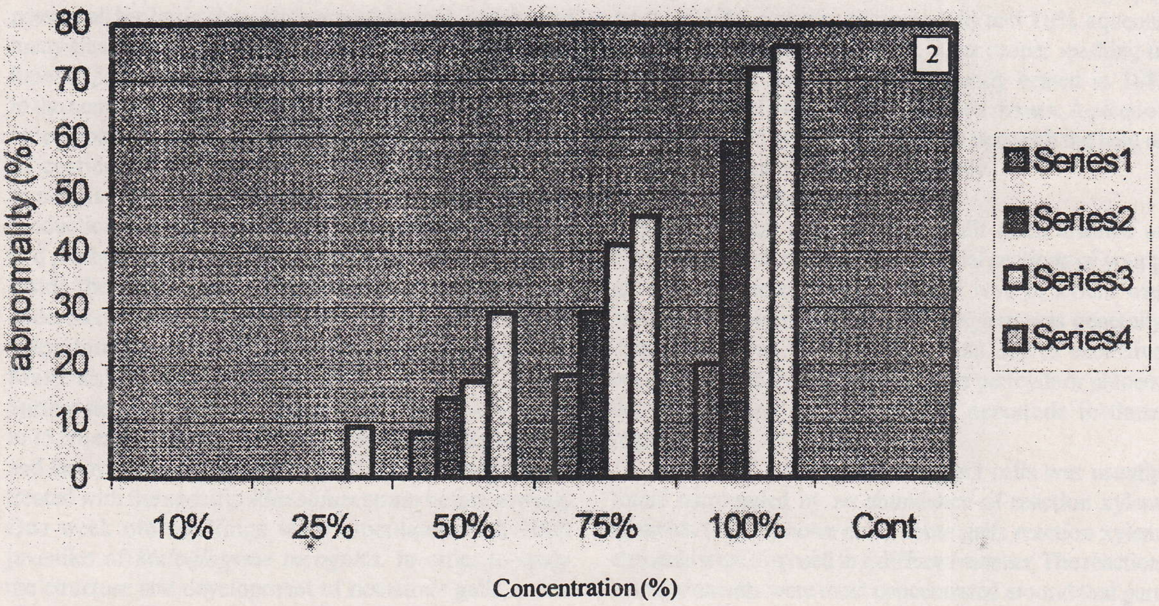


Fig. 1-2. 1. Impact of Amruth Distillery effluent on mitotic index.
2. Impact of Amruth Distillery effluent on cytological abnormality.

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