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EVALUATION OF CYTOTOXIC EFFICACY OF DAIRY EFFLUENT ON ROOT MERISTEM CELLS OF GARLIC (*Allium sativum* L.)

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Abstract: In the present investigation an attempt as been made to assess the cytotoxic effects of dairy effluent in the root tip cells of Garlic (*Allium sativum* L.). The Garlic bulbs were treated with different concentrations of dairy effluent (5, 10, 25, 50, 75 and 100%) at room temperature. The percentage mitotic index was found to be decreased significantly as the concentration of the effluent increased, except in 10% concentration where the mitotic index is higher than control. The chromosomal aberrations were found to be increased as the concentration of the effluent increased when compared to control. The observed chromosomal abnormalities were chromosomal bridge, nuclear lesion, scattered chromosome, fragmented anaphase, fragmented metaphase, micronuclei, scattered chromosome, sticky chromosome and sticky metaphase. According to our findings we can say that 10% concentration of the effluent will promote the cell division when compare to control on the other hand higher concentration of the effluent will produce negative side effects on mitotic division in root tip cells of Garlic.
Keywords: Dairy effluent, cytotoxicity, garlic, chromosomal abnormalities.

INTRODUCTION

Environmental pollution has become an important global issue as water is directly used for various purposes (Naik *et al.,* 2007). In India, over last few decades' rapid industrialization, population explosion and more urbanization have created pollution in environment by generation of variable quantity and quality of solid and liquid wastes (Arora *et al.,* 1985).To economize the irrigation water, industrial effluents are now a day's used for irrigating agricultural fields (Sharma, 2011). Effluent reuse can provide considerable benefit when used under controlled conditions to establish protection of farm workers and consumers of the produce (Aleem and Malik, 2003). However, despite the treatment to the effluent being employed by some industries, it is still not possible to remove all the undesirable properties from effluent (Egbenni *et al.,* 2009).

Some industrial and sewage effluents are the rich source of plant nutrients and soils being logical sink for their disposal. But many untreated and contaminated sewage and industrial

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waste water may have high concentration of heavy metals such as Cd, Ni, Pb and Cr (Arora *et al.,* 1985; Narwal *et al.,* 1993). Their continuous disposal on agricultural soils have resulted in soil sickness (Narwal *et al.,* 1988) and accumulation of toxic metals in soil (Adhikari *et al.,* 1993; Narwal *et al.,* 1993) which may affect human and animal health. The dairy industry in India on an average have been reported to generate 6- 10 litres of waste water per litre of the processed milk (Chakraborty and Saha, 2011). The dairy effluent have high loads as milk being major constituent high levels of BOD, COD, oil and grease, nitrogen and phosphorous (Bario, 2007).

Garlic is a perennial that grows to a height of 2 feet or more. The leaves are strap like, 1-2 feet long which surrounds a central flower stalk or scape, which develops a tiny white cluster as it blossoms. Leaves are six to twelve in number, flat, linear, longitudinally folded along the midrib. Leaf bases form pseudostem, which remains stiff until the bulb matures. A head of garlic is composed of a dozen of cloves which are thick leaf bases together forms a bulb, which lies underground and forms propagating structure The flowers are produced in a small cluster at the top of the stem, often together with several bulblets, and surrounded by a papery basal spathe; each flower is white, pink or purple, with six petals 3-5 millimetres long. The flowers are often abortive and rarely produce any seeds (Medina and Garcia, 2007).

MATERIALS AND METHODS

The dairy effluent sample was collected from Mysore- Chamarajanagar District Co-operative Milk Producers' Societies' Union Limited. , Siddarthanagar, Mysore, brought to laboratory and refrigerated for further analysis. Garlic bulbs (*Allium sativum* L., 2n=16) of white and healthy, average size (25-30mm diameter) were obtained from Mysore market. The dried roots present at the base of the bulbs were removed off with a sharp razor to expose the root primordia (Fiskesjo, 1985). For root growth inhibition and evaluation of root apical meristems, the garlic bulbs were exposed to 0, 5, 10, 25, 50, 75, 100% (v/v, dairy effluent/ tap water) of the test sample and distilled water as control. The base of each garlic bulb was suspended in different concentrations of effluents taken in glass vials and respective concentrations of effluents were added when required. Similar replicates were used and left at low light intensity for a week.

CYTOLOGICAL STUDIES

The roots were excised from respective cloves and fixed in 3:1 ethanol: acetic acid. After 24hours of fixation, the tips were preserved in 70% ethanol, were kept in refrigeration 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 slide and mounted in 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 prepared 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) was calculated using formula

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

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

The increasing discharge of hazardous chemicals into the environment has affected the balance of natural ecosystems and has drawn attention of several researchers and governmental agencies to the health of living organisms (Leme *et al.,* 2009). 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, 1994).

The effect of different concentration of effluent on mitotic index is represented in the table 1. As could be observed in the table there was a significant reduction in the mitotic index in the root tip cells. As the effluent concentration increased the mitotic index found to be decreased when compare to control except in 10% concentration. Maximum value of the mitotic index was observed in the 10% concentration (15.06%). While the minimum value of the mitotic index was observed at 100% concentration (2.73%). The mitotic index decreased from 11.58 to 2.73% at dosage25 to 100% concentration of effluent respectively.

In root tip cells of garlic, at higher concentration effluent inhibits the mitosis at metaphase and anaphase (Table 2). In control prophase was maximum (59.35%), while it was minimum (29.65%) in 100% concentration, however as the concentration of the effluent increased the percentage of prophase was found to be decreased significantly from 46.48 to 29.65% in 5 to 100% effluent concentrations respectively. The maximum value of metaphase (27.09%), anaphase (36.43%) and telophase (16.08%) were observed at 10% concentration and the

minimum value of metaphase (9.43%), anaphase (8.12%) and telophase (6.81%) were observed at 100% concentration of effluent, when compare to control.

In the present investigation the mitotic index was found to be decreased as the concentration of the effluent increased when compared to control except in 10% concentration. However the mitotic cells observed in treated root tips at higher concentrations was relatively lower than the 10% and control sets. 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* meristematic cells, showing cytotoxicity to dairy effluent.

The different types of chromosomal abnormalities induced by different concentration of dairy effluent are presented in the table 3. The percentage of chromosomal aberrations was found to be increased as the concentration of the effluent increased, except in 10% concentration when compared to control. The most common types of abnormalities observed were Chromosomal bridge, fig. A, fragmented metaphase, fig. B, fragmented anaphase, fig. C, nuclear lesion, fig D, scattered chromosome, fig. E, fragmented prophase, fig. F, micronuclei, fig. G, sticky chromosome, fig. H and sticky metaphase, fig. I.

Nuclear lesion was observed in all the concentration of effluent except in control, on the other hand the percentage of the nuclear lesion was found to be increased as the effluent concentration increased. Highest frequencies of nuclear lesion were observed at 100% concentration (18.54%), while it was not observed in control. Nuclear abnormality (NA), in the present study was found to be nuclear lesion, resulting in decrease in mitosis or cell division. Similar results were observed by Leme *et al.,* (2008). Effluent is effective in the formation of the chromosomal bridge at 25% concentration, while control did not show any chromosomal bridge. Occurrence of chromosomal bridge may be due to stickness or formation of dicentric chromosome caused by breakage and reunion (Jabee et al., 2008). Chromosomal bridge mainly arises due to the non disjunction of sticky chromosome or breakage and reunion during separation at anaphase (Koduru and Rao, 1981). Occurrence of fragmented metaphase were maximum (0.73%) at 50% concentration, while it was minimum (0.0.2%) at10%concentration of effluent. Fragmented anaphase and fragmented prophase were maximum (0.46%) and (0.01%) at 50% concentration respectively. 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, 1978).

Sticky metaphase were found to be maximum (0.67%) at 50% effluent concentration. The maximum value of sticky anaphase (0.03%) observed in 100% concentration. Chromosomal stickiness arises from improper folding of the chromosome fiber in to a single chromatids and chromosomes. As a result there is a intermingling of the fibers; the chromatids become attached to each other by means of subchromatid bridges (Grant, 1978). The maximum value of scattered chromosome (0.54%) was observed at 50% concentration.

The maximum value of micronuclei (1.82 %) and was observed at 50% and 5% concentration of effluent, when compare to control. Micronucleus has been considered as simplest end point of cell division as a result of mutagenic effect of chemicals. The presence of micronuclei in the present study has also been observed by Leme *et al.,* (2008) in onion root meristem cells indicating clastogenic and aneugenic effects. Chromosomal aberration (CA) are characterized by change in either a chromosomal structure or total number of chromosomes, which occur spontaneously due to the exposure of physical or chemical mutagens. The chromosomalaberrations such as chromosome bridges and chromosome breaks, are results of aneugenic effects obtained in the present study, is in correlation with the works of Leme *et al.,* (2008), Fernendes *et al.,* (2007), Ateeq *et al.,* (2002), Bolle *et al.,* (2004).

The study revealed that the presence of chemicals which altered the water quality. There was reduction in mitotic index upon increased concentrations of effluent treatments, except in 10% where it was maximum. In support to reduction in cell division, there was increase in chromosomal abnormalities with increased effluent concentrations employed in treatment of garlic and was maximum in 100% concentration. The results obtained from this study reveals, that the dairy effluent having potential cytotoxic efficacy by inhibition of cell divisions in garlic root tip cells.

Table 1: Effect of different concentrations of dairy effluent on mitotic index of root meristem cells of *Allium sativum.*

Effluent concentration (%)	Total Number of Cells	Number of Dividing Cells	Mitotic index (%)
Control	3811	516	13.53 ± 0.32^{b}
5	3282	393	11.98 ± 0.26^{c}
10	3402	512	15.06 ± 0.30^{a}
25	3434	381	11.58 ± 0.56^{c}

50	3138	227	7.23 ± 0.18^{d}
75	2709	149	5.51 ± 0.10^{e}
100	2068	54	2.73 ± 0.31^{f}

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

Table 2: Effect of different concentrations of dairy effluent on different stages of mitosis in root meristem cells of *Allium sativum.*

Effluent concentration (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
Control	59.35 ± 0.43^{a}	16.80 ± 0.34^{c}	23.45 ± 0.57^{b}	9.78 ± 0.59^{d}
5	46.48 ± 1.53^{b}	20.43 ± 1.03^{b}	22.45 ± 1.03^{c}	10.97 ± 0.48^{b}
10	49.62 ± 1.13^{c}	27.09 ± 0.28^{a}	36.43 ± 0.59^{a}	16.08 ± 0.51^{a}
25	47.76 ± 0.74^{d}	17.04 ± 0.70^{d}	22.08 ± 0.15^{c}	10.50 ± 0.23^{c}
50	44.86 ± 1.20^{e}	15.41 ± 0.29^{e}	18.68 ± 1.51^{d}	7.52 ± 0.28^{e}
75	37.87 ± 1.00^{f}	13.21 ± 0.06^{f}	14.05 ± 0.68^{e}	7.36 ± 0.33^{e}
100	29.65 ± 1.31^{g}	9.43 ± 1.60^{g}	8.12 ± 0.40^{f}	6.81 ± 0.62^{f}

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

Table 3: Somatic chromosomal abnormalities (%) in root tip cells of *Allium satium* induced by different concentrations of Dairy effluent

Chromosomal Abnormalities (%)	Effluent concentration (%)						
	control	5	10	25	50	75	100
Chromosomal bridges	0.00 ± 0.00^{f}	0.28 ± 0.03^{d}	0.39 ± 0.67^{c}	0.94 ± 0.05^{a}	0.60 ± 0.02^{b}	0.14 ± 0.02^{e}	0.14 ± 0.03^{e}
Fragmented metaphase	0.00 ± 0.00^{e}	0.05 ± 0.01^{c}	0.02 ± 0.03^{d}	0.01 ± 0.01^{e}	0.73 ± 0.11^{a}	0.50 ± 0.05^{b}	0.00 ± 0.00^{e}
Fragmented anaphase	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.01 ± 0.02^{c}	0.00 ± 0.00^{d}	0.46 ± 0.06^{a}	0.00 ± 0.00^{d}	0.02 ± 0.02^{b}

Nuclear lesion	0.00 ± 0.00^{g}	0.65± 0.01^{f}	2.33 ± 0.11^{e}	6.43 ± 1.86^{d}	7.38 ± 0.37^{c}	14.64 ± 0.65^{b}	18.54 ± 3.90^{a}
Scattered chromosome	0.00 ± 0.00^{b}	0.00± 0.01^{b}	0.00 ± 0.01^{b}	0.00 ± 0.00^{b}	0.54 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Micronuclei	0.00 ± 0.00^{d}	0.00± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	1.82 ± 0.22^{a}	0.51 ± 0.13^{b}	0.02 ± 0.02^{c}
Sticky chromosome	0.00 ± 0.00^{b}	0.03± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Sticky metaphase	0.00 ± 0.00^{c}	0.00± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.02 ± 0.01^{b}	0.00 ± 0.00^{c}	0.03± 0.02^{a}

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

Figure 1: Chromosomal abnormalities in root meristem of *Allium sativum* treated with different concentrations of Dairy effluent.

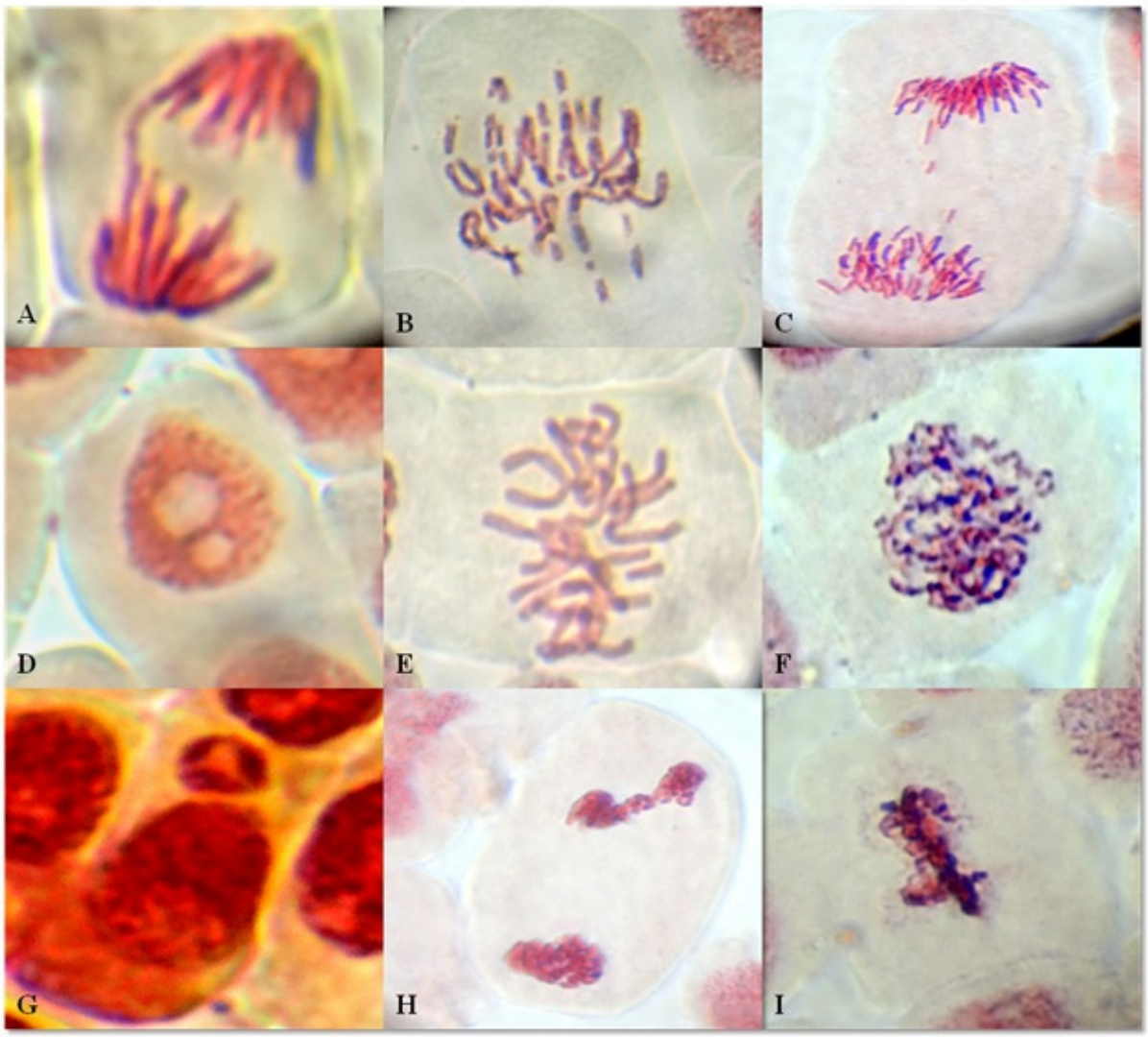

Figure 1: A-chromosomal bridge, B-fragmented metaphase, C-fragmented anaphase, D-nuclear lesion, E- scattered chromosome, F- fragmented prophase, G- micronuclei, H-sticky chromosome and I- sticky metaphase.

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