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Clastogenicity of sugar factory effluent using *Allium* assay

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In the current study, an attempt has been made to assess the physicochemical parameters of distillery effluent and its cytotoxic effects on root tip cells of onion (*Allium cepa* L.). The Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS) were found to be very high in the effluent. The onion bulbs were treated with different concentrations of the distillery effluent (25, 50, 75 and 100%) at room temperature. The percentage mitotic index and relative division rate were found to be decreased significantly as the concentration of the effluent increased. On the other hand, mitotic inhibition percentage and relative abnormality rate were found to increase as the effluent concentration increased. The chromosomal abnormalities were found to be increased as the concentration of the effluent increased when compared to the control. The observed chromosomal abnormalities include sticky metaphase, disturbed chromosome, sticky telophase, multipolar chromosome, laggards, fragmented metaphase, fragmented anaphase, scattered anaphase and chromosomal bridge. From the present study, it can be concluded that higher concentrations of the effluent inhibit cell division when compared to the control.

Keywords: Sugar effluent, onion, chromosomal aberrations

Sugar industry is one of the most important agro-based industries in India. Many industries are directly or indirectly dependent on sugar industry which in turn plays a important role to the overall development of India (Saranraj and Stella, 2014). Sugar industry is responsible for creating noteworthy impact on rural economy. India is a major producer of sugar in the world. Apart from producing sugar and alcohol, these factories generate many by-products and waste materials. The industry also generates about 7.5 million tonnes of molasses and 45 million tonnes of bagassae as valuable by-products. A significant amount of waste is generated during the manufacture of sugar and contains high amounts of production load particularly in items of suspended solids, organic matters, effluent, sludge, press mud and bagassae. The waste water from sugar mills with its high organic load rapidly depletes available oxygen when discharged into water body, consequently rendering the water unfit for drinking and domestic purposes (Saranraj and Stella, 2014).

Pollution with xenobiotics from various industrial activities is the important pollution issue, which will increase in an unprecedented manner due to augment in population growth and accelerated industrialization. Water pollution by industrial effluents has been one of the vital issues of environmental concern in countries

around the world. Due to continual disposal of waste water into the water bodies, water quality become deteriorated because of the mixing of rich organic load of the effluent with water (Fayaza et al., 1996). Water pollution can also happened indirectly as an off shoot of soil pollution – through surface runoff and leaching to ground water. In recent years, due to expansion of distilleries in the sugar cane growing countries, the indiscriminate disposal of spent wash in sugarcane cultivation lands adjacent to different industries is affected by heavy metal toxicity (Om et al., 1994).

Allium cepa L. (onion) is widely used in all parts of the world as flavouring vegetable. The use of the *A. cepa* root length inhibition bioassay as a sensitive, cost effective and valid indicator of toxicity test for the routine monitoring of water pollution is well documented (Fiskesjo, 1985). Most of the studies indicate that there is an excellent correlation between chromosome abnormalities and mutagenic activity found in root-tip systems and those found in mammalian cell systems. Observation of the root tip system of plants therefore constitutes a rapid and sensitive method for environmental monitoring (Majer et al., 2005).

Materials and Methods:

Collection of effluent sample: The sugar factory effluent sample was collected from Pandavapura Sugar Factory P S S K Ltd" Srirangapattana, Mandya District. Karnataka state, India. The treated effluent sample was collected in hard plastic screw capped can and brought to laboratory, stored in a refrigerator for further tests. Onion bulbs (*Allium cepa* L. 2n=16) pinkish and healthy average sized (30-40mm in diameter) were brought from local market. The dried roots present at the base of the bulbs were removed off with a razor to expose the root primordia to effluent. For root growth inhibition assay, the onion bulbs were exposed to 25, 50, 75, 100% (v/v, sugar effluent/ distilled water) of the test sample and distilled water served as control (Fig. 1). The base of each onion bulbs were suspended in different concentrations of effluents taken in glass vials and respective concentrations of effluent was added when required.

Figure: 1. Germination of onion bulbs in different concentration of effluent along with control

Physicochemical analysis: Treated distillery effluent sample was analysed for a standard physicochemical properties, including TDS, total hardness, sulphates, phosphates, nitrates, BOD, and dissolved oxygen (DO) according to methods described by APHA (2005).

Cytological studies: After treatment the root tips were excised from respective bulbs and fixed in 3:1 ethanol: acetic acid. After 24 hours of fixation, the root tips were preserved in 70% ethanol and kept in refrigerator for further studies. The fixed root tips were placed on a clean watch glass to which 9 drops of aceto-orcein and 1 drop of 1N HCl was added. The watch glass was warmed till emergence of fumes and kept for an hour. A single root tip was taken on to a clean glass slide and mounted with 45% acetic acid. Placing coverslip on the root tip was squashed by applying uniform pressure. The slides were sealed with paraffin (Fiskesjo, 1985). The slides were observed under microscope. The number of cells at dividing phase, abnormal cells and chromosomal aberrations were recorded in each concentration and Mitotic Index (MI), Mitotic Inhibition Percentage (MIP), Relative Division Rate (RDR) and Relative Abnormal Rate (RAR) were calculated following the methods of Rekha et al. (2004).

Statistical analysis

The obtained data were subjected to statistical analysis using SPSS package Ver. 14.0 according to Tukey's mean range test at 5% level significance.

Results and discussion

Physicochemical analysis: The physico-chemical parameters of treated distillery effluent is shown in the Table 1. The colour and odour remained light brown and unpleasant respectively. The pH of the treated effluent was 7.9 and alkaline in nature. The BOD of the treated effluent was found to be 30 mg/L while the COD was found to be 248 mg/L. The TDS and Total Suspended Solids (TSS) were 2010 mg/L and 38 mg/L respectively. The chloride content and sulphate content were found to be 426 and 36 mg/L respectively. The sulphate content was found to be very less when compared to chloride. Oil and grease contents were found to be nil in treated effluent. Higher plants that make them excellent genetic models to assess environmental pollutants, being frequently used in monitoring studies. The chemical mutagens cause damage in organisms when exposed, making possible to assess genetic end points from point mutations to chromosome damage in cells of several organs, tissues of leaves, roots and pollen (Grant, 1978). The effect of different concentration of sugar effluent on the mitotic index, mitotic inhibition percentage, relative division rate and relative abnormal rate of root meristem of *Allium cepa* L. is represented in Table: 2. A significant reduction in the mitotic index was observed as the concentration of the effluent increased when compared to control. Mitotic index decreased from (6.33 to2.43%) in control and 100% effluent concentration respectively. Mitotic inhibition percentage (MIP) and relative abnormal rate (RAR) was maximum and minimum in 100% and 25% (87.35%, 65.98 %and 0.917% to 2.36%) respectively. The relative division rate (RDR) was found to be decreased with increasing effluent concentration. The RDR decreased from 144.91% to 57.94 % from 25 to 100% concentration respectively.

The effect of different concentrations of effluent from sugar factory on different stages of mitosis is represented in Table 3. The maximum and minimum values for prophase (fig. 2 A) were observed in control and 100% concentration 62.12% and 23.14% respectively. However as the concentration of the effluent increased the percentage of prophase was found to be decreased significantly from 51.31% to 23.14% in 25 to100% effluent concentrations respectively. The maximum and minimum value of metaphase (fig.2 B) (47.16% and 17.18%), anaphase (fig. 2. C) (38.14%and 7.06%) and telophase (fig. 2. D) (23.84% to 1.82%) were observed from control to 100% effluent concentration respectively. The mitotic index

was found to be decreased as the concentration of the effluent increased when compared to control. However the mitotic cells observed in treated root tips at higher concentrations was relatively lower than control. The decrease in mitotic index in the present investigations with increased effluent concentration is in accordance with Kincl et al. (1996), where they showed reduction in mitotic index of *A. cepa* meristamatic cells, showing cytotoxicity to dairy effluent.

The decrease in mitotic index indicated a mitodepressive effect of spent wash treatment on cell division activities in root-tip cells of sugarcane (Srivastava and Jain, 2010). The present investigation showed decrease in the mitotic index as the concentration of effluent increased. This indicates the cytotoxic effect of sugar effluent. The rate of aberration increased as the concentration increased (Michael et al., 2009). Such a reduction in mitotic activity could be due to inhibition of DNA synthesis. The reduction in the mitotic index clearly indicates the mitodepressive and cytotoxic effects of the fungicide on the present test system. This might have been achieved by the inhibition of DNA (Pulate and Tarar, 2014). The mitotic index was decreased significantly in all treated groups as the concentration of effluent increased when compared to control. The reduction in mitotic activity was more significant when the concentration of the herbicide increased and the exposure time prolonged (Yuzbasioglu et al., 2003). Significant reduction in mitotic index may be due to the inhibition of DNA synthesis or the blocking in the G2 phase of the cell cycle (Khanna and Sharma, 2013).

A strong dosage effect is obvious from a decline in the mitotic index values with the increase in concentration and exhibiting a marked decrease at highest concentration (Shanthamurthy and Rangaswamy, 1979). The high dose of chromium supply has a toxic effect on cell division attributes. Similar type of abnormalities is due to loss of microtubule of spindle fibres (Pickett-Heaps and Trimothy, 1982). Similar mitodepressive response has been observed in *Allium cepa* root cells in response to insecticides, herbicides, pesticides and chemical mutagens treatment. The percentage of chromosomal abnormalities in root tip cells of *Allium cepa* induced by different concentrations of sugar effluent is represented in Table: 4. The chromosomal abnormalities were found to be increased as the effluent concentration increased from 25 to 100% when compared to control, however in control sets chromosomal abnormalities were found to be completely absent. The most common types of abnormalities observed were Fragmented anaphase with bridges (fig. 2 E), sticky metaphase (fig. 2 F), disturbed chromosome (fig. 2 G), scattered anaphase (fig. 2 H), chromosomal bridge with laggards (fig. 2 I), scattered chromosome (fig. 2 J), multipolar chromosome (fig. 2 K), fragmented metaphase (fig. 2 L), fragmented anaphase (fig. 2. M) and sticky telophase (fig. 2 O). These abnormalities probably were due to the effect of effluent on spindle apparatus (Shanthamurthy and Rangaswamy, 1979).

The abnormalities such as chromosomal bridge, laggard and stickiness gradually increased as the effluent concentration increased when compared to control. Similar findings were observed in *Allium cepa* due to the action of alkalies, acids and bleaching agents present in the paper mill effluents (Jayaprakash et al., 1994). It could be synergistic or individual or both (Shanthamurthy and Rangaswamy, 1979). The formations of chromosomal stickiness could also be observed at high frequency owing to the disturbance in nucleic acid metabolism of the cell (Chidambaram et al., 2009). The sticky chromosomes have resulted in abnormal uncoiling of chromosomes during anaphase to telophase (Pulate and Tarar, 2014). The sticky nature of chromosome may be due to delay in

chromosome movement by pesticide treatment. Thus the chromosome could not reach the poles and remained scattered in the cytoplasm and appeared condensed and sticky. Chromosome stickiness arises from improper folding of chromosomes fibre into single chromatids and chromosomes as a result there is an intermingling of fibres and the chromosomes become attached to each other by means of subchromatid bridges. Stickiness may be produced by the action of the herbicide on the polymerization process, or may be resulted in the fragmentation of chromosomes and bridges form at anapahase-telophase stages (Yuzbasioglu et al., 2003). Chromosome fragmentation results from multiple breaks of chromosome in which there is loss of chromosome integrity. Fragmentation can range from partial to total disintegration of the chromosome .chromosome fragmentation in plant cells has been observed only rarely after treated with effluents, herbicides, pesticide and wastewater.

Table: 1 Physico-chemical parameters for treated sugar effluent

Sl. No.	Parameters	Results
1	Color	Light brown
2	Odour	unpleasant
3	pH	7.9
4	BOD	30 mg/L
5	COD	248 mg/L
6	Total dissolved solids	2010 mg/L
7	Total suspended solids	38 mg/L
8	Oil and grease	Nil
9	Chloride	426 mg/L
10	Sulphate	36 mg/L

Table: 2. Effect of different concentrations of sugar effluent on mitotic index, mitotic inhibition percentage, relative division rate & relative abnormal rate of root meristem cells of *Allium cepa* L.

Effluent Concentration	Total no. of cells	No. of Dividing cells	Mitotic index	Mitotic inhibition percentage	Relative division rate	Relative abnormal rate
Control	2507	482	19.22 ± 0.144^{a}	-	-	-
25%	2398	152	6.33 ± 0.308^{b}	65.98 ± 2.00^{d}	144.91 ± 5.263^{a}	0.917 ± 0.031^{d}
50%	2854	131	4.59 ± 0.436^{c}	76.13 ± 2.34^{c}	108.83 ± 9.603^{b}	1.296 ± 0.115^{c}
75%	2579	92	3.56 ± 0.218^{d}	81.48 ± 1.361^{b}	84.76 ± 7.373^{c}	1.822 ± 0.075^{b}
100%	2011	48	2.43 ± 0.291^{e}	87.35 ± 1.414^{a}	57.94 ± 7.368^{d}	2.386 ± 0.241^{a}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package version 14.0 according to Turkey's mean range test at 5% level significant

Chromosomal bridges were found to be the result of stickiness of chromosome. The bridge formation can be due to the general stickiness of the chromosome at metaphase stage, or breakage and reunion of chromosomes. Lagging chromosomes arise mainly due to abnormal spindle formation and as a results spindle fibre failed to carry the respective chromosomes to the polar regions and resultantly lagging chromosomes appeared (Pulate and Tarar, 2014). Lagging chromosomes resulted due to failure of the

chromosomes to get attached to the spindle fibre and to move to either of the two poles. The formation of C-mitosis, lagging chromosome, multipolarity and polyploidy may be due to the disturbance in the spindle formation affected by the herbicide. Chromosomal fragmentation formed as a result of multiple breaks of the chromosome in which there is a loss of chromosomal integrity. Fragmentation can range from partial to total disintegration of chromosome. Fragmentation occurs in prophase, metaphase and anaphase (Grant, 1994).

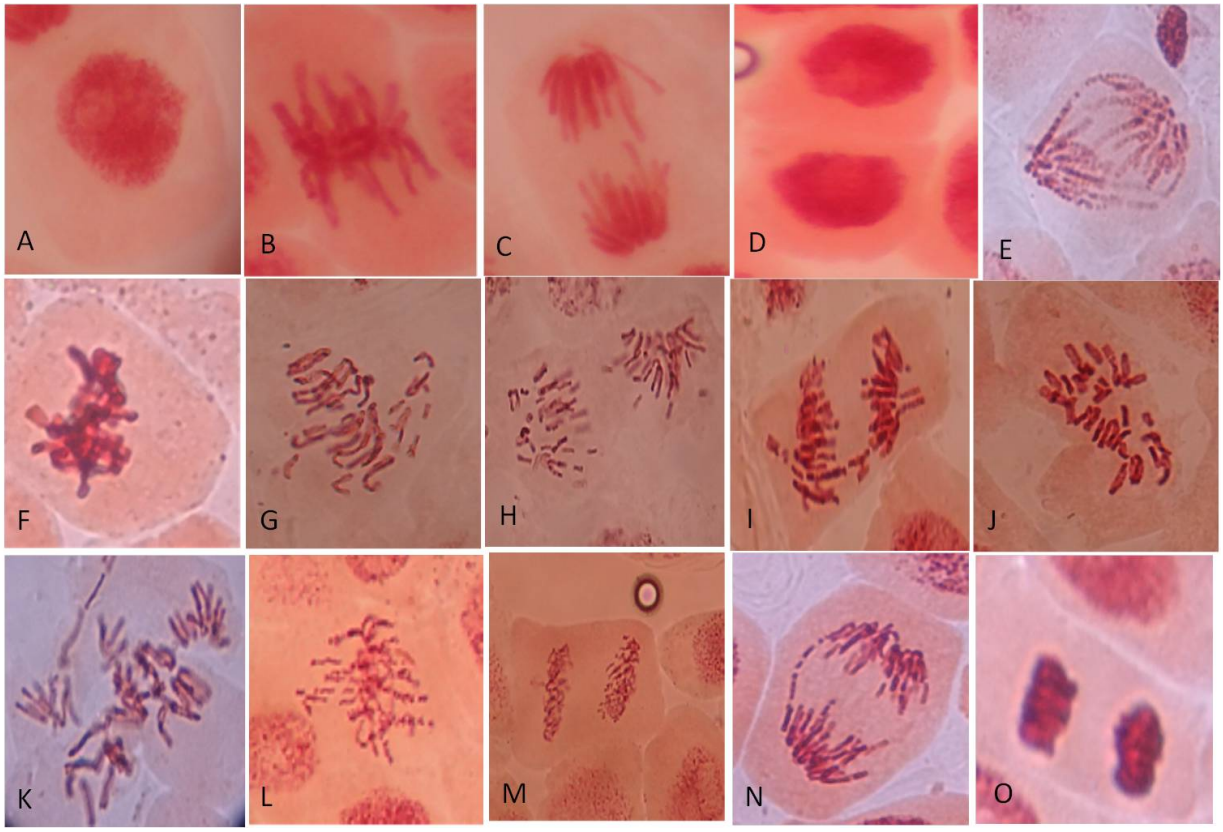

Fig.2. A. Prophase B. Metaphase C. Anaphase D. Telophase E. Fragmented anaphase with bridges F. Sticky metaphase G. Disturbed chromosome H. Scattered anaphase I. Chromosomal bridge with laggard J. Scattered chromosome K. Multipolar chromosome L. Fragmented metaphase M. Fragmented anaphase N. Anaphase with bridges O. Sticky telophase.

Table 3: Effect of different concentrations of sugar effluent on Mitosis of root meristem cells of *Allium cepa* L.

Effluent concentration (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
Control	134.12 ± 3.44^{a}	119.16 ± 2.98^{a}	127.14 ± 1.78^{a}	102.84 ± 1.97^{a}
25%	51.31 ± 1.69^{b}	45.36 ± 2.03^{b}	35.32 ± 1.21^{c}	21.06 ± 1.23^{b}
50%	43.18 ± 1.51^{c}	29.18 ± 2.01^{c}	47.14 ± 1.03^{b}	12.18 ± 1.02^{c}
75%	32.69 ± 1.21^{d}	36.03 ± 1.92^{d}	18.18 ± 1.01^{d}	6.18 ± 0.32^{d}
100%	23.14 ± 1.03^{e}	17.18 ± 1.01^{e}	7.06 ± 0.74^{e}	1.82 ± 0.13^{e}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver.14.0 according to Turkey's mean range test at 5% level significance

Table: 4 Somatic chromosomal abnormalities (%) in root tip cells of Allium cepa induced by different concentrations of sugar effluent:

		Effluent Concentration (%)			
Abnormalities	**Control**	**25%**	**50%**	**75%**	**100%**
Total abnormal cells	-	22.14±4.15^{d}	37 ± 5.08^{c}	47 ± 5.14^{b}	48 ± 5.24^{a}
Sticky Metaphase	-	3.42±0.14^{c}	5.34± 0.04^{a}	2.03± 0.01^{d}	4.09 ± 0.02^{b}
Disturbed chromosome	-	0.00 ± 0.00^{d}	3.86 ± 0.03^{c}	5.14± 0.03^{b}	7.14 ± 0.13^{a}
Sticky telophase	-	1.12±0.01^{c}	2.08 ± 0.01^{b}	2.04 ±0.06^{d}	4.12 ± 0.12^{a}
Multipolar chromosome	-	0.00 ± 0.00^{c}	5.03 ± 0.03^{a}	2.14 ± 0.01^{b}	2.18 ± 0.02^{b}
Laggards	-	4.14 ± 0.14^{b}	4.18 ± 0.05^{b}	5.13 ± 0.05^{a}	6.17 ± 0.04^{a}
Fragmented Metaphase	-	6.02± 0.17^{b}	6.82 ± 0.07^{b}	7.18 ± 0.05^{a}	5.85 ± 0.03^{c}
Fragmented Anaphase	-	5.18 ± 0.12^{d}	7.14 ± 0.03^{c}	10.42 ± 0.07^{b}	11.73 ± 0.13^{a}
Scattered chromosome	-	1.14 ± 0.01^{c}	0.00 ±0.00^{d}	8.19 ± 0.06^{a}	2.14 ± 0.04^{b}
Chromosomal Bridge	-	0.00 ±0.00^{d}	2.14 ±0.02^{c}	5.32 ± 0.04^{b}	6.81 ± 0.07^{a}
Scattered anaphase	-	2.08 ±0.12 ^{b}	3.18 ± 0.04^{a}	1.32 ± 0.01^{c}	1.12 ±0.01^{c}

Mean ± SE followed by the same superscript are not statistically significant between the concentration when subjected to SPSS package ver.14.0 according to Turkey's mean range test at 5% level significance.

Conclusion

Based on the present study it can be concluded that there was a significant reduction in the mitotic index of the dividing cells and the chromosomal abnormalities were found to be increased as the concentration of the effluent increased when compare to control.

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