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Short Communication

Genotoxicity and mutagenicity caused by of polluted surface water in the National fertilizer limited (NFL), Bathinda (Punjab, India) using plant bioassays

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Abstract

Factories release pollutants into water bodies that cause deleterious effect on meiotic behavior, genome damage and infertility. By taking into consideration the present investigation is being done to know about cytotoxic potentials of the polluted water using in vivo bioassay and to study the impact of pollution on genetic systems of the inhabitant plant species. The results has shown that chromosome abnormality increases with increase in concentration of pollutants and plants growing in polluted water showed meiotic abnormality including laggard, stickiness, vagarant etc.

Keywords: Meiosis, mitosis, pollution, chromosomal abbreations.

Introduction

Water is an indispensable natural product used in all aspects of human life. The pollution of water bodies is a global problem because of the danger that polluted waters may contain mutagenic and carcinogenic substances that may cause or promote the occurrence of human diseases[1]. Majority of water bodies are getting polluted by addition of the domestic wastage, industrial waste, and a lot of other pollutant that are badly effecting human health as well as living beings of water bodies. The main source of contamination includes ceramic industries, mining, waste of paper industry, smoldering units, PVC plastics, battery, pigments, mining etc[2].

Genotoxic pollution of water body is the introduction of contaminant having mutagenic, teratogenic as well as carcinogenic action into the principal media including genome of resident flora and fauna[3]. Genotoxicity has deleterious effect, which affects a genetic material of cell and affect its integrity[4]. Genotoxic material include heavy metals[5,6], microbial toxins[4], polycyclic aromatic hydrocarbons[7,8]. These are reported to lead to mutations in chromosomes due to their ability to form covalent bonds with the DNA, and form DNA adducts that prevent replication[9].

The Allium cepa test being efficient is used analyzing in situ genotoxic potential of several materials[10]. It is fact that green plant are also main source of the antimutagens, toxic as well as genotoxic agents[11]. This assay being low cost, is easy to use and provides similar results to animal testing because of similarity in their gene compositions, so similar response to the mutagens. The presence of metacentric chromosomes in *A. cepa* cells allows easier and better microscopic assessment.

Material and methods

In situ assay: For meiotic analysis, unopened, young flower buds from the affected area were collected and were fixed in Carnoy' fixative (Absolute alcohol: Chloroform: Glacial acetic acid: 6:3:1) for 24 hours. The materials were subsequently transferred to 90% Alcohol and kept at 4˚C in refrigerator till use. The developing anther were squashed in 1-2 drops of 2% Acetocarmine, prepared by dissolving 2gm of standard strain Carmine (BDH) in 100ml of 45% Acetic acid. A number of freshly prepared slides were examined for mitotic analysis. The incidence of various types of meiotic abnormalities like late disjunction, laggards, bridges at Anaphase-1 or Telophase-1 etc., were scored from various slides at random and microsporogenesis was studied.

In vivo allium assay: Water extract preparation: water is taken and further diluted with different volumes of water to prepare the concentrations.

Genotoxicity assay: Allium bioassay was done to study the cytotoxicity action of extracts of collected polluted water, following the procedure[12,13].

Test organism and growing conditions: Healthy and equal sized bulblets of *Allium sativum* (2n=16) was used as test organism. Outer scales of the bulblets were removed.

Test tubes filled with tap water were taken and bulblets were placed over the test tubes with root primordial dipping in water. Water was changed every 24 hrs. The whole set was placed in the above condition for 48hrs at 25˚C, till the roots attain the size of about 2cm then bulbils were treated with polluted water of various concentration for 24 hours. After treatment rootlets were cut and transferred in carnoy's fixative and then stored in rectified spirit in cold place and used for further analysis.

Microscopic analysis: The various calculations were made as follows:

$$\text{Mitotic index} = \frac{\text{Total number of dividing RTCs}}{\text{Total number of observed RTCs}} \times 100$$

$$\text{Percent Mitotic phase} = \frac{\text{Number of cells at mitotic phase}}{\text{Total number of dividing cells observed}} \times 100$$

$$\text{Percentage aberration} = \frac{\text{Total number of abnormal RTCs}}{\text{Total number of observed RTCs}} \times 100$$

$$\%\ \text{micronuclei} = \frac{\text{Total number of cells with micronuclei}}{\text{Total number of observed RTCs}} \times 100$$

Results and discussion

Data regarding allium assay, meiotic behavior and pollen fertility has been shown in Table-1,2,3 respectively. From the data it is clear that Plants growing in polluted water showed more incidences of cytological meiotic aberrations frequency of abnormal tetrads as well as pollen fertility than the plants growing on non-polluted area. Similarly *In vivo Allium* assay conducted on the aqueous extract of polluted showed moderate level of genotoxicity. Clastogenic alterationss have been assigned to induce break in DNA treated with various reagents. Presence of bridges at anaphase and telophase is due to breakage and reunion of chromatids[14]. Non clastogenic abnormalities induction by treatment with polluted water includes stickiness, vagrant chromosomes, multipolarity etc. These abnormalities are also called physiological aberrations[15]. During Aberrations in chromosomal proteins changes the surface nucleo-protein that cause stickiness and or abnormal spindle action[16].

Conclusion

Genotoxic as well as mutagenic potential of the polluted water of NFL suggest that a serious threat to flora and fauna of the locality. However, further study is needed for exploration of the biological results of DNA damage in aquatic organisms due to harmful effect of polluted water of NFL and to formulate future strategies for safeguarding aquatic organisms as well as nearby environment.

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Table-1: Mitotic analysis of RTCs of *Allium sativum*, treated with extracts of polluted water.

Treatment	Total RTCs Observed	Mitotic index ± SD	Bdg %	Fg %	Bd+ Fg %	Vag %	Lag%	Stic %	MNC %	Total %
Control	943	11. 77	0.23	-	-	-	-	-	-	0.21
1 % SE	1024	10..86	0.8	0.7	0.38	-	0.18	-	0.11	2.41
5 % SE	903	9 .87	1.11	-	0.89	0.12	0.23	0.67	-	2.98
10 % SE	894	8.38	1.12	1.11	-	0.34	0.88	0.24	0.54	4.10
20 % SE	796	7.80	0.76	0.89	1.04	-	0.13	0.27	0.63	3.64
50 % SE	1214	7.18	1.42	0.91	0.76	0.42	0.26	0.33	0.09	4.12
100 % SE	789	5.22	1.29	1.16	0.65	0.90	0.40	0.77	0.27	5.34

Abrebbattions: Bdg- bridge, Fg- fragment, vag- vagarant, stic- stickiness, MNC- micronucleus.

Table-2: Meiotic Behaviour and Data on Microsporogenesis of Plants from polluted site.

Name of Species	Locality	Chr No.	Chromosomal aberrations (%)			Total (%)	Cytomixis	Tetrads with MNC	Polyad
			Late disjunction	Bridge at Anaphase	Laggards				
Boerhaavia diffusa	P NP	n=26 n=26	- -	- -	6.16 0.20	6.16 2.10	- -	- -	4.45 -
Cannabis sativa	P NP	n=10 n=10	1.13 -	11.52 1.03	7.14 2.10	11.6 1.04	- -	- -	8.34 -
Cenchrus ciliaris	P NP	n=16 n=16	1.02 -	6.9 -	14.64 -	22.5 -	- -	1.61 -	2.95 -
Croton bonplandianum	P NP	n=10 n=10	- -	- -	5.47 -	5.47 -	- -	- -	6.43 1.07
Nasturtium aquaticum	P NP	n=16 n=16	- -	16.7 2.34	1.10 -	17.6 2.34	- -	- -	- -
Parthenium hysterophorus	P NP	n=17 n=17	- -	- -	16.68 3.10	16.68 3.10	6.27 1.24	5.78 0.35	2.83 0.04

Abrebbations: P- Polluted, NP- Nonpolluted.

Table-3: Data on pollen fertility of plant species from polluted and non polluted area.

Name of Spp.	Pollen fertility (%)	
	Polluted	Nonpolluted
Boerhaavia diffusa	94.16	98.02
Cannabis sativa	86.12	94.03
Cenchrus ciliaris	88.57	92.21
Croton bonplandianum	93.42	98.27
Nasturtium aquaticum	95.88	98.47
Parthenium hysterophorus	93.30	97.43

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