

Genotoxic effect of distillery effluent on root tip cells of *Allium sativum* L.

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ABSTRACT

In the current study an attempt has been made to evaluate the cytotoxic efficacy of distillery effluent on somatic cells of *Allium sativum*. The garlic bulbs were treated with different concentrations of effluent viz. 2.5, 5, 7.5 and 10% at room temperature. The percentage mitotic index was found to be decreased significantly as the concentration of the effluent increased, except in 2.5% concentration, where the mitotic index is higher than control. The chromosomal abnormalities were found to be increased as the effluent concentration increased when compared to control. The observed chromosomal aberrations were laggards, fragmented chromosome, fragmented anaphase and metaphase, chromosomal bridges, micronuclei, lobed nucleus, scattered chromosome, disoriented anaphase, Polyploidy cells. According to present findings 2.5% concentration of the effluent was found to enhance the rate of cell division when compare to control on the contrary higher of the effluent showed negative effects on mitotic division in somatic cells of Garlic.

1. INTRODUCTION

Pollution is the undesirable change in our surroundings that have harmful effects on plants, animals and human beings. Any alteration to air, water, soil or food threatens the health, survival capability or activities of human or other living organisms in the environment, this undesirable changes in the environment is called Environmental Pollution [1]. Environmental pollution is becoming the most challenging problem to life forms due to rapid industrialization. Industrial and domestic discharges are the major sources of toxic chemicals present in the environment and are reported to be mutagenic [2]. In India land application of wastewater is considered as a valuable source of irrigation and has been practiced since many years [3]. Although utilization of wastewater for crop irrigation overcomes the scarcity of freshwater, irrational use poses potential health hazards to farm workers and consumers. Soil can also lose productivity from increased salinity, sodicity [4] and adds up toxic compounds to irrigated water and increase dissolved residues which tremendously increase the total amount of sediments which contains various types of long chain detergents, plastics, phenolic chemicals, glass, heavy metals, toxic gases, pesticides etc. [5]. Apart from these toxic compounds they also contains high organic load and moderate level of plant nutrients. Improper management of wastewater irrigation may provide the crops with nutrients beyond their specific requirement and subsequently accumulates them at

undesirable high levels in crop which leads to reduction in yield and its quality [6]. Despite the treatment being employed by some industries, it is still impossible to remove all undesirable properties from the effluents [7]. The effluent samples from Oil, Refinery [8], steel, distillery, tannery, thermal power station [9], flash light factory [10], sewage waste water [11], Pulp and Paper mill factory [12] are used in research to study the effect of their harmful contents on living organisms. Among the major industries in India, distillery is the one that contribute to the pollution. Distilleries are the agro-based revenue earning industries that produce alcohol. India is the second largest producer of Ethanol in Asia [13]. Presently there are 319 distilleries in India producing 3.25 billion liters of alcohol annually [14]. Higher Plants are useful, reliable and economical bioassays for biomonitoring environmental pollutants, since the plant chromosomes are excellent biomarkers for mutagenic studies [15]. Bio-indicators are the biological responders to an external hazardous agents that give a measure of exposure and that can be used to indicate harmful effects and to predict future harm [16]. Cytogenetic test analyses the frequency and type of chromosome aberrations in mitotic cells because it reflects early warning signs of adverse long term effect in the populations [17]. *Allium cepa* L. and *Allium sativum* L. are the important bioassays that are routinely used for the detection of environmental mutagens. *A. cepa* L. has been recommended as a standard assay for environmental monitoring [18]. Both the test systems possess large chromosomes and are easy to culture under laboratory conditions and hence *A. sativum* L. can also be used as a standard model for environmental monitoring [15], hence in the resent investigation an attempt has been made to assess the cytotoxic effect of distillery effluent on *Allium sativum*.

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2. MATERIALS AND METHODS

2.1 Collection of Effluent Sample

The effluent sample was collected from 'Chamundi distilleries' (Maliyuru, T. Narsipura taluk) Mysore. Sample were collected in a plastic containers and the samples were brought to lab and stored in refrigerator.

2.2 Collection of Garlic Bulbs

The garlic bulbs of similar size were collected from Srinidhi Stores, KR Market, Mysore. The effluent sample was diluted with distilled water and different concentrations viz, 15%, 25%, 50%, 75%, 100% of the effluent were taken in separate test tubes. One tube with distilled water was taken as Control. The garlic bulbs of similar size were cleaned by removing old roots and placed directly on the tube containing test liquids [18]. The test tubes were left for a week in low intensity of light but the formation of root were not observed in any of the concentrations. The tubes were again replaced by lower concentration of the effluent like 2.5%, 5%, 7.5% and 10% new garlic bulbs were placed again. After 4 days the germination of root was observed.

2.3 Cytological Studies

The root tips were cut from the respective bulbs and fixed in 3:1 alcohol: acetic acid mixture. After 24hrs root tips were transferred and stored in 70% ethanol until further use. The root tips were squashed following the method of [18], in brief root tips were taken in a watch glass and stained with 9 drops of 2% Aceto-orcein stain and 1 drop of 1N HCl. The watch glass was warmed and kept for an hour. A single root tip was taken on a clean slide and mounted in 45% acetic acid, by placing cover glass the root tip was squashed by applying uniform pressure with thumb and by tapping with a pencil. The cover slip was sealed with paraffin wax or nail polish and observed under microscope for chromosomal abnormalities. Photographs were taken wherever necessary. More than 2000 cells were counted in slide at different field. The number of cells at division phase, abnormal cells and chromosomal aberrations were noted in each concentration and mitotic index was calculated using the formula

$$MI = \frac{\text{Total number of cells in division}}{\text{Total number of cells observed}} \times 100$$

2.4 Statistical analysis

The data was subjected to statistical analysis using SPSS package (ANOVA) Ver.16 according Tukey's HSD significant test at 5% level.

3. RESULTS AND DISCUSSION

Mitotic index is a best biomonitor to assess the effects of various chemicals/effluents/herbicides on cell division [18, 2]. The effect of distillery effluent on mitotic frequency is showed in Table 1. The mitotic index was observed for more than 2000 cells. It was found that the mitotic index decreased with the increasing concentration of the effluent sample except at 2.5 % (11.36) when

compared to control (10.63) and had a significant decrease in mitotic index in 2.5 %, 5%, 7.5% and 10% (11.36, 11.14 , 9.256, 7.710) respectively. In the present study the mitotic index was found to be decreased with increasing concentration and declined in the order 2.5% > 5% >7.5% >10%. Cytotoxicity is defined as a decrease in mitotic index and as increase in the fraction of cells with C-Mitosis, multipolar anaphase, sticky chromosomes and laggards [19, 20]. The decrease in mitotic activity and mitotic index could be due to the inhibition of DNA synthesis and also indicates the loss of cell division. The distillery wastes contains many undesirable cytotoxic compounds with high BOD, COD, TDS, TSS may cause cell death which may appear as decline in the mitotic index. These results are in agreement with the earlier studies conducted on Sugarcane exposed to distillery effluent that inferred heavy metals like Cadmium, chromium, nickel and lead jointly affect the normal sequences of mitosis leading to disturbance of spindle function which lead to decrease in mitosis [21]. The results of the mitosis show that the proportion of the cells in metaphases, anaphases and telophases taken together steadily decreased from 11.36 at 2.5% of effluent to as low as 7.710 at 10% concentration. This is due to the arrest in the cell cycle before metaphase to restore the integrity of DNA [22]. The chromosomal aberrations were observed in all the concentrations except control. The observed chromosomal abnormalities include Binucleate cell with lobed nucleus (fig.1, J), polyploid cells (fig.1, N and O), multinucleated cell (fig.1, K) and micronucleus (fig.1, H) Fragmented chromosomes observed in Metaphase (fig. 1, C) The Anaphases were characterized by Chromosomal bridges (fig.1, B and A), Fragmented anaphase (fig. 1, C), Laggards (fig.1 I), C-mitosis (fig.1 G), multipolar Anaphase (fig. 1,E, and F) in all the concentrations. Micronucleus, (fig. 1, H) and Binucleate cell (fig. 1, H) formation was found in Telophase. The of high frequency of chromosomal aberrations reflects that the distillery effluent acts primarily on mitotic spindle, which results in disorientations of chromosomes at various stages of cell cycle [23]. The chromosomal aberrations like micronucleus, chromosome bridge, polyploidy cells, multinucleate cells, fragmented metaphase and anaphase, lobated nucleus, laggards, multipolar anaphase were common. Micronucleus formation was determined by the acentric fragments or whole chromosomes that were not incorporated to the main nucleus during cell division cycle [24]. Chromosome bridges is either due to chromosome stickiness producing abnormal anaphase separation or may be attributed to unequal translocation or inversion of chromosome segments and also due to breakage and fusion of chromosome or chromatids [25]. The chromosomal fragments are more common in metaphase and anaphase. This can be derived from chromosomal breakages in anaphase bridges and metaphase, which can originate from cohesive chromosomal translocations. The chromosomal fragments also result from multiple breakages of the chromosome in which there is a loss of chromosome integrity [26]. Binucleate cells arise as a consequence of the inhibition of cell plate formation. These form a distinct sub population of easily detected cells.

Table 1: showing mitotic index at different concentration of effluent sample.

Effluent concentration	Total no. of Cells	No. of dividing Cells	Mitotic Index
Control	4921	520	10.63 ± 0.361 ^c
2.5 %	5144	594	11.36 ± 0.350 ^a
5 %	5034	563	11.14 ± 0.060 ^b
7.5 %	4564	421	9.256 ± 0.040 ^d
10 %	3936	298	7.710 ± 0.151 ^e

Mean ± SE followed by same superscript are not statistically significant between the concentration, when subjected to SPSS package Ver.16.0 according to Tukey mean range test at 5% level significance.

Table 2: showing chromosomal abnormalities at different concentration of the effluent (%).

Abnormalities	Control	Concentration (%)			
		2.5 %	5 %	7.5%	10 %
Abnormal Cells	0.00±0.00e	30.26±1.65d	34.45±1.96c	37.46±2.01b	41.46±3.24a
Fragmented Metaphase	0.00±0.00	5.52±0.42	2.85±0.54	3.54±0.68	10.54±0.84
Fragmented Anaphase	0.00±0.00	3.75±0.24	3.25±0.64	0.00±0.00 ^a	1.23±0.25
Chromosome Bridge	0.00±0.00	0.00±0.00 ^a	5.25±0.67	10.49±0.85	6.49±0.62
Binucleate Cells	0.00±0.00	0.00±0.00 ^a	5.56±0.69	0.00±0.00 ^a	0.00±0.00 ^a
Multinucleate cells	0.00±0.00	10.46±0.95	6.25±0.58	6.89±0.69	6.25±0.63
Polyploid cells	0.00±0.00	0.00±0.00 ^a	0.00±0.00 ^a	4.25±0.35	12.25±0.97
Micronucleus	0.00±0.00	0.00±0.00 ^a	8.28±0.46	0.00±0.00 ^a	0.00±0.00 ^a
C – Mitosis	0.00±0.00	2.32±0.12	1.23±0.05	0.00±0.00 ^a	2.05±0.45
Multipolar Anaphase	0.00±0.00	3.85±0.56	2.23±0.09	4.58±0.65	1.45±0.08
Lobed Nucleus	0.00±0.00	3.49±0.45	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Laggards	0.00±0.00	4.78±0.68	2.02±0.50	10.04±0.95	3.15±0.24

Mean ± SE followed by same superscript are not statistically significant between the concentration, when subjected to SPSS package Ver.16.0 according to Tukey mean range test at 5% level significance.

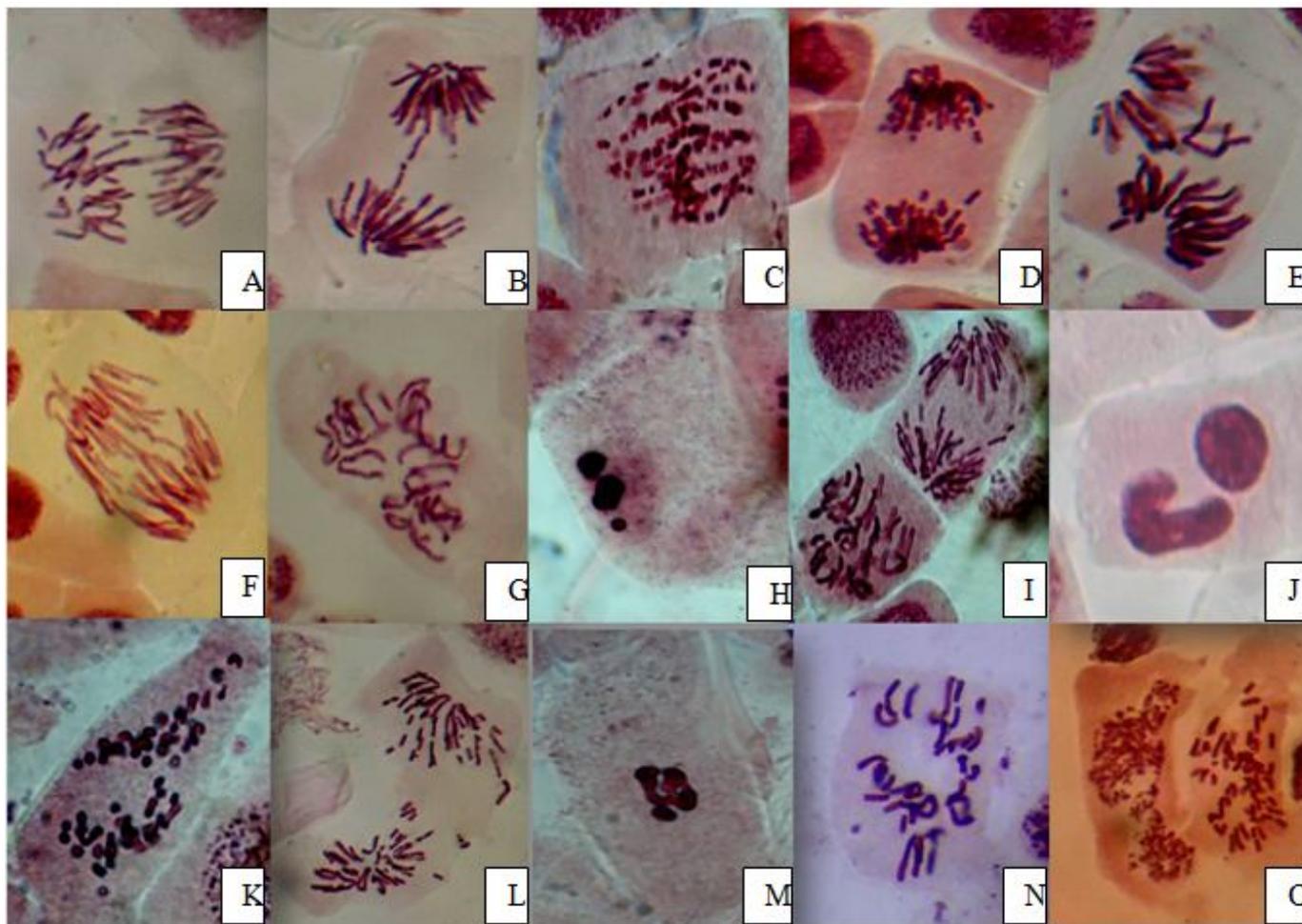


Fig.1: , A, B-Chromosomal bridge (5% and 10%), C-Fragmented metaphase, D-Fragmented anaphase, E, F-Multipolar anaphase (7.5% and 10%), G- C mitosis, H- Binucleate cell with micronucleus, I-Laggards, J-Binucleate cell with lobed nucleus, K-Multinucleate cell, L-Laggards with fragmentation, M-Lobed nucleus, N,O- polyploidy cells (7.5% and 10%).

Thus the effluent have an effect on phragmoplast. Phragmoplast is a plant cell specific structure forms during cytokinesis and serves as a scaffold for cell plate assembly and subsequent formation of a new cell wall separating two daughter cells. It is a complex assembly of microtubules, microfilament and endoplasmic reticulum [27]. Multinucleate cells were common in all the concentrations. It is due to failure in cell plate formation in already binucleate cells. The mitotic irregularities such as incompleated anaphase or unequal distribution of chromosomes to daughter cells results in polyploidy cells [26]. Multipolar anaphases were observed in 2.5%, 5% and 10% concentration. It is caused as a result of malfunction of mitotic spindle which leads to unbalanced chromosome distribution heading them for more than two poles onto the cells [28] and are indicating the inhibition of cytokinesis [29]. C-Mitosis is a result of which all anaphase chromosome lie on the metaphase plate instead of moving towards their respective poles [20].Laggards are lagging chromosomes observed in 2.5% and 5% concentration may be attributed on the failure of spindle apparatus to organize in a normal way [30].

4. CONCLUSION

Distillery effluent significantly influences all aspects of growth and development. The current analysis evidently showed that there was a significant lessening in the mitotic index of the dividing cells in higher concentrations and the chromosomal aberrations were found to be increased as the concentration of the effluent increased.

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