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RESEARCH ARTICLE

EFFECT OF ARSENIC AND MANGANESE ON CELL DIVISION OF GREEN GRAM

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ABSTRACT

In this study, the effects of arsenic and manganese on the cell division in root tips of green gram were conducted. For this purpose, *Vigna radiata* were germinated into various concentrations of As and Mn (5, 10, 25, 50 100 mg/l) at 23-24°C for 72 h. Chromosome aberration assay was used to determine the mitotic indices and rate of chromosome aberration in green gram root tip cells due to As and Mn treatment. The results showed that the mitotic indices were complicated due to different concentrations of As and Mn. However, the increase in As concentration has led to a gradual increase in the percentage of chromosomal aberration and than manganese on the root tip cells during mitotic index. They exhibited many chromosomal abnormalities such as formation of chromosome bridges, laggards, stickiness, precocious movement, formation of binucleate structure and other abnormal behaviors. There was no considerable change in 2n number of chromosome with the increase in As concentrations. It is concluded that the As and Mn has significant mutagenic effect on the root tip cells of green gram.

INTRODUCTION

Heavy metal pollution of soils received considerable attention as a consequence of the increased environmental pollution from industrial and agricultural sources. Arsenic (As) is a widespread natural element, this is not a bioorganic element to plants and animals. The increased soil content of arsenic is a result of the technogenic contamination. Arsenic in groundwater and its transport in the environment have become a matter of great concern in India and several other countries. Arsenic (As) is not essential for plant growth. Because of chemical similarities to P, As is able to replace P in many cell reactions and it shows many harmful toxicities to plants including wilting of new-cycle leaves and retardation of root and top growth (Aller et al., 1990). Metal contamination issues are becoming increasingly common in elsewhere. Metals are natural part of terrestrial systems occurring in soil, rocks, air, and water in organisms. A few metals including Cu, Mn and Zn are however essential to plant metabolism in trace amounts. It is only when metals are present in bio available forms at excessive levels that they have the potentiality to become toxic to plants. Metal toxicity issues in plants and soils are a significant problem through out the world. Most metal toxicity occurs as a result of anthropogenic disturbance, such as mining, where unnaturally high amounts of metals are released during various processes.

These industrial processes discharge large quantities of heavy metals in liquid, solid and gaseous wastes into the environment and can ultimately have significant adverse biological and ecological effects (Zou *et al*, 2006). Water pollution is defined as the addition of any thing to water which alters the natural quality. Water is mostly polluted by the industrial wastewaters released from various industries (Kudesia, 2000). Heavy metals are the main constituents of many industrial effluents. The industrial, agricultural and municipal wastes are the key sources of these toxic heavy metals in the wastewater (Kirupalakshmi *et al.,* 2004). Their common feature in relation to biological life is that in excess quantities they are poisonous and cause death of most living organisms. They can neither be created nor destroyed or any one heavy metal can be transformed into another. It therefore, means that once a metal is mobilized in the environment, its total amount remains the same (Sankar Ganesh, 2008). Heavy metals Arsenic and Manganese are considered as the most toxic to plants when it is present in irrigated water at higher concentration. There are several reports on the toxic effects of heavy metals on plants (Chaugh and Sawney, 1996, Panta and Patra, 1997, Srivastava *et al,* 1997, Lalitha *et al*, 1999 and Sankarganesh, 2008). The present investigation has been find out the effect of different concentrations of heavy metals on cytological studies of Green gram (*Vigna radiata* (L.) Wilczek) var. Vamban 1.

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MATERIALS AND METHODS

The experimental plant *Vigna radiata* belongs to the family Fabaceae. It is one of the important pulse crops in India. The seeds of *Vigna radiata* were obtained from the National Pulses Research Centre, Vamban, Pudukkottai district of Tamil Nadu. Seeds of uniform in size, colour and weight were choosing for experimental purpose.

Preparation of As and Mn solution

Sodium arsenate (Na_2HASO_4-$7H_2O$) and Manganous sulphate ($MnSO_4H_2O$) salt is used for the present study. A known weight (2.66 g) of sodium arsenate and weight (2.92 g) of manganese sulphate was dissolved in 1000 ml of distilled water to obtain the standard solution. From the standard solution, the different concentrations (5, 10, 25, 50 and 100 mg/l) of arsenic and manganese were prepared and used for the germination studies.

Germination study

The seeds were surface sterilized with 0.1% mercuric chloride solution for 2 minutes and washed thoroughly with tap water and then by distilled water for 30 minutes. The seeds were placed in sterilized petriplates lined with filter paper. Each petriplate was moistening uniformly by various concentrations of different heavy metal solutions. The seeds were irrigated with distilled water was treated as control. All the petriplates were kept under diffused sunlight at room temperature (28±2°C). The number of seeds germinated in each treatment was counted on 7^{th} day.

Cytological and mitotic studies

Root tips from the green gram plants were collected, washed with distilled water and fixed in 1:3 acetic alcohols for 24 hours and then stored in 70% alcohol for subsequent use. The root tips were fixed between 8.45 AM to 9.25 AM. The green gram seeds were treated with various concentration of different heavy metal (arsenic and manganese) solutions (5, 10, 25, 50 and 100 mg/l) in petriplates. After, two days the root tips collected from green gram which were not given the heavy metal treatment were taken as control. The root tips after treatment were washed in distilled water and fixed in 1:3 acetic alcohol. They were kept for over night in the fixative and were stored in 70% alcohol for subsequent use. Then root tip squashes were made by using iron alum, haematoxylin squash technique of Marimuthu and Subramaniam (1960). This haematoxylin squash technique was found to be suitable for the cytological investigation.

RESULTS

The effect of different concentrations of selected heavy metals (arsenic and manganese) on induced somatic chromosomal abnormalities in green gram seedlings was observed. They exhibited many chromosomal abnormalities such as formation of chromosome bridges, laggards, stickiness, precocious movement, formation of binucleate structure and other abnormal behaviors. The control seedlings showed normal mitotic division and the 2n number was 22. Various concentrations of selected heavy metals initiated the seedlings to exhibit various chromosomal abnormalities.

Cytological studies in arsenic and manganese treated seedlings

The detailed study of chromosome morphology, size, type and number was observed in 5, 10, 25, 50 and 100 mg/l of arsenic and manganese concentrations. In these cases of arsenic, the chromosomal aberrations, which revealed the diploid complement of chromosome 2n = 2 and the manganese, metaphase chromosome revealed the diploid complement with 2n = 22 chromosomes. The total chromosome length of As was 75.2µm and Mn was 78.7µm, absolute chromosome length of As was 37.6 µm and Mn was 39.35□µm, average chromosome length of As was 3.41 µm and Mn was 3.6□µm were observed at 5 mg/l concentration. The total chromosome length of As was 68.1 µm and Mn was 73.1□µm, absolute chromosome length As was 34.05 µm and Mn was 36.55□µm, average chromosome length of As was 3.11 µm and Mn was 3.3□µm were recorded in 10 mg/l concentration. The total chromosome length of As was 65.4□µm and Mn was 71.4□µm, absolute chromosome length As was 32.7 µm and Mn was 35.7□µm, average chromosome length As was 2.97µm and Mn was 3.2 µm were recorded in 25mg/l Concentration. The total chromosome length As was 57.8□µm and Mn was 67.5□µm, absolute chromosome length As was 28.9□µm and Mn was 33.75□µm, average chromosome length As was 2.63□µm and Mn was 3 µm were recorded in 50 mg/l. The total chromosome length As was 51.7□µm and Mn was 65.5□µm, absolute chromosome length As was 25.85□µm and Mn was 28.25□µm, average chromosome length As was 2.35□µm and Mn was 2.57□µm were observed in 100 mg/l concentrations (Table 1 to 4 and Plate 1, 2).

The different concentrations (5-100 mg/l) of heavy metals on green gram seedlings showed different spectrum of abnormal cells and frequency of abnormalities. The abnormal cells in the control plant showed less in number (5) and frequency (1.92). This was followed by the successive concentrations (5, 10, 25, 50 and 100 mg/l) of various heavy metals. It was revealed that number of abnormal cells increased with increased in concentration of heavy metals. Among the heavy metals, arsenic showed more toxic effect than other heavy metals tested. So, the higher (100 mg/l) concentrations of arsenic provided more number of abnormal cells (25) and frequency (11.74) than that of the other concentrations and the control.

DISCUSSION

Heavy metals are one of the most important groups of pollutants of aquatic environment, which originate from domestic sewage, industrial effluents and agricultural run off etc. Addition of heavy metals such as As, Al, Cr, Mn, Mo, Ni, Cu, Pb, etc., into the environment causes toxic and carcinogenic effects on flora and fauna and create great ecological crisis at the global level. Heavy metal accumulation in soil and its importance on the morphological, biochemical and cytological aspects of plants have received more attention in recent times by many workers (Abbasi *et al.,* 1992; Premkumar *et al.* 2001; Prakash *et al.,* 2004). Rapid urbanization and industrialization have enhanced levels of toxic heavy metals in the environment posing a potential health hazard for all living organisms. Heavy metals have some

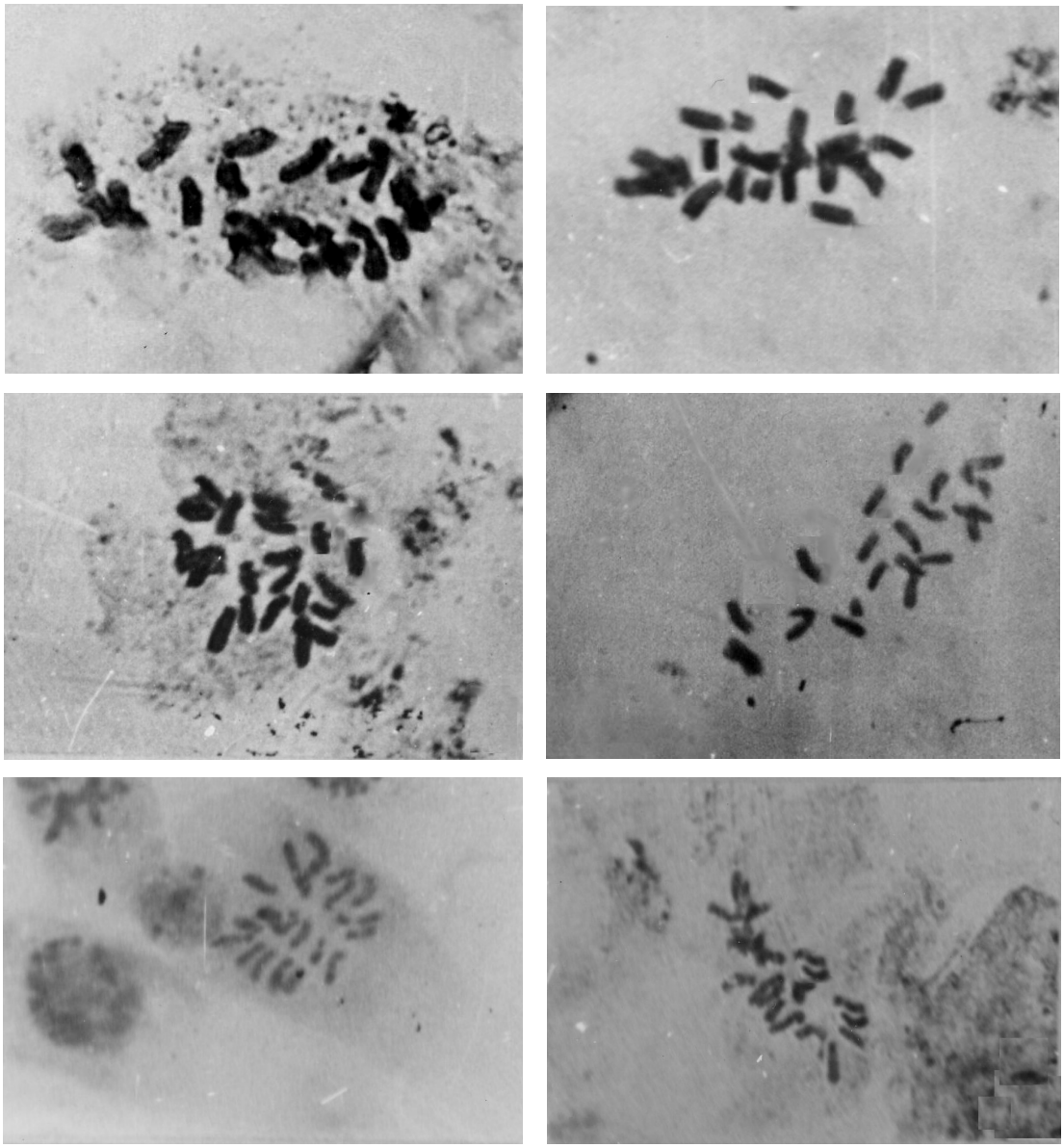

PLATE I
Mitosis (Arsenic)
Microphotographs x 1250

genotoxic potential. Growth inhibition at higher concentrations may be linked with lower mitotic activity in the root meristematic zone or to an inhibition of cell enlargement in the elongation zone as a consequence of decreased cellular turgor (Gabbrielli *et al.*, 1990). From the cytological studies, the researcher can understand that the chromosomal aberrations are more in arsenic treatment and less in manganese treatment seedlings when compared to the control. All kinds of chromosomal variations are found to be increased with increase in heavy metal concentrations.

Light microscopic studies showed that heavy metal treated plants had much smaller leaf cells and more reduced 'intercellular spaces in leaves than in the control. The reduction in cell sizes and the decrease in intercellular spaces seemed to be largely responsible for the decrease of the leaf area. This is supported by previous reports of several authors (Vasquez *et al.*, 1986; Di Domenico *et al.,* 1989). The formation of bridges could be attributed to chromosome stickiness and to chromosome breakage and reunion. The induction of lagging could be attributed to the failure of the normal organization and function of the spindle apparatus. Such type of abnormalities is due to the loss of microtubule of spindle fibers. The micronuclei observed at higher doses of both salts, may originate from a lagging chromosome or from a chromosome fragment. This was supported by previous reports of several authors (Badr and Ibrahim 1987; El-Khodary *et al.*, 1990; Haliem, 1990; Patil and Bhat, 1992; Salam *et al.*, 1993). Nagpal and Grover (1994) classified induced chromosomal abnormalities into

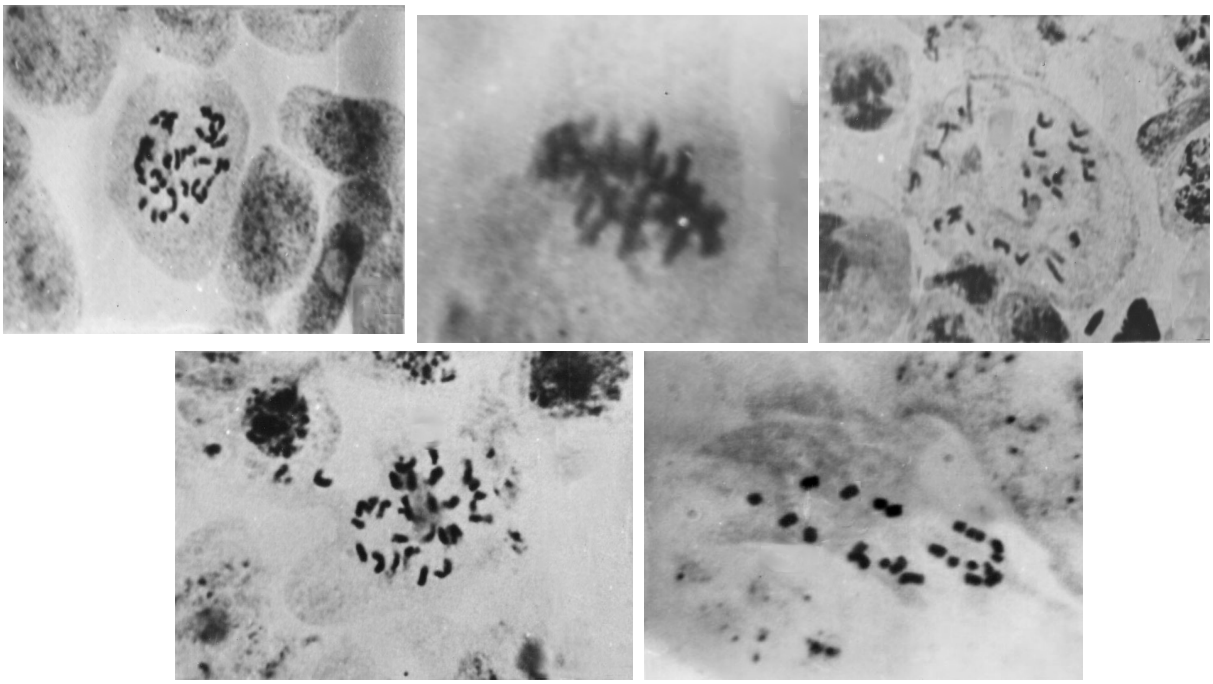

PLATE II
Mitosis (Manganese)
Microphotographs x 1250

Table 1. Effect of different concentrations of arsenic on cytological behaviour of greengram (*Vigna radiata* (L.) Wilczek)

Con. of Arsenic (mg/l)	Chromosome		Chromosome length in μm				L/S ratio	Position of centromere	Chromosome length
	Type	No.	Long arm (L)	Short arm (S)	Satellite (S)	Total length (L + S + S)			
	J	3	3.9	2.7	-	20.4	1.4	Submedian	
	V	7	1.9	1.9	-	28	1	Median	Total chromosome length = 80.5 μm
Control	S	2	4.4	3.3	0.2	16.2	1.3	Submedian	Absolute chromosome length = 40.25 μm
	I	6	1.8	0.2	-	13.2	9	Subterminal	Average chromosome length = 3.66 μm
	I	4	1.5	0.2	-	9.5	7.5	subterminal	
	J	3	3.9	3	-	14.6	1.36	Submedian	
	S	2	3.8	2.3	0.2	13.4	1.52	Submedian	Total chromosome length = 68.9 μm
5	V	8	1.5	1.5	-	25.6	1	Median	Absolute chromosome length = 34.45 μm
	I	6	1.2	0.2	-	10.2	6.5	Subterminal	Average chromosome length = 3.13 μm
	I	3	1.3	0.2	-	5.1	6.5	Subterminal	
	S	2	3.5	2.1	0.2	12	1.68	Submedian	
	J	3	3.7	2.4	-	18.9	1.44	Submedian	Total chromosome length = 68.1 μm
10	V	7	1.3	1.3	-	19.6	1	Median	Absolute chromosome length = 34.05 μm
	I	6	1.4	0.2	-	10.8	7	Subterminal	Average chromosome length = 3.11 μm
	I	4	1.3	0.2	-	6.8	6.5	Subterminal	
	S	2	3.1	2.0	0.2	11	1.55	Submedian	
	J	3	3.2	2.3	-	17.1	1.39	Submedian	Total chromosome length = 65.4 μm
25	V	8	1.3	1.3	-	22.4	1	Median	Absolute chromosome length = 32.7 μm
	I	5	1.3	0.2	-	8.5	6.5	Subterminal	Average chromosome length = 2.97 μm
	I	4	1.2	0.2	-	6.4	6	Subterminal	
	S	2	2.9	1.9	0.2	10.4	1.53	Submedian	
	J	2	3.0	2.1	-	10.6	1.43	Submedian	Total chromosome length = 57.8 μm
50	V	8	1.2	1.2	-	20.8	1	Median	Absolute chromosome length = 28.9 μm
	I	6	1.2	0.2	-	9.6	6	Subterminal	Average chromosome length = 2.63 μm
	I	4	1.2	0.2	-	6.4	6	Subterminal	
	S	2	2.2	1.7	0.2	8.6	1.3	Submedian	
	J	3	2.8	1.9	-	14.7	1.47	Submedian	Total chromosome length = 51.7 μm
100	V	8	0.9	0.9	-	16	1	Median	Absolute chromosome length = 25.85 μm
	I	5	0.8	0.2	-	6	4	Subterminal	Average chromosome length = 2.35 μm
	I	4	1.2	0.2	-	6.4	6	Subterminal	

Table 2. Effect of different concentrations of arsenic (mg/l) in greengram (*Vigna radiata* (L.) Wilczek) on total number of abnormal cells, frequency of abnormalities and percentage of mitotic abnormalities

Con. of Arsenic (mg/l)	Total No. of cells analysed	Total no. of abnormal cells	Number of abnormal cells				Frequency of total abnormalities	% of mitotic abnormalities			
			Bridge	Laggard	Stickiness	Binucleate cells		Bridge	Laggard	Stickiness	Binucleate cells
Control	260	5	2	1	1	1	1.92	0.77	0.38	0.38	0.38
5	256	12	5	3	2	2	4.69	2	1.17	0.78	0.78
10	243	17	7	5	3	2	7	7	2.05	1.23	0.82
25	231	19	9	5	3	3	8.2	8.2	2.16	1.3	1.29
50	224	23	7	9	4	3	10.27	10.27	4.02	1.8	1.3
100	213	25	9	7	5	4	11.74	11.7	3.29	2.35	1.9

Table 3. Effect of different concentrations of manganese on cytological behaviour of greengram (*Vigna radiata* (L.) Wilczek)

Con.of Manganese (mg/l)	Chromosome		Chromosome length in μm				L/S ratio	Position of centromere	Chromosome length
	Type	No.	Long arm (L)	Short arm (S)	Satellite (S)	Total length (L + S + S)			
Control	J	3	3.9	2.7	-	20.4	1.4	Submedian	Total chromosome length = 80.5 μm Absolute chromosome length = 40.25 μm Average chromosome length = 3.66 μm
	V	7	1.9	1.9	-	28	1	Median	
	S	2	4.4	3.3	0.2	16.2	1.3	Submedian	
	I	6	1.8	0.2	-	13.2	9	Subterminal	
	I	4	1.5	0.2	-	9.5	7.5	subterminal	
	S	2	4	3	0.2	14.8	1.33	Submedian	
5	J	3	3.7	2.5	-	19.2	1.48	Submedian	Total chromosome length = 78.7 μm Absolute chromosome length = 39.35 μm Average chromosome length = 3.6 μm
	V	7	1.7	0.2	-	25.2	1	Median	
	I	5	1.6	0.2	-	10	8	Subterminal	
	I	5	1.5	0.2	-	9.5	7.5	Subterminal	
	S	2	3.7	2.8	0.2	13.8	1.32	Submedian	
10	J	3	3.6	2.2	-	18	1.6	Submedian	Total chromosome length = 73.1 μm Absolute chromosome length = 36.55 μm Average chromosome length = 3.3 μm
	V	6	1.7	1.7	-	21.6	1	Median	
	I	5	1.5	0.2	-	9.5	7.5	Subterminal	
	I	6	1.3	0.2	-	10.2	6.5	Subterminal	
	S	2	3.3	2.5	0.2	12.4	1.17	Submedian	
25	J	3	3.5	2.7	-	19.2	1.37	Submedian	Total chromosome length = 71.4 μm Absolute chromosome length = 35.7 μm Average chromosome length = 3.2 μm
	V	6	1.6	1.6	-	20.4	1	Median	
	I	6	1.5	0.2	-	11.4	2.14	Subterminal	
	I	5	1.2	0.2	-	8	3	Subterminal	
	S	2	3.0	2.1	0.2	10.6	1.43	Submedian	
50	J	3	3.2	1.9	-	15.9	1.68	Submedian	Total chromosome length = 67.5 μm Absolute chromosome length = 33.75 μm Average chromosome length = 3 μm
	V	8	1.5	1.5	-	25.6	1	Median	
	I	5	1.4	0.2	-	9	7	Subterminal	
	I	4	1.3	0.1	-	6.4	13	Subterminal	
	S	2	2.5	1.9	0.2	9.6	1.32	Submedian	
100	J	3	3	2.1	-	15.9	1.43	Submedian	Total chromosome length = 56.5 μm Absolute chromosome length = 28.25 μm Average chromosome length = 2.57 μm
	V	7	1.1	1.1	-	16.8	1	Median	
	I	6	0.9	0.2	-	7.8	4.5	Subterminal	
	I	4	1.3	0.1	-	6.4	13	Subterminal	

animal and plant cells have been studied by Paton and Allison (1972); Ruposhev (1976); Somashekar and Arekal (1983) and Sharma *et al.* (1988). The most abundant of them were stickiness, breakage, lagging, bridges and disturbed phases at the level of M_2 seed storage protein. The metal treatment caused changes in protein banding patterns especially at the high molecular weight regions (George, 1999).The chromosomal aberration like stickiness, laggards, chromosome bridges, irregular metaphase, fragmentation and binucleate cells were increased with the increasing concentrations of metals. Similar results were reported by Kumar and Tripathi (2003). The present study reveals that the presence of heavy metal, in the irrigated water causes many variations in germination, growth, biochemical and cytological behaviour of greengram. It can be concluded that the heavy metal containing wastewater are toxic to crops. So, this polluted water should be properly treated to remove the heavy metals and treated water with suitable dilution may be used for irrigation purpose.

Table 4. Effect of different concentrations of manganese (mg/l) in greengram (*Vigna radiata* (L.) Wilczek) on total number of abnormal cells, frequency of abnormalities and percentage of mitotic abnormalities

Con. of Manganese (mg/l)	Total No. of cells analysed	Total no. of abnormal cells	Number of abnormal cells				Frequency of total abnormalities	% of mitotic abnormalities			
			Bridge	Laggard	Stickiness	Binucleate cells		Bridge	Laggard	Stickiness	Binucleate cells
Control	260	5	2	1	1	1	1.9	0.77	0.38	0.38	0.38
5	253	10	5	3	2	1	4	2	1.19	0.79	4
10	246	14	7	3	2	2	5.69	2.85	1.22	0.81	0.81
25	232	17	8	4	3	2	7.33	3.4	1.72	1.3	0.86
50	229	19	9	5	3	2	8.3	4.4	2.18	1.31	0.87
100	218	21	11	5	3	2	9.6	5.04	2.29	1.37	0.92

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