J. Phytol. Res. 8 (2): 107-114, 1995

GENOTOXIC EFFECTS OF LEAD NITRATE ON PEA PLANT

R.K. SENGUPTA and P. GHOSH

Cytogenetics and Tissue Culture Laboratory, Department of Botany, University of Kalyani, Kalyani 741235, Nadia, West Bengal, India.

Cytogenetical effects of lead nitrate were investigated through seed soak treatment of *Pisum sativum* L. Effects were steadily increased with the increase in dose and duration. The probable mechanism resposible for producing chromosomal anomalies and phenotypic alterations have been discussed in detail. Effects were diluted in successive generation but it is difficult to interprete that it is entirely safe from genotoxic point. The appearance of chromosome breakage, cytomiotic cells and anaphasic bridges that chemical can affect genetic recombination which may lead to loss of important factors or gain undesirable characters. It can be concluded that lead nitrate is a cytotoxic chemical mutagen reduces plant growth as well as yield.

Keywords : Germination; Lead nitrate; Meiotic anomalies; Phenotypic effect; *Pisum sativum* L.

Introduction

Metals and their compounds form a major component which pollute soil, water, air in major cities in India. Heavy metals have for long been known to be important constituent of aerosol causing air pollution. The mutagenic effects of heavy metals and their salts have been reported in certain animal and plant system[1-8]. Chromosome damage is an efficient reliable and economical criterian to measure genetic toxicity. The objective of the present paper is to examine the genotoxic effects of lead nitrate on pea plant.

Material and Methods

50 seeds of *Pisum sativum* L. in each set were treated with 10^{-2}M, 10^{-3}M, 10^{-4}M, 10^{-5}M concentration of $Pb(NO_3)_2$ solution for 2,4 and 5 hours respectively. They were grown individually in experimental field, Department of Botany, University of Kalyani and sown in the lines keeping a distance of 15 cm between the plants and 25 cm between the lines laid in the randomised block design for each set of treatments. The germination percentage was recorded in each along with the control. The rate of induced variabilities of quantitative characters in M_1 and M_2 generation was analysed statistically. For meiotic study, the flower buds of suitable size from the treated and control plants were fixed in Carnoy's fluid (Abs. alcohol : Chloroform : Glacial acetic acid - 6:3:1) and smeared in 2% aceto carmine solution. Photomicrographs were taken from the suitable plates.

Results and Discussion

a. *Effect on germination* : The $Pb(NO_3)_2$ induced lethality for different concentration on the pea seeds was presented in the table (Table 1). In this experimental set the lower concentration did not affect the germination percentage and restored survival ability upto maturity as compared to the control. The effects were found to vary with the time and concentration of treatments. Seed germinability at different concentrations (10^{-5}M, 10^{-4}M, 10^{-3}M, 10^{-2}M) for 6 hours

duration of treaement showed 38.66, 29.33, 29.83, 21.66 percent respectively whereas the percentage of germination in the control set was higher i.e. 82.26 percent.

b. *Phenotypic effects of $Pb(NO_3)_2$ toxicity on M_1 and M_2 generation* : In M_1 and M_2 generation, the effects regarding the plant height, number of branches per plant, number of leaves per plant, number of flowers per plant, number of pods per plant, pod length, number of seeds per pod, yield per plant were highly reflected with the increase in time and concentration of the treatments (Table 2,3). Mean values representing those M_1 parameters were 45.00 cm, 28.00, 102.28, 10.25, 8.50, 6.50 cm, 5.25, 15.50 g and in M_2 parameters were 46.40 cm, 29.60, 112.38, 10.86, 11.25, 6.25, 6.05, 10.64 g at low concentration and time (10^{-5}M for 2 hours). At higher concentration (10^{-2}M for 6 hours), mean values representing those phenotypic characters were manifested by 22.60 cm, 8.50, 39.85, 3.75, 4.75, 3.35 cm, 2.50, 7.00 g for M_1 and 22.80 cm, 8.50, 37.86, 3.75, 4.80, 3.36 cm, 3.00, 7.25 g for M_2. Effects at different concentration and durations were comparable to the control set (Table 2,3). Coefficient of variabilities (C.V.) for all the characters found were irregularly decreased or increased with the increase in concentration and time.

c. *Metiotic study* : Meiotic anomalies from the flower buds from M_1 of the treated sets $Pb(NO_3)_2$ were observed 12.81% at 10^{-2}M concentration for 6 hours duration. The chromosomal anomalies like clumping, grouping, stickiness, C-metaphase with bivalents and univalents were of common occurrence besides multipolarity, laggards, early separation, unequal condensation and fragmentation. Cytomictic cells were found 1.24% and highest among all the treated series (Table 4, Fig.1 A-F).

In M_2 generation, meiotic study of the flower buds exhibited maximum percentage of anomalies at 10^{-2}M for 6 hours and gave the value 11.18% (Table 5). It has also been found that the effects for all the treated series were less than that of M_1. Clumping, grouping and stickiness were common and cytomictic cells though found in natural condition increased with the higher concentration of lead nitrate solution.

An analysis of the values on germination, (Table 1), revealed a striking feature with lead nitrate treatment at different concentration on pea plant. There is a established linear dose response relationship showing the gradual declining tendency with the increase in concentration and time. It may be inferred from the results that the metalic compound, $Pb(NO_3)_2$ at low concentration does not hamper so much in germination. At higher concentraion, the effects are manifested

Table 1. Effect of Pb $(No_3)_2$ on germination in *Pisum sativum* L. Cv Local.

Duration	Germination Percentage in different doses on soil				
	Control	10^{-5}M	10^{-4}M	10^{-3}M	10^{-2}M
2 hours	81.66±0.354	55.66±0.982	49.66±0.125	49.33±0.905	42.00±.0.494
4 hours	83.25±0.624	50.66±0.544	29.00±0.666	39.00±0.666	29.00±0.882
6 hours	82.26±0.544	38.66±0.544	29.33±0.440	29.83±0.448	21.66±0.364

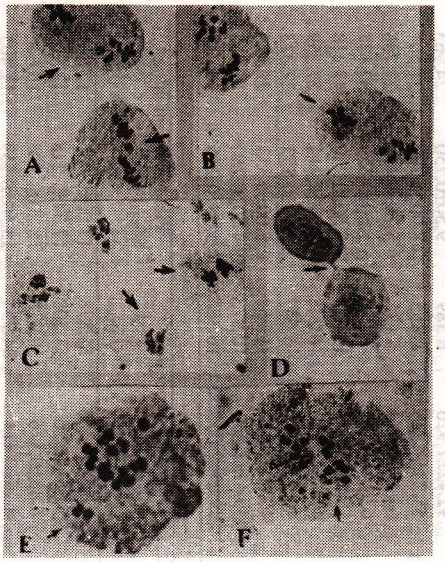

Fig.1 (A-F). Meiotic abnormalities induced by Pb $(No_3)_2$ in *Pisum sativum* L.
A. Anaphasic laggard; B. Unequal condensation at Anaphase-I; C. Clumping and grouping; D. Cytomixis; E. Early separation at Anaphase; F. C-Metaphase with fragments.

severely not only during germination but also in the growth. Dey *et al.*,[9] recorded similar results in conidial germination of microbe following the treatment with Cobalt II and Cobalt III complexes. It may however, be proposed that lead nitrate has affected some cellular reaction pathways for inhibition of the phenomenon of germination. Similar opinions were also mentioned by other researchers as well indicating retardation of growth at higher concentration of physical and chemical mutagen treatment [10-12].

In M_1 generation, plant height, number of branches per plant, number of leaves per plant, number of pods per plant, pod length, number of seeds per pod, yield per plant decreased with the increase in time and concentration of lead nitrate treatments (Table 1). The decrease in the number of pods per plant, seeds per pod showed that either the flower primordia could not reach upto the maturity and exist a direct correlation as indicated by the mean values. Similar results were reported in *Vicia faba* due to spray and seed soak treatments with environmental chemical mutagen by Amer and Farah,[13] in *Pisum abyssinicum, Hordeum sativum* and *Beta vulgaris* due to cadmium toxicity by Mukherjee *et al.*[14] and in *Solanum melongena* L. var pusa purple long due to mercury, cadmium and lead toxicity by Sengupta and Ghosh[8]. In M_2 generation also, the number of seeds per pod as well as yield per plant beside the other characters generally reduced and restored the effectivity of heavy metal compound. From the experiments, it can be inferred that lead is a nonessential toxic element probably inhibiting numerous enzymes and affects the natural growth

Table 2. Phenotypic effect of heavy metal [Pb $(No_3)_2$] toxicity on M_1 parameters in *Pisum sativum* L. CV. Local.

Dose/ treatment	Duration	Plant height in cm		No. of branches per plant		No. of leaves per plant		No. of flowers per plant	
		Mean ± S.E.	C.V. Value	Mean ± S.E.	C.V. Value	Mean ± S.E.	C.V. Value	Mean ± S.E.	C.V. Value
Control	2 hrs	58.76±1.386	7.458	29.00±1.250	13.629	115.75±5.389	14.712	13.25±1.386	33.075
	4 hrs	58.85±1.414	7.492	30.24±1.216	14.348	118.55±4.344	13.396	13.44±0.892	34.018
Pb $(No_3)_2$	6 hrs	58.54±1.284	7.522	30.55±1.018	14.355	120.48±3.488	10.422	14.55±0.784	34.302
10^{-5} M		45.00±1.544	10.849	28.00±0.353	3.986	102.28±1.534	4.742	10.25±0.544	16.781
10^{-4} M	2 hrs	40.00±1.525	10.047	24.75±1.916	24.478	92.57±3.181	10.865	10.50±0.750	22.585
10^{-3} M		35.75±1.634	14.452	23.25±1.515	20.604	92.42±1.926	6.589	9.50±0.750	24.963
10^{-2} M		40.25±1.192	9.364	10.50±0.450	13.551	55.28±1.723	9.855	8.00±0.797	31.501
10^{-5} M		40.00±0.612	4.837	11.25±0.572	16.077	64.28±1.044	5.135	5.75±0.414	22.766
10^{-4} M	4 hrs	38.75±1.138	9.286	11.25±0.572	16.077	63.14±1.491	7.466	5.50±0.559	32.137
10^{-3} M		39.75±0.252	2.017	11.25±0.789	22.176	56.28±2.371	13.321	5.50±0.750	43.118
10^{-2} M		36.75±1.582	13.611	10.50±0.559	16.833	47.42±1.537	10.248	4.75±0.414	27.559
10^{-5} M		35.00±0.935	8.447	9.50±0.559	18.605	46.42±2.212	15.067	5.00±0.353	22.323
10^{-4} M	6 hrs	33.75±1.192	11.164	9.25±0.414	14.152	40.00±1.512	11.952	5.00±0.353	22.323
10^{-3} M		32.00±0.985	9.733	9.00±0.559	19.639	39.42±1.368	10.973	4.50±0.250	17.566
10^{-2} M		22.60±0.935	13.081	8.50±0.252	9.374	39.85±2.065	16.385	3.75±0.216	18.213

Dose/ treatment	Duration	No. of pods per plant		pod length in cm		No. of seeds per pod		Yield per plant in g	
		Mean ± S.E.	C.V. Value	Mean ± S.E.	C.V. Value	Mean ± S.E.	C.V. Value	Mean ± S.E.	C.V. Value
Control	2 hrs	11.50±0.829	22.792	6.25±0.216	10.727	5.75±0.216	11.878	17.00±0.935	17.391
	4 hrs	12.24±0.534	20.564	6.08±0.144	10.826	5.55±0.326	11.508	17.34±1.218	17.525
Pb $(No_3)_2$	6 hrs	12.38±0.792	20.684	6.34±0.542	10.388	5.85±0.526	10.328	18.20±1.206	16.646
10^{-5} M		8.50±0.960	35.712	6.50±0.250	12.161	5.25±0.414	24.934	15.50±0.559	11.403
10^{-4} M	2 hrs	8.75±1.292	46.689	6.00±0.353	18.603	6.00±0.353	18.603	12.50±0.961	24.309
10^{-3} M		8.25±1.138	43.616	5.00±0.353	22.323	5.50±0.559	32.137	12.50±1.030	26.054
10^{-2} M		8.00±0.353	13.952	5.00±0.252	15.936	4.50±0.353	24.804	11.50±0.559	15.370
10^{-5} M		8.50±0.559	20.794	6.50±0.559	17.193	6.00±0.559	29.459	10.50±0.559	16.833
10^{-4} M	4 hrs	8.50±0.559	20.794	5.50±0.559	32.137	5.75±0.414	22.766	10.00±0.353	11.161
10^{-3} M		8.25±0.414	15.867	5.00±0.353	22.323	5.50±0.250	14.372	9.75±0.739	23.966
10^{-2} M		8.00±0.353	13.952	5.00±0.353	22.323	4.75±0.414	27.559	8.75±0.414	14.960
10^{-5} M		7.50±0.250	10.540	5.00±0.353	22.323	5.00±0.438	27.699	9.00±0.353	12.402
10^{-4} M	6 hrs	7.00±0.790	35.685	4.50±0.250	17.566	3.50±0.438	39.570	8.50±0.790	29.388
10^{-3} M		5.25±0.414	24.934	4.00±0.353	27.904	3.00±0.353	37.206	7.75±0.544	22.195
10^{-2} M		4.75±0.414	27.559	3.35±0.216	21.015	2.50±0.255	32.252	7.00±0.612	27.644

Table 3. Phenotypic effect of heavy metal [$Pb(No_3)_2$] toxicity on M_2 parameters in *Pisum sativum* L. CV. Local.

Dose/ treatment	Duration	Plant height in cm Mean ± S.E.	C.V. Value	No. of branches per plant Mean ± S.E.	C.V. Value	No. of leaves per plant Mean ± S.E.	C.V. Value	No. of flowers per plant Mean ± S.E.	C.V. Value
Control	2 hrs	59.72±1.488	7.878	30.25±1.268	13.254	124.74±4.184	10.605	15.76±0.814	16.331
	4 hrs	59.44±1.382	8.224	31.25±1.018	13.356	125.54±3.296	10.518	14.38±0.644	16.688
$Pb(No_3)_2$	6 hrs	58.62±1.216	7.948	31.35±1.244	12.414	128.39±3.014	11.249	15.52±0.546	15.355
10^{-5} M		46.40±1.879	12.804	29.60±0.516	5.512	112.38±1.58	4.468	10.86±0.514	14.965
10^{-4} M	2 hrs	41.25±1.224	9.382	26.36±0.339	4.066	94.48±3.185	10.659	10.54±0.752	22.560
10^{-3} M		38.75±1.664	13.578	24.18±1.518	19.850	90.34±2.336	8.176	9.80±0.356	11.486
10^{-2} M		35.25±1.414	12.683	10.36±0.559	17.061	65.48±1.787	8.629	8.25±0.336	12.877
10^{-5} M		40.38±0.414	3.241	13.26±0.616	14.689	69.36±2.144	9.774	6.80±0.228	10.602
10^{-4} M	4 hrs	38.68±1.183	9.670	12.27±0.336	8.658	58.35±1.338	7.250	5.75±0.336	18.477
10^{-3} M		35.58±1.544	13.721	10.25±0.787	24.277	56.88±2.216	12.318	5.50±0.414	23.801
10^{-2} M		34.48±2.144	19.661	10.25±0.418	12.894	48.85±2.252	14.576	4.85±0.514	33.510
10^{-5} M		34.48±0.414	3.796	10.50±0.216	6.504	52.26±2.018	12.209	5.30±0.344	20.523
10^{-4} M	6 hrs	35.08±1.218	10.978	9.60±0.138	4.545	48.40±2.116	13.823	5.25±0.414	24.934
10^{-3} M		30.50±0.789	8.179	9.25±0.262	8.956	40.50±2.018	15.755	4.80±0.544	35.836
10^{-2} M		22.80±0.336	4.659	8.50±0.448	16.665	37.86±2.856	23.852	3.75±0.328	27.656

Dose/ treatment	Duration	No. of pods per plant Mean ± S.E.	C.V. Value	pod length in cm Mean ± S.E.	C.V. Value	No. of seeds per pod Mean ± S.E.	C.V. Value	Yield per plant in g Mean ± S.E.	C.V. Value
Control	2 hrs	12.58±0.544	13.673	6.75±0.289	13.443	6.25±0.216	10.927	17.50±0.987	17.833
	4 hrs	10.52±0.264	14.014	6.85±0.244	13.506	6.55±0.524	10.422	17.58±0.864	17.826
$Pb(No_3)_2$	6 hrs	12.39±0.216	14.322	6.85±0.252	13.268	6.55±0.328	10.526	17.56±0.318	17.458
10^{-5} M		11.25±0.216	6.071	6.25±0.216	10.927	6.05±0.544	28.431	16.64±0.335	6.365
10^{-4} M	2 hrs	7.88±1.287	51.643	6.25±0.316	15.987	6.00±0.336	17.707	14.46±0.787	17.209
10^{-3} M		8.28±1.384	52.852	5.25±0.336	20.236	5.80±0.336	18.317	12.38±1.183	30.313
10^{-2} M		7.25±0.544	23.725	5.05±0.778	48.713	4.70±0.216	14.531	11.86±0.315	8.398
10^{-5} M		8.80±0.556	19.978	6.70±0.418	19.727	5.05±0.483	25.243	11.25±0.339	9.528
10^{-4} M	4 hrs	8.25±0.338	12.954	5.80±0.216	11.775	5.78±0.414	22.648	11.05±0.514	14.708
10^{-3} M		8.25±0.414	15.867	5.60±0.348	19.649	5.25±0.336	20.236	9.80±0.338	10.905
10^{-2} M		8.05±0.789	30.991	5.50±0.353	20.294	4.25±0.356	26.486	8.66±0.448	16.357
10^{-5} M		7.80±0.292	11.837	5.50±0.336	19.316	5.75±0.448	24.636	9.25±0.474	16.203
10^{-4} M	6 hrs	7.24±0.336	14.674	5.28±0.228	13.654	4.50±0.414	29.090	8.60±0.514	18.898
10^{-3} M		6.36±0.559	27.791	4.45±0.414	29.417	3.75±0.556	46.881	7.85±0.343	13.816
10^{-2} M		4.80±0.414	27.272	3.36±0.254	23.903	3.00±0.318	33.517	7.25±0.216	9.420

system. Dhir *et al*[4]., have put forth that the action of metal on the metabolism of eukaryotic system is influenced by the presence of other metals in the substrate in the organism concerned.

The study of meiosis reflected the number of univalents at higher concentration of lead treatment and furnished that the metallic compound affected the normal pairing of chromosomes. Studies of the later stages revealed that the treatment with the chemicals have resulted various types of clastogenic changes such as stickiness, laggards, anaphasic bridge, cytomictic cells, unequal separation, early separation of anaphase, fragmented telophase and multipolarity, clumping and grouping with varying frequencies. Clumping, grouping and stickiness were the dominant among the anomalies. The stickiness and anaphasic bridge produced due to the hindrance of chromosome movement. The cytological effects induced by the heavy metal depends on the mode of action of the metal itself with the bio-organic molecule[15]. The occurrence of univalents and new orientation seems to be outcome of some disturbances during pairing of homologous chromosomes which may be due to the chromosome breakage in PMCS of treated plants. The hindrance of movement of the bivalent to the equatorial plate usually resulted in monorientation of chromosomes. The retardation of movement exhibited unequal separation of chromosomes and laggards. It is difficult to logically conclude and establish any cause and effect relationship concerning the cytomixis phenomenon. The result of cytomixis is obviously a change in the amount of chromatin and/or number of chromosomes in the cells during mitosis or meiosis and in normal and cytogenetically imbalanced plants, it has been reported[16-18]. From the perusal of literature it has been concluded that most of the metals when administered to the higher organism are clastogenic and mutagenic in nature at certain doses and durations of treatments[7]. The initiation of effects and their degrees depend upon the number of factors including the rate and mode of administration, the solvent used, rate of detoxification with the increasing error of cell free DNA synthesis, excretion and interaction with foreign and endogenous substances and electronegativity [7,19].

In our observations, it has been found that the cytogenetical effects though persisted in M_1 but diluted in M_2. Varner[20] suggested that the plant growth hormone, kinetin, naturally present in meristimatic tissue seems to be responsible for the recovery of cellular irregularities and have autoreplicating mechanism. Hence it may be concluded that the heavy metal interferes with the divisional process ultimately leads to various irregularities and brings about severe effect on the productivity of agricultural crops.

Acknowledgement

Financial assistance provided by the University Grants Commission, New Delhi is gratefully acknowledged. The authors are grateful to the Head, Department of Botany for providing necessary facilities and encouragements.

References

1. Fiskesjo G 1969, *Heriditas* **62** 314
2. Bauchinger M, Schmid E, Embroot H J and Dresp J 1976, *Mutat. Res.* **40** 57.
3. Tribedi S, Biswas A K, Konar D, Ray S and Dey

Table 4. Meiotic chromosomal anomalies in percentage (Mean ±S.E.) on M_1 induced by the effect of Pb $(No_3)_2$ in *Pisum sativum* L. CV. Local.

Dose/ treatment	Duration	Clumping or grouping or stickiness	Bivalents with uni-valents	Multipola-rity	Laggards	Early sepa-ration of anaphase	Unequal con-densations of anatelophase	Fragmented telophase	Cytomixis	Total anomalies
Control	2 hrs	0.08	-	-	-	-	-	-	0.58	0.66±0.056
	4 hrs	0.04	-	-	-	-	-	-	0.55	0.59±0.064
	6 hrs	0.06	-	-	-	-	-	-	0.48	0.54±0.048
Pb $(No_3)_2$										
10^{-5} M	2 hrs	2.01	1.71	0.57	-	-	-	-	-	4.29±0.172
	4 hrs	2.33	1.66	-	-	-	-	-	-	3.99±0.072
	6 hrs	3.18	-	-	0.93	-	-	-	-	4.01±0.174
10^{-4} M	2 hrs	2.71	0.72	0.79	-	-	-	-	-	4.22±0.118
	4 hrs	3.12	-	-	1.17	-	-	0.55	1.06	5.90±0.116
	6 hrs	4.10	1.18	-	1.05	0.56	0.34	-	1.22	8.45±0.106
10^{-3} M	2 hrs	2.82	0.57	-	0.59	-	-	-	-	3.98±0.144
	4 hrs	4.18	1.16	0.67	-	-	-	-	0.54	6.55±0.128
	6 hrs	5.27	1.21	-	0.66	0.71	-	-	1.04	7.14±0.214
10^{-2} M	2 hrs	2.77	0.46	1.21	-	0.66	-	-	-	5.10±0.274
	4 hrs	5.66	1.72	0.66	0.82	-	0.47	0.71	0.56	10.60±0.242
	6 hrs	7.78	1.55	-	1.17	0.48	0.59	-	1.24	12.81±0.236

Table 5. Meiotic Chromosomal anomalies in percentage (Mean ±S.E.) on M_2 induced by the effect of Pb $(No_3)_2$ in *Pisum sativum* L. CV. Local.

Dose/ treatment	Duration	Clumping or grouping or stickiness	Bivalents with uni-valents	Multipola-rity	Laggards	Early sepa ration of anaphase	Unequal con-densations of anatelophase	Fragmented telophase	Cytomixis	Total anomalies
Control	2 hrs	0.04	0.61	-	-	-	-	-	0.47	1.12±0.142
	4 hrs	0.02	0.59	-	-	-	-	-	0.39	1.01±0.328
	6 hrs	0.05	0.57	-	-	-	-	-	0.47	1.09±0.314
Pb $(No_3)_2$										
10^{-5} M	2 hrs	2.72	0.54	-	-	0.33	-	-	-	2.99±0.216
	4 hrs	3.76	1.08	-	0.45	-	-	-	-	5.29±0.144
	6 hrs	3.87	1.26	0.47	-	0.52	-	0.71	-	6.83±0.057
10^{-4} M	2 hrs	2.08	0.76	-	-	-	-	-	-	2.84±0.082
	4 hrs	4.38	1.27	0.52	-	0.28	0.76	-	-	7.21±0.114
	6 hrs	4.87	1.31	-	0.39	-	0.82	0.47	0.63	8.49±0.141
10^{-3} M	2 hrs	2.34	0.48	-	-	-	-	-	-	2.82±0.172
	4 hrs	4.41	1.33	0.44	0.56	-	0.77	-	1.22	8.73±0.206
	6 hrs	5.82	1.71	-	-	0.56	0.63	1.08	0.79	10.59±0.166
10^{-2} M	2 hrs	2.81	0.59	-	-	-	-	-	-	3.40±0.144
	4 hrs	4.77	1.21	0.37	0.77	-	-	0.48	1.06	8.66±0.116
	6 hrs	5.68	1.77	1.13	0.46	0.38	-	0.55	1.21	11.18±0.121

K 1984, *Int. J. Environ. Stud. Review of Cytology* **24** 99

4. Dhir H, Talukdar G and Sharma A 1985, *The Nucleus* **28** 68
5. Sathaiah V, Athma P and Reddy P T 1984, *Pers.in Cytol. and Genet.* (G K Manna and U Sinha eds) **4** 265
6. Chakraborty I, Sharma A and Talukdar G 1989, *The Nucleus* **32(1,2)** 12
7. Sharma G P and Sobti R C 1989, *Prs. In Cytol. and Genet.* (G K Manna and U Sinha eds.) **6** 465
8. Sengupta R K and Ghosh P 1992, *Cell and Chromosome Research* **15(1)** 7.
9. Dey K, Maiti R K, Bhar J K, Banerjee R D, Sarkar G M, Malakar A, Datta S and Banerjee P 1981, *Agent and Actions*, **11(617)** 761
10. Gunckel J E 1954, *Brookhaven Symp. Biol.* **6** 252
11. Singh B B 1974, *Red Bot.* **14** 195
12. Datta A K and Biswas A K 1983, *Cytologia* **48** 293
13. Amer S M and Farah O R 1976, *Cytologia* **41** 597
14. Mukherjee A, Sharma A and Talukder G 1984, *The Nucleus* **27** 121
15. Venugopal B and Luckey T D 1978, *Metal Toxicity in mammals.* Plenum Press, New York, USA.
16. Gottschalk W 1970, *The Nucleus* **13** 1
17. Bobak M and Herich R 1978, *The Nucleus* **21(1)** 22
18. Datta A K and Biswas A K 1984, *Cytologia* **49** 437.
19. Levan A and Tjio J H 1951, *Hereditas* **37** 307
20. Varner J E 1964, *Plant Physiol.* **39** 413.