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EXCESSIVE ARSENIC CONTENT IN THE SOIL MAY BE INJURIOUS TO HEALTH : A GENOTOXIC STUDY FROM JAJJAL, PUNJAB

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ABSTRACT

Occurrence of high frequency of malignancy among the residents of this cotton growing region of Punjab and the presence of arsenic in the soil have necessitated the assessment of its genotoxic potential. This study has been carried out to test the genotoxicity of the soils from three sites, i.e. tube-well irrigated field, canal irrigated field and non-irrigated area, of Jajjal village of Talwandi Sabo Block of Punjab (India) using *Allium* assay. Bulblets (cloves) of *Allium sativum* were treated with 1, 5, 10, 20, 50 and 100% W/V of the soil extracts for 48h and tap water was used for control. In the chromosome aberration assay, root tip cells of *Allium sativum* were scored for the presence of various mitotic abnormalities. Bridges and fragments were scored as indicators of clastogenicity and laggards or vagrant chromosomes were indicators of spindle poisoning. Tubewell irrigated soils were observed to be more genotoxic than the other soil samples.

Key words: *Allium* assay, genotoxicity, chromosome aberrations, micronuclei, Arsenic in the soil.

Introduction

The Malwa region of Punjab, India has seen tremendous increase in the incidence of dreaded disease cancer during last decade virtually making it a cancer region of Punjab. A recent study conducted in the cotton belt of Punjab indicted a cocktail of risk factors which were more common use of tobacco and alcohol, consumption of non-vegetarian and spicy food, high levels of heavy metals in water and above all excessive pesticides use as the possible reasons for higher cancer frequency in the area (Thakur *et al.,* 2008). Punjab consumes about 15% of the pesticides used in India and out of this more than 90% are used in the cultivation of cotton, rice and vegetables (Singh, 2002). High agricultural output demands more applications of these chemicals, which in turn are being accumulated in the soils of such areas. Hundal *et al.* (2007, 2009) have reported the presence of excessive arsenic in the Bathinda district. The human population is exposed to such pollutants either directly through dust inhalation, consumption of contaminated water and physical contact with the soil or indirectly through consumption of plants raised over these soils.

The soil is a complex mixture and chemical analyses are of limited utility to characterize its genotoxic potential. However, bioassays can be conveniently used for assessing the genotoxicity of such mixtures without prior knowledge about its chemistry. Many attempts have been made to assess genotoxicity of soil by using a variety of assays (Smith 1982, Brown *et al.* 1985, Knize *et al.* 1987, McDaniels *et al.* 1993, Watanabe *et al.* 2000, Saggoo *et al.,* 2007).

Present study is aimed at assessing the genotoxic potential of the soil from one of the village (Jajjal) of the infamous cancer belt of Talwandi Sabo block, Distt. Bathinda of Malwa region of Punjab. Samples from three types of soil (non-irrigated area, canal irrigated fields and tubewell irrigated fields) have been studied with an aim to investigate their genotoxic potentials.

Material and Methods

Sampling

Village Jajjal is located in Block Talwandi Sabo, Bathinda, Punjab. Three sites in the village were selected for sampling, i.e. tubewell irrigated field, canal water irrigated field and barren area soil without irrigation. The soil samples were homogenized with a pestle and mortar. These were then subjected to various tests for physical and elemental characterization and genotoxicity testing.

Element-Analysis

The soil samples were digested using triacid mixture (HNO3: H2SO4: HClO4:: 10: 1: 4) and subjected to elemental analysis. The amounts of copper, iron, manganese and zinc were estimated following Lindsay and Norevell (1978).

The arsenic content of the soil was analysed at 193.7 nm using Hydride generation system having a lamp current of 8.0 mA.

Allium Assay

Extract preparation

Water extracts of the samples was prepared by adding the sample (Weight/Volume) in distilled water to obtain 1, 5, 10, 20, 50 and 100 % solutions. The solutions thus obtained were thoroughly mixed and kept for 24 h at room temperature. The undissolved solids were later removed by centrifugation and the supernatant was used for experimental purposes.

Test organism and growth conditions

Medium sized healthy bulblets of common garlic (*Allium sativum*), procured from local market, were used as the test organism. Outer scales were removed carefully to expose root primordia. Test tubes filled with distilled water were taken and bulblets were placed over the test tubes in such a way that the lower portion of the bulblets were dipping in the water. The whole setup was placed at 28±2°C till the roots reach the size of nearly 2 cm in length. Water in the test tubes was changed every 24 hours.

Treatment of roots

The germinating bulblets, having 2-3 cm long roots, were placed over the test tubes containing different concentrations of treatment solution, i.e. water extracts of soil. Tap water was used for control treatment. Treatments were given for 48h, thereafter the bulblets were removed from the treatment setups, rinsed in water and the root tips were excised and fixed in Carnoy's fixative (Absolute alcohol: Chloroform: Glacial Acetic acid :: 6:3:1).

Staining and scoring of slides

Mitotic preparations were made by hydrolyzing the root tips in a mixture of 1N HCl and 2% acetocarmine (1:9), at 60ºC for 2 h. After maceration the root tip squashes were prepared in 2% acetocarmine and observed under microscope. Each root tip preparation

was scanned, taking 8-10 observations of cells at random under the microscope and counting nearly 1000 cells. The observations included, analysis of the mitotic index, total number of dividing cells at various stages and scoring of cytological abnormalities like fragments, bridges, micronuclei, multipolarity and vagrants indicating various clastogenic and physiological disturbances.

Observations and Discussion

The *Allium* anaphase – telophase chromosome aberration assay was deployed as a method for rapid screening of chemicals and environmental samples. The most important advantage of the *Allium* test is that it is a 'low budget' method, which besides being fast and easy to handle, also gives reliable results. Presently the assay has been used to investigate the genotoxic effect of the various soil samples from village Jajjal. The aqueous extracts of soil samples were used for treatment of *Allium* roots. Tap water was used as control.

The data on mitotic index and various cytological aberrations induced as a result of different treatments are presented in Table 1. The type of aberrations detected were micronuclei at prophases, vagrant chromosomes, laggards, bridges, etc. at anaphases and telophases and multipolar

TABLE 1— MITOTIC ABERRATIONS IN THE ROOT MERISTEM CELLS OF *ALLIUM SATIVUM* EXPOSED TO SOIL FROM JAJJAL VILLAGE.

Soil Extract	Mitotic Index± SD	Total Cells Observed	Percentage Aberrations						
			Br	Fr	Br+ Fr	Vag.	Multi.	MNCs	Total Aberrations
Control	15.8±0.42	1026	0	0	0	0	0.20	0	0.20
Tubewell irrigated soil									
1%	11.7±0.36	972	1.65	1.03	0.35	0.78	1.72	0.81	6.34
5%	9.35±0.51	1142	1.23	0.25	0.19	0.97	1.49	2.36	6.49
10%	8.51±0.61	1231	0.22	0.45	0.08	0.81	1.65	4.28	7.49
20%	7.19±0.92*	862	0.67	0	0	1.26	0.48	8.21	10.62
50%	6.25±0.83**	1210	0.19	0	0.83	0.29	1.09	9.98	12.38
100%	5.82±0.69**	991	1.89	0.66	2.52	0.97	0.31	8.32	14.67
Canal irrigated soil									
1%	9.7±0.53	991	1.05	1.01	0.38	0.62	1.56	0.69	5.31
5%	8.55±0.61	1023	1.12	0.22	0.15	0.86	1.31	2.08	5.74
10%	7.42±0.41*	1201	1.41	0.56	0.12	0.91	1.45	3.01	7.46
20%	7.01±0.32**	985	0.59	0.03	0	1.07	0.39	6.07	8.15
50%	6.09±0.71**	1233	0.15	0	0.76	0.31	1.14	7.82	10.18
100%	5.31±0.53**	1107	1.73	0.84	1.34	0.85	0.52	8.03	13.31
Non irrigated soil									
1%	9.31±0.41	1171	0.99	1.23	0.22	0.58	1.39	0.46	4.87
5%	8.45±0.79	975	1.02	0.26	0.12	0.78	1.22	1.68	5.08
10%	7.26±0.69*	1102	1.33	0.48	0.09	0.86	1.24	2.82	6.82
20%	6.92±0.57**	1013	0.42	0.01	0.01	1.03	0.24	5.98	7.69
50%	5.87±0.94**	1252	0.11	0	0.52	0.28	1.12	6.65	8.68
100%	5.63±0.93**	1139	1.12	0.33	1.01	0.77	0.48	7.99	11.7

Abbreviations: Br- Bridges, Fr- Fragments, Vag- Vagrants, Multi.- Multipolarity, MNCs- Micronuclei.

* Significant at 0.05 level, ** Significant at 0.01 level.

anaphases. The incidence of aberrations increased with the increase in the concentration of the soil extracts in dose dependant manner. Of the three soil samples from village Jajjal, the sample collected from Tubewell irrigated fields caused highest frequency of total aberrations followed by canal irrigated soil. The barren land showed minimum genotoxicity in *Allium* assay.

Plant based assay systems are recognized as sensitive biomonitors of the cytotoxic and genotoxic effects of the environmental chemicals and can be used both *in situ* and in the laboratory (Grant, 1999). *Allium* test has been used earlier for determination of cytotoxic and genotoxic effects of various environmental hazards, wastes, etc. (Samka-aakinel *et al*., 1996). In the present study, the treatment of *A. sativum* roots with soil extracts caused alterations in the mitotic index, induced micronuclei and other cytological aberrations. The extracts of all the three samples of soil showed mitodepressive nature. Many cytotoxic agents like heavy metals, pesticides, etc. may block the cell division resulting in the decline in the mitotic index. The reduction in mitotic index may also be attributed to the affect of toxins on DNA/ protein synthesis (Chauhan *et al*., 1998).

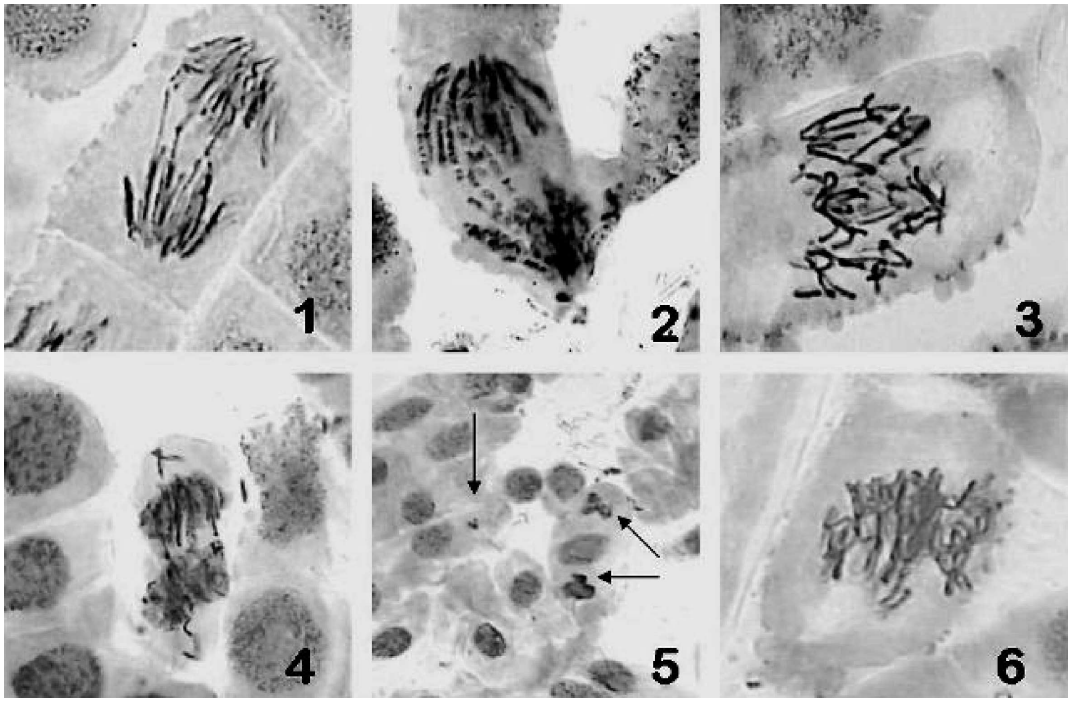
The increased incidence of micronuclei observed in the root tip cells exposed to soil extracts may be result of increased chromosome breaks and fragments that lag and are not included in the nuclei. Micronuclei are considered an indication of a true mutagenic effect (Auerbach, 1962; Ma, 1983; Grover and Kaur, 1999). The increase in percentage of aberrant cells in the treated garlic roots and formation of micronuclei confirm the genotoxic potential of the soils.

Toxic heavy metals and pesticides are the common environmental contaminants resulting from agricultural activities. Interaction among metals or with other elements, even at low levels present in the soil can have synergistic action and exhibit genotoxicity (Chandra *et al*., 2005). The presence of heavy metals and other pollutants always pose a risk of their passage into food chain (Suhreeyapongse *et al*., 2002) and further manifest their genotoxicity (Saggoo and Kour, 2003; Saggoo and Grewal, 2003, 2006; Gill and Saggoo, 2009).

The elemental analysis of the soil (Table 2) revealed the presence of arsenic, a toxic metalloid, much above the permissible limit of 10 ppb (WHO, 1981). The content of arsenic was found to be higher in the tubewell irrigated soils (3.2 ppm), followed by canal irrigated (2.2 ppm) and then the soils without irrigation (0.84 ppm). Indisputably the arsenic content in the water must be containing higher grades of dissolved arsenic pollutant. This is as expected due to increased genotoxicity evidenced by increased occurrence of aberrations in the mitotic RTCs thus indicating a relationship between the two (r= 0.9992). Epidemiological study by Thakur *et al* (2008) has indicted presence of As, Cr, Cd, Hg in drinking water along with pesticide residues in the environment, alcoholism and smoking and spicy food habits as possible reasons for the high rate of cancer cases in the region. Studies by Hundal *et al.* (2007, 2009) have indicated the presence of excessive arsenic in the Talwandi Sabo region.

TABLE 2 — ELEMENTAL ANALYSIS OF SOILS OF JAJJAL VILLAGE.

Element	**Content in different types of soils (ppm)**		
	Tubewell irrigated	**Canal irrigated**	**Non irrigated**
Copper	0.40	0.34	0.22
Iron	1.68	2.76	2.58
Manganese	1.92	2.28	3.12
Zinc	0.16	2.22	0.30
Arsenic	3.20	2.20	0.84



Explanation to the figures:
Figures 1-6. Cytological aberrations induced in RTCs of *Allium sativum* following exposure to various soil extracts.
Fig. 1. Three chromatid bridges at anaphase.
Fig. 2. Anaphase showing laggards and chromosomal breaks.
Fig. 3. Multipolarity at anaphase.
Fig. 4. Sickiness, multiple bridges and vagrant chromosome at anaphase.
Fig. 5. Prophases showing micronuclei.
Fig. 6. Stickiness of chromosomes at metaphase.

The present findings of genotoxicity in soil serve as a warning of an underlying environmental hazard. There is urgent need for large scale monitoring of all environmental agents to chalk out an action plan for its remediation.

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