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Arsenic Cytotoxicity: A bioassay using Barley

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ABSTRACT

Barley, *Hordeum vulgare* (2*n*=14) has been used by many cytologists, to determine cytotoxicity of environmental pollutants like heavymetals, industrial effluents, agricultural chemicals *etc.* In the present study, Arsenic(in the form of arsenic trioxide of concentrations100,250, 500,750and1000ppb), cytotoxicity was analyzed on Barley root tip mitosis, to understand its toxic effects on cells and chromosomes of a plant-basedsystem. The results show that Arsenic can induce various Mito depressive and clastogenic chromosomal aberrations like chromosomal breakages, disturbed spindle organization and other cellular metabolic defects. Generally the cytological anomalies were found to be dose-dependent.

Figures : 09 References : 42 Table : 01
KEY WORDS : Aberrations, Arsenictrioxide, Cytotoxicity,*Hordeum vulgare,*Mito depression

Introduction

Arsenic (As) is chemically a metalloid, having properties intermediate between metals and non-metals. It has been reported by various workers that it bears excessive toxicity for all living organisms[34]. World Health Organization[40] and Environment Protection Agency[9] of the US, have determined 10 ppb, as the threshold limit of Arsenic in drinking water. But if we go through the reports of various workers, it becomes evident that many countries, especially those located in the South and South East Asia, have much higher levels of Arsenic in their drinking water[3]. The main reason for such elevated levels of Arsenic contamination seems to be the rampant use of underground water. With receding water levels, boring for groundwater is becoming deeper and deeper. This leads to the use of water which has heavy metals accumulated in several thousand years. Moreover, the geochemistry of deep rocks is such that it contributes significant amounts of heavy metal contaminants in water.

In 1984, workers[11] discovered groundwater arsenic contamination for the first time in Gangetic plains of West Bengal. It was later also reported by others[6]. UP Jal Nigam and UNICEF published a combined report in 2011 and identified 18 districts where Arsenic levels were above the 50 ppb (BIS standard) and 31 districts where it was above 10 ppb[7]. The India Science Wire reported in 2019, that as many as 2.34 crore people in 40 districts of rural areas Uttar Pradesh are exposed to high levels of arsenic by groundwater. Ballia, Barabanki, Gorakhpur, Ghazipur, Gonda, Faizabad and Lakhimpur Kheri are the worst affected districts[15]. Majority of them are in the flood plains of Ganga, Rapti and Ghaghara rivers. Ten other districts with moderate risk of arsenic contamination are Shahjahanpur, Unnao, Chandauli, Varanasi, Pratapgarh, Kushinagar, Mau, Balrampur, Deoria and Siddharthnagar. Some workers, reported similar situation in districts like Bahraich, Ambedkar Nagar, Bareli, Basti, Bijnaur, Meerut, Sant Ravidas Nagar, Shahjahanpur, Sitapur and Kanpur *etc*[41].

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TABLE-1: Active mitotic index and mitotic abnormalities in treated and control sets of barley roots

Treatment (ppm)	TAd	AMI (%)	MIn (%)	Metaphase abnormalities (%)						Anaphase abnormalities (%)				Tab (%)
				Un	Sc	Pm	Fr	St	Cl	Lg	Dp	Br	Cl	
CONTROL	442	8.84		0.28				0.48			0.16			0.92
As_2O_3														
100 ppb	284	5.68	35.75	1.76	2.82			0.95	0.48	0.32	0.16		0.63	7.12
250 ppb	209	4.18	52.71	2.87	3.83	0.48		1.69	0.72	0.48	0.24		0.72	11.03
500 ppb	198	3.96	55.20	4.54	4.04		0.51	2.53	1.01	1.26	0.25	0.51	1.52	16.17
750 ppb	56	1.12	87.33	3.57	8.93	0.52	1.31	3.65	1.57	2.09	0.26	1.04	1.57	24.51
1000 ppb	38	0.76	91.40	4.05	7.50	1.02	0.82	4.10	1.06	2.05	0.50	0.95	1.62	23.67

TAd=Total number of Actively dividing cells; AMI=Active mitotic Index; MIn=Mitoinhibition; Un=Unorientation of chromosomes; Sc=Scattering of chromosomes; Fr=Fragmentation of chromosomes; Pm=Precocious movement of chromosomes from the Metaphase plate; St=Stickiness of chromosomes; Cl=Clumping of chromosomes; Lg=Lagging chromosomes; Dp=Disturbed polarity of chromosomes; Br=Chromatin bridge between the poles; Tab (%)=Total percentage of abnormal cells.

Arsenic has been proved to be a carcinogen by the International Agency of Research on Cancer[16]. Its toxic effects on human health have been thoroughly studied by various workers[24,28]. It has been shown to induce various disorders related to cardiovascular, gastrointestinal, respiratory, endocrine, renal and other systems[31]. Arsenic is usually found in nature, in two inorganic forms *viz*. Arsenite and Arsenate. Some organic forms are known *viz.* Monomethyl Arsenic Acid and Dimethyl Arsenic Acid[42].

As far as plants are concerned, various studies have shown that it affects plants as well. The effects range from defoliation, root growth retardation, stunting and even death. All these have long been attributed to Reactive Oxygen Species (ROS) which are built up by presence of Arsenic. This causes oxidative damage to membranes[10]. Toxicity may manifest in disturbances in nutrient uptake, photosynthesis, carbohydrate, protein and lipid metabolism[7].

Keeping in view, the harmful effects of As and its increasing levels in groundwater, the present work envisages to describe the cytotoxic effects induced by arsenic trioxide on the root tip meristematic cells of *Hordeum vulgare*L. The objective of the work is to determine the nature of cytotoxicity induced by the pollutants in a plant-based system to get an insight into the mechanism of harmful effects on chromosomes.

Materials and Methods

Based on its availability, solubility and effectiveness, Arsenic Trioxide (As_2O_3) was used as the salt providing Arsenic to the plant roots of Barley. The root tip mitosis is a very sensitive stage and any change in the ambient conditions has marked effect on the chromosomal morphology and division cycle. Barley has 2n=14 chromosome number and large sized chromosomes which makes it an ideal material for a bioassay on toxicity of Arsenic.

1. **Selection of dose of treatment:** The concentrations of the solutions to be prepared were chosen on the basis of earlier reports of levels of As contamination in different districts of the state which range from 100 to over 1000 ppb.
2. **Preparation of Solutions:**0.01 mg of arsenic trioxide (As_2O_3, Merck-AR grade) was dissolved in 100 ml of deionized water, to make the concentration of 0.1 mg/l or 100 ppb and subsequently, different concentrations like 250, 500, 750 and 1000 ppb were prepared following dilution with deionizedwater.
3. **Treatments:**Healthy and same sized seeds of Barley (procured from CSAU, Kanpur)were washed in distilled water and soaked for 5 hours in distilled water to initiate the germination process. The seeds were then taken out and dipped in different Arsenic solutions for 8 hours. A suitable control was also maintained in distilled water. The seeds were then taken out and germinated in petridishes having wet autoclaved cotton wool. The roots emerged within next 12 h. When the root length were 0.5 to 1.0 cm, they were cut at the time of mitosis (around 11 am) and preserved in Carnoy's solution (3:1 Ethyl alcohol: Glacial Acetic Acid) for 12 h and then stored in 70% alcohol at $4^{O}C$.
4. **Determination of cytotoxicity:** As_2O_3 treated roots were cytologically assessed. The treated root tips from each set were boiled in N HCl and squashed in 1% Acetocarmine. Each set was repeated in triplicate. The slides were observed and micro-photographed under Olympus Scientific Microscope. On an around 5000 cells were observed per set. Active Mitotic index (number of dividing cells/ total cells estimated×100) and chromosomal aberrations recorded with their frequencies. Untreated control roots were also studied in similar manner. The results were statistically analyzed.

Results and Discussion

The results of the cytological analysis have been presented in Table 1. The Mitotic Index (MI) in control set was around 34%. However, a clear-cut dose-based decrease in MI. At the highest dose set, the MI went down upto 7.34%. A few workers have attributed Mito depression caused by As_2O_3 to suppression of DNA Synthesis in S-phase[26,35]. Heavy metals may also disturb microtubule organization to delay division[5]. Mito depression as a consequence of heavy metal treatment, has been reported by many workers[2,4,8,21,30].

A few cells of Control sets showed clumping, however the percentage of Total Abnormal Cells (TAb%) remained extremely low. Common abnormalities in chromosomal structure, in all treated sets, included Stickiness and Clumping, Fragmentation, Precocious movement, Disturbed polarity, Lagging chromosomes, Cytomixis, Micronuclei, Multinucleate cells, Polyploids *etc.*

Chromosome agglutination characterized by chromosome stickiness and clumping was significantly higher than most other anomalies. The biochemical mechanism for chromosome agglutination is not precisely understood. In case of metals, it seems probable that the positively charged metals might interact with non-histone and the DNA itself, resulting in structural changes and stickiness. McGill and Klasterska have attributed stickiness and clumping to improper folding of chromatin fibers leading to creation of sub-chromatid bridges between the chromosomes[19,25]. Workers

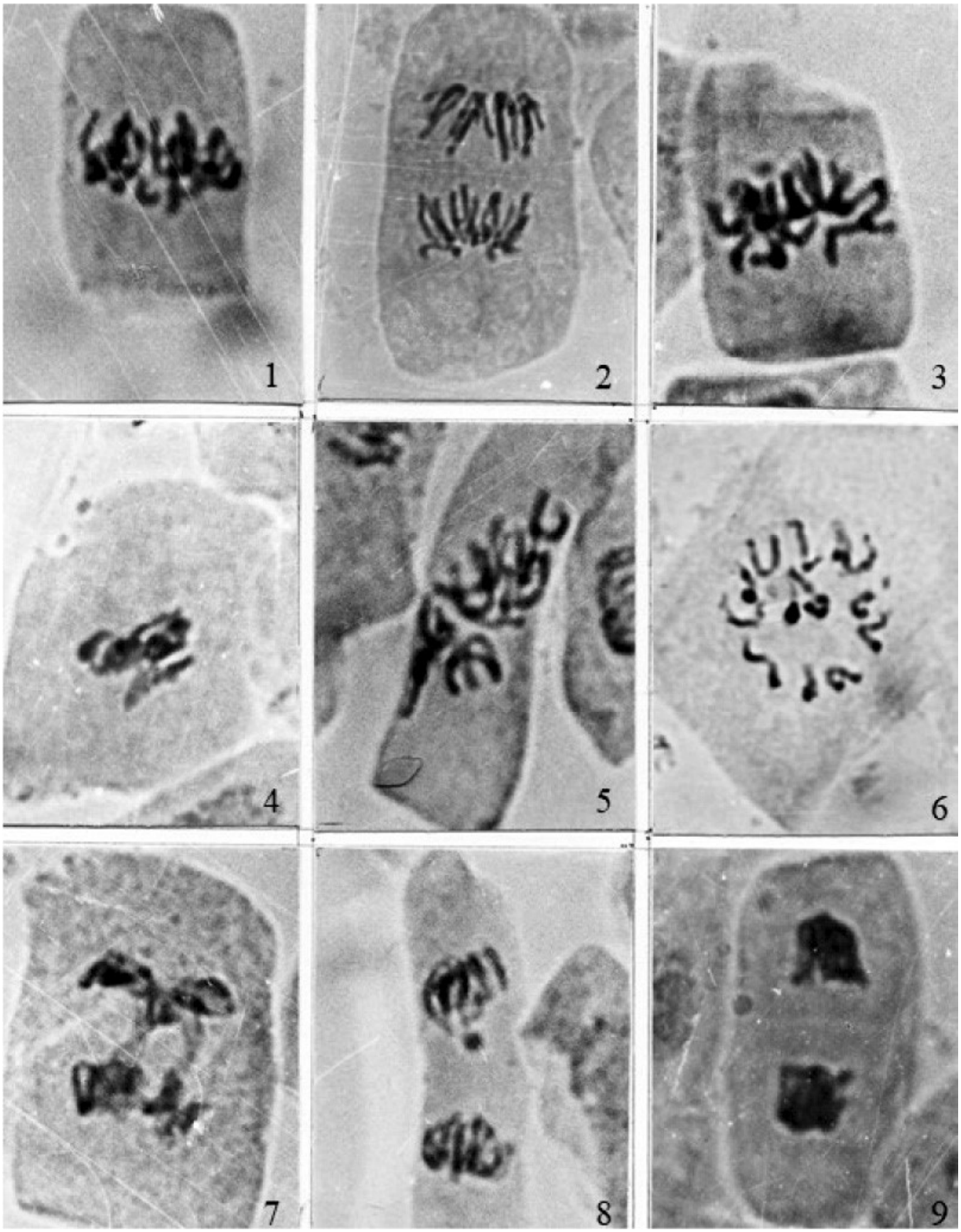

Fig.1-9 : Normal mitosis and chromosomal abnormalities by different treatments in Barley root meristem. 1: Normal metaphase; 2: Normal Anaphase; 3: Stickiness; 4: Clumping; 5: Unorientation; 6: Disturbed polarity; 7: Chromatin bridge; 8: Laggards; 9: Clumping at anaphase (Scale bar: 1cm=4μ)

attributed stickiness to changes in cytochemically-balanced reactions, which in turn lead to changes in cytoplasmic viscosity[18].

Inhibition of spindle organization by various chemicals, can lead to conditions of scattering of chromosomes, also known as C-metaphase. It can be said that the signal given after prophase for organization of spindle had been blocked[36]. Metals have high affinity for the –SH group of the spindle monomers. The attachment of metal ions with these groups, leads to inability of the monomers to polymerize and form fibres[39]. Some protein kinases, which play an important function in organization of the spindle, might also get inactivated by unavailability of ATP for phosphorylation or direct interaction with the toxins.

Spindle dysfunction also produced anomalies like lagging chromosomes at anaphase. Workers used the term 'chromosomes with inactivated centromeres' for lagging chromosomes, as they linked this anomaly to absence of centromeres, localized stickiness at the centromeric portions of the chromosomes or to inability of centromere to condense microtubules[12]. Similarly, precocious movement, another spindle anomaly can be attributed to spindle dysfunction[23].

Chromosome fragmentation was observed in almost all sets at higher doses. This may be assigned to the failure of broken chromosomes to recombine or due to mis-repair of DNA. Some workers suggested that the upset of nucleic acid metabolism ultimately results in disturbed protein re-duplication causing chromosomes to break at several loci[33]. However no definite mode of action for causing fragmentation can be given to pesticide or heavy metal.

Chromatin bridges may arise due to stickiness in localized portions of chromosomes leading to retardation in chromatin disjunction[1]. Bridges might also be viewed as indicators of exchange between the chromosomes involving breakage and proximal reunion[37].

Among other abnormalities were chromosome erosion, multipolarity, polyploid cells *etc.* Pool established that the chemicals may lead to less localized inhibition of DNA synthesis[29]. This may in turn cause less or unstained regions on the chromosomes and giving an eroded appearance. It was reported that pole formation in dividing cells depends upon the number of points of RNA and polysaccharide assemblage[20]. Interaction of some chemicals leads to disturbed assemblages and multipolar condition. Formation of polyploid cells may result due to failure of scattered chromosomes to separate into two nuclei[17]. Micronuclei at Telophase represent the remnants of laggards and fragments of earlier phases, which fail to reach the poles.

Some slides showed a few giant cells which may possibly be due to the abnormal mitotic activity under physiological stress induced by the treatment or may be the artifacts of errors in squash preparation. However, the 'giant cells are the outcome of chaotic mitotic activity due to physiological stresses'[14]. Furthermore, such cells are not observed in any squash preparation of untreated control.

It is evident that Arsenic can induce both clastogenic as well as spindle aberrations which might be the cause of cells with polyploid chromosome number. Cytotoxic effects of heavy metals (copper, cadmium, lead,mercury and arsenic) are also reported in various plant species[4,13,21,22,30,38].

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