 Cytologia **70**(2): 119–128, 2005

Cytological Effect of the Potassium Metabisulphite and Potassium Nitrate Food Preservative on Root Tips of *Allium cepa* L.

Ayşe Nihal Gömürgen*

Hacettepe University, Science Faculty, Botany Department, 06532, Beytepe.Ankara, Turkey

Received September 27, 2004; accepted October 30, 2004

Summary The effects of the food preservatives potassium metabisulphite (PMB) and potassium nitrate (PN) have been studied on root tips of *Allium cepa* L. (variety Kantartopu-3). Roots of *A.cepa* were treated with a series of concentrations, ranging from 50 ppm to 100,000 ppm for 3, 6, 12, 24 and 48 h. Examinations of roots were done in permanent root tip squash preparations stained by the Feulgen technique. PMB and PN effect on the relative duration of each mitotic stage as compared with the control. They are also caused reduction in the mitotic index, indicating mitotic inhibition and increased frequency of abnormal mitosis.

The type of abnormalities induced are chromosome stickiness, c-metaphase, anaphase and telophase bridges, disturbed chromosomes of anaphase and telophase stages, anaphase lagging and forward chromosomes at anaphase and telophase and micronuclei formation at intephase cells.

Key words Potassium metabisulphide, Potassium nitrate, Food preservative, Chromosome abnormalities.

The population of the word is increasing everyday. Therefore it is essential to find new food sources and ways to make productive use of these food sources. Many methods have been developed storing food for long periods and also many chemical substances have been used for the preservation of food, as an antimicrobial agents. It has been reported many of chemicals are genotoxic effects in different test systems (Gömürgen 2000, Matsuoka *et al*. 1979, Luca *et al*. 1987) especially the antimicrobial agents (Mukherjee *et al*. 1982). Since 1983 when Levan used Allium test for the first time, it has been the standard material for studying the effects of various chemical pollutants on the chromosomes.

Food preservatives, sodium benzoate and sodium sulphite, inhibit DNA synthesis, induce anaphase bridges and chromatin erosion in interphase nuclei in *Vicia faba* root (Nyagi and Gopalan 1982). Sasaki *et al*. (2002) determined the genotoxicity of 39 chemicals currently in use as food additives mostly they induced DNA damage in gastro intestinal organs. Sorbic acid and its potassium salt, sodium sorbate (Hasegewa *et al*. 1984) and also potassium sorbate have induced sister chromatid exchanges in Chinese hamster cells (Abe and Sasaki 1977). Although fresh solution of sodium sorbate and potassium sorbate did not induce sister chromatid exchange or micronuclei in bone marrow cells of mice (Ishidate *et al*. 1984) and was not mutagenic in the salmonella/microsome assay (Akin and Sümer 1991). Meng and Zhang (1992) stated that sodium bisulphate caused sister chromatid exchanges and micronuclei in human blood lymphocytes in a dose-dependent manner. Sodium metabisulphite, a food preservative, induced chromosome aberrations and sister chromatid exchanges also decreased replication index and mitotic index in cultured human lymphocytes (Rencüzoğulları *et al*. 2001a). Sodium metabisulphite significantly decreased mitotic index and increased the mitotic abnormalities dose dependently in root tip cells of *Allium cepa* (Rencüzoğulları

* Corresponding author, e-mail: gomurgen@hacettepe.edu.tr

et al. 2001b). However there are many food preservatives whose genotoxic effects are unknown.

Potassium metabisulphite and potassium nitrate are used as preservative in various kinds of food products. The Ministry of Health of Turkey (Anonymous 1990) has suggested that PMB and Potassium nitrate may be used as 500 mg/kg. PMB is used as food preservative, to reduce discoloration of light-colored fruits and vegetables, such as dried apples and dehydrated potatoes. It is also used in wine-making because it inhibit bacterial growth but do not interfere with the desired development of yeast (Saldamli 1985). Nitrate and nitrites have been used for centuries in curing and preserving meat and fish. It is also used in the manufacture of certain cheeses (Binkerd and Kolari 1975).

The aim of this study was to investigate the cytogenetic effects and cytotoxicity of PMB and PN in *Allium cepa* L. root tip cells.

Materials and methods

Bulbs of *Allium cepa*, variety Kantartopu-3 ($2n$=16) were used as plant test material. Bulbs were placed in jars with their basal ends dipping in distilled water and germinated at room temperature (20±2°C). After 72 h, when the roots were 1.5 cm in length, transferred to jars containing potassium metabisulphide and potassium nitrate with a series of concentrations, ranging from 50 ppm to 100,000 ppm. The treatment was done for 3, 6, 12, 24 and 48 h. Root tips were fixed in acetic alcohol (1 : 3). Squashes were made by Feulgen technique. Root tips were hydrolyzed in 1 N HCl for 10 min at 60°C. Slides were made permanent with Canada balsam. Three replicates were performed for each treatment and the control and scoring was made from the 3 roots of each replicate. The mitotic index (MI) was calculated for each treatment as a number of dividing cells/100 cells.

Cytological abnormalities documented with photomicrographs.

Results and discussion

The effects on MI and the frequency of mitotic phases are given in Tables 1 and 2 for the treatment of PMB and PN respectively. MI was reduced as the concentration increased and the period of treatment prolonged both the treated cells of PMB and PN. In roots treated with the highest concentrations (100,000 ppm) of PMB at all periods mitosis had completely ceased, so this concentration did not show in Table 1. Same effect of PN at the highest concentration also observed, especially at 48 h treatment of PN at 10,000 ppm to 100,000 ppm concentrations there were no cell division but at almost all of the cells nuclear vacuolation was determined (Fig. 1). Nucleolar vacuolation was also observed on 100,000 ppm concentration of PN nitrate while at 48 h nucleolar vacuolation was observed on 1,000, 50,000 and 100000 ppm concentrations.

MI reflects the frequency of cell division. The MI decreased with increased PMB and PN concentrations and treatment duration (Tables 1 and 3). The degree of mitotic inhibition is clearly dose dependent. Mitotic activity was reduced as the concentration increased and the period of treatment prolonged. Several other chemicals and food preservatives have been reported to inhibit mitosis (Gömürgen 2000, Bushra *et al*. 2002, Rencüzoğulları *et al*. 2001a, 2001b). Reduction of the mitotic activity seems to be common effect of some food preservatives (Rencüzoğulları *et al*. 2001a, 2001b). Such a drop in the mitotic index indicates that PMB and PN interfere in the normal sequence of mitosis thus preventing a number of cells from entering prophase, state at interphase. Reduction in the mitotic activity could be due to inhibition of DNA synthesis (Schneiderman *et al*. 1971). Beu *et al*. (1976) also showed that exposing the root tips of *Vicia faba* to high concentration of the herbicide, led to inhibition of DNA synthesis. This may suggest that PMB and PN may cause inhibition of DNA synthesis.

The food preservatives PMB and PN caused a change in the frequencies of different stages.

Table 1. Number of total cells examined and total mitosis, percentage of total and abnormal mitotic phases, mean of mitotic index after treating *A. cepa* root tips with different concentrations of Potassium metabisulphide

Time of treatment	Concentrations (ppm)	Examined total cells	Total mitosis	Prophase %		Metaphase %		Anaphase %		Telophase %		Micronuclei	MI means±SE
				Total	Abnormal	Total	Abnormal	Total	Abnormal	Total	Abnormal		
3 hrs	control	8864	606	54	0	16	0	10	0	20	0		6.836±0.51
	50	11870	680	40	0	6	26	7	14	7	0	0	5.729±0.28
	100	5420	260	46	0	0	42	0	0	12	0	0	4.797±0.14
	500	9110	760	33	0	16	12	0	9	30	0	0	8.342±0.04
	1000	5890	240	92	0	0	0	0	0	8	0	0	4.075±0.10
	5000	5310	230	17	4	13	35	9	5	17	0	3	4.331±0.20
	10000	8421	390	64	0	5	13	3	3	7	5	1	4.631±0.07
	50000	15380	890	30	9	5	17	3	3	33	0	3	5.787±0.25
6 hrs	control	9014	610	46	0	20	0	8	0	26	0		6.767±0.06
	50	12000	650	20	5	0	67	0	0	8	0	11	5.417±0.06
	100	9560	500	22	6	44	14	0	2	12	0	5	5.230±0.03
	500	7548	363	38	0	22	11	5	5	19	0	1	4.809±0.59
	1000	7252	336	44	6	4	26	8	0	12	0	1	4.633±0.26
	5000	6884	248	18	0	15	21	5	3	38	0	11	3.602±0.34
	10000	9042	447	33	1	14	11	7	10	24	0	1	4.944±0.78
	50000	7190	390	23	14	34	3	3	14	9	0	4	5.424±0.45
12 hrs	control	8472	306	41	0	20	0	12	0	27	0		3.612±0.02
	50	9258	453	17	2	7	59	4	5	6	0	25	4893±0.10
	100	11802	381	14	5	13	60	2	2	4	0	3	3228±0.17
	500	9244	264	21	0	30	11	8	6	24	0	13	2855±0.26
	1000	7020	220	32	0	9	9	0	5	45	0	0	3.134±0.29
	5000	9072	363	36	0	5	28	0	7	23	1	3	4.001±0.37
	10000	8242	226	31	4	15	18	8	12	11	1	2	2.742±0.14
	50000	9298	226	25	2	16	16	4	10	25	2	1	2.431±0.51
24 hrs	control	9520	330	45	0	20	0	8	0	27	0	0	3.466±0.05
	50	8400	262	42	1	21	9	6	5	14	2	0	3.119±0.24
	100	6950	200	16	4	17	4	34	8	15	2	2	2.878±0.13
	500	6640	189	22	4	14	9	26	6	15	3	1	2.846±0.40
	1000	4640	118	33	3	19	9	11	4	20	1	3	2.543±0.60
	5000	10060	269	22	6	18	21	4	3	20	6	6	2.664±0.33
	10000	3212	68	46	4	11	8	19	5	7	0	5	2.118±0.15
	50000	2790	40	21	4	9	15	34	0	17	0	9	1.433±0.04
48 hrs	control	9788	471	50	0	15	0	9	0	25	0	0	4.812±0.12
	50	8362	196	28	0	11	42	0	7	8	4	22	2.344±0.02
	100	7880	242	22	3	13	17	1	13	29	2	28	3.071±0.08
	500	9900	350	45	5	8	42	0	0	0	0	33	3.535±0.22
	1000	6718	119	17	0	8	49	6	7	13	0	0	1.771±0.31
	5000	10647	199	19	1	6	37	10	8	18	1	5	1.869±0.18
	10000	10555	246	39	0	21	5	4	9	22	0	5	2.331±0.41
	50000	11893	213	28	2	12	11	7	10	28	2	4	1.791±0.36

Table 2. Number of total cells examined and total mitosis, percentage of total and abnormal mitotic phases, mean of mitotic index after treating *A. cepa* root tips with different concentrations of Potassium nitrate

Time of treatment	Concentrations (ppm)	Total cells examined	Total mitosis	Prophase %		Metaphase %		Anaphase %		Telophase %		Micronuclei	Nucleolar vacuolation	MI means±SE
				Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal			
3 hrs	control	8854	407	54	0	16	0	10	0	20	0	0	0	4.597±0.28
	50	3131	127	37	2	18	1	6	1	35	0	0	0	4.056±0.09
	100	4125	134	36	16	9	24	0	10	5	0	0	0	3.197±0.52
	500	8434	177	34	20	9	14	2	2	18	1	13	0	2.099±0.16
	1000	3518	78	23	5	5	26	1	19	21	0	1	0	2.217±0.08
	5000	3972	111	30	11	11	14	13	2	16	3	2	0	2.795±0.27
	10000	3027	50	14	12	16	22	6	8	8	14	0	0	1.652±0.43
	50000	4021	39	4	3	5	7	5	6	3	6	0	0	0.969±0.19
	100000	2746	6	0	0	0	6	0	0	0	0	0	2740	0.218±0.25
6 hrs	control	9014	340	46	0	20	0	8	0	26	0	0	0	3.772±0.42
	50	4975	111	23	5	14	20	14	4	20	0	0	0	2.231±0.16
	100	5933	148	30	10	12	17	12	6	11	2	3	0	2.495±0.32
	500	5124	93	22	17	9	23	9	11	9	0	0	0	1.815±0.12
	1000	3504	46	17	26	7	23	7	4	9	7	3	0	1.276±0.41
	5000	2415	209	33	7	6	11	9	3	7	24	60	0	1.683±0.23
	10000	2009	196	24	12	3	22	3	2	17	17	176	0	1.547±0.32
	50000	4011	50	0	0	0	0	0	80	0	20	10	0	1.246±0.21
	100000	4379	42	3	0	0	60	0	5	16	16	65	0	0.959±0.05
12 hrs	control	8472	306	41	0	20	0	12	0	27	0	0	0	3.612±0.11
	50	7027	224	32	11	14	6	14	6	17	0	0	0	3.188±0.25
	100	10138	371	24	3	22	5	22	6	17	1	0	0	3.659±0.16
	500	12779	435	26	5	21	12	21	6	9	0	0	0	3.404±0.44
	1000	13337	252	28	6	15	9	15	4	13	10	23	0	1.889±0.07
	5000	10235	176	15	10	11	21	12	4	24	3	6	0	1.719±0.25
	10000	5000	79	0	0	0	0	0	100	0	0	0	0	1.580±0.51
	50000	8581	72	0	0	4	68	0	6	6	16	0	0	0.874±0.18
	100000	4000	0	0	0	0	0	0	0	0	0	0	4000	0.000±0.00
24 hrs	control	9520	330	45	0	20	0	8	0	27	0	0	0	3.466±0.32
	50	10787	408	30	5	9	18	9	9	20	0	0	0	3.782±0.06
	100	11772	296	17	7	4	19	4	12	20	17	52	0	2.514±0.08
	500	10828	297	27	6	8	23	8	9	16	3	9	0	2.743±0.16
	1000	15112	393	18	6	11	20	11	8	25	1	4	0	2.601±0.07
	5000	13260	108	32	20	0	33	0	0	9	6	3	0	0.814±0.26
	10000	2850	18	11	6	17	0	28	11	22	6	190	0	0.632±0.56
	50000	3800	21	14	10	19	19	10	14	5	9	2	0	0.552±0.43
	100000	8086	43	37	30	0	28	0	0	5	0	28	0	0.532±0.23
48 hrs	control	9788	471	50	0	15	1	9	0	25	0	0	0	4.812±0.05
	50	11141	458	20	12	14	18	7	3	22	4	17	0	4.111±0.33
	100	11875	381	15	8	16	22	10	15	14	0	7	0	4.101±0.41
	500	16737	541	19	6	18	15	7	12	21	2	56	0	3.232±0.08
	1000	12783	320	13	19	9	18	4	10	22	5	20	0	2.503±0.26
	5000	8052	179	1	0	0	3	0	96	0	0	15	0	2.223±0.06
	10000	3500	0	0	0	0	0	0	0	0	0	0	3500	0.000
	50000	2450	0	0	0	0	0	0	0	0	0	0	2450	0.000
	100000	3000	0	0	0	0	0	0	0	0	0	0	3000	0.000

Table 3. Frequencies of different types of pro-metaphase, anaphase and telophase abnormalities after treating *A. cepa* root tips with different concentrations of potassium metabisulphite

Time of treatment	Concentration (ppm)	% of metaphase abnormalites				% of anaphase abnormalities					% of telophase abnormalities					
		Sticky metaphase	c-metaphase	Disturbed metaphase	Metaphase with forward	Early anaphase	Disturbed anaphase	Anaphase with laggard	Anaphase bridge	Anaphase with forward	Tetraploid anaphase	Tripolar anaphase	Telophase bridge	Telophase with laggard	Telophase with forward	Micronucleus
3 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	4	60	4	0	0	15	0	10	4	3	0	0	0	0	0
	100	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0
	500	44	13	0	0	0	0	0	31	0	12	0	0	0	0	0
	1000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	5000	73	0	0	0	0	0	0	9	0	0	0	0	0	0	18
	10000	48	12	0	2	0	4	2	5	1	2	4	8	6	4	1
	50000	0	76	0	5	0	0	0	10	0	0	0	0	0	0	9
6 hrs	control	0	51	25	0	0	13	0	0	0	0	0	0	0	0	11
	50	0	81	3	0	0	0	0	0	0	0	0	0	0	0	16
	100	0	47	20	0	0	0	0	0	9	0	0	0	0	0	24
	500	20	25	20	0	0	20	0	10	0	0	0	0	0	0	5
	1000	79	0	17	0	0	0	0	0	0	0	0	0	0	0	4
	5000	56	5	0	0	0	0	0	5	5	0	0	0	0	0	29
	10000	33	7	12	0	3	11	10	21	0	0	0	0	0	0	3
	50000	0	14	0	0	0	39	0	25	0	0	0	0	0	0	22
12 hrs	control	0	0	0	0	0	0	0	0		0	0	0	0	0	0
	50	12	63	0	0	0	2	0	5	0	0	0	0	0	0	18
	100	33	8	51	3	0	0	0	2	0	0	0	0	0	0	3
	500	43	0	0	0	0	13	0	8	0	0	0	0	0	0	36
	1000	67	0	0	0	0	0	0	33	0	0	0	0	0	0	0
	5000	69	2	2	0	0	0	0	16	0	2	0	3	0	0	6
	10000	35	4	14	4	4	6	9	15	0	0	2	4	0	0	3
	50000	37	8	8	3	0	12	0	27	0	0	0	0	2	2	1
24 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	33	14	5	4	6	6	1	9	4	4	1	5	1	1	0
	100	6	27	7	5	4	8	19	4	10	0	1	4	3	2	2
	500	32	17	2	8	0	5	0	2	9	6	5	3	6	5	1
	1000	41	23	4	5	6	0	0	12	0	3	0	4	2	0	3
	5000	39	27	5	4	0	0	4	2	0	1	1	5	5	7	6
	10000	42	30	0	3	3	6	7	0	3	6	0	0	0	0	5
	50000	37	36	14	13	0	0	0	0	0	0	0	0	0	0	9
48 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	22	28	7	0	0	0	0	9	0	0	0	8	0	0	26
	100	3	32	3	0	0	16	0	11	0	0	0	3	0	0	32
	500	51	0	0	0	0	0	0	0	0	0	0	0	0	0	49
	1000	64	11	10	2	0	2	0	11	0	0	0	0	0	0	0
	5000	68	7	2	0	0	8	0	7	2	0	0	3	0	0	3
	10000	21	0	0	7	0	0	0	48	0	0	0	0	0	0	24
	50000	33	6	0	0	0	0	0	38	0	0	0	6	0	0	17

Their frequencies depend on the duration of treatment and concentration of PMB and PN applied.

PMB and PN exerted a marked mitodepressive action on mitosis and induced a number of chromosomal aberrations. The different kinds of chromosome aberrations were presented in Table 3 for PMB and in Table 4 for PN.

Late prophase appeared at the cells treated with PN while there was no abnormal prophase appeared at the cells treated with PMB (Fig. 2).

The most common type of abnormality observed with all concentrations and periods of treat-

Table 4. Frequencies of different types of pro-metaphase, anaphase and telophase abnormalities after treating *A. cepa* root tips with different concentrations of potassium nitrate

		% of pro-metaphase abnormalities						% of anaphase abnormalities									% of telophase abnormalities			
Time of treatment	Concentration (ppm.)	Late prophase	Sticky metaphase	c-metaphase	Disturbed metaphase	Metaphase	Tetraploid c-metaphase	Early anaphase	Disturbed anaphase	Forward anaphase	Anaphase with laggard	Anaphase bridge	Anaphase with forward	Tripolar anaphase	Tripolar Anaphase with laggard	Multipolar anaphase	Telophase bridge	Telophase with laggard	Telophase with forward	Micronucleus
3 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	60	20	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0
	100	32	6	35	6	0	0	0	21	0	0	0	0	0	0	0	0	0	0	0
	500	44	16	4	8	0	0	0	4	0	0	2	0	2	0	0	2	0	0	18
	1000	10	13	26	13	0	0	0	21	0	0	9	0	2	2	2	0	0	0	2
	5000	35	17	25	6	0	0	0	0	0	0	2	2	0	0	0	0	5	2	6
	10000	21	14	18	7	0	0	0	11	0	0	4	0	0	0	0	11	14	0	0
	50000	14	10	14	10	0	0	0	10	0	0	14	0	0	0	0	14	14	0	0
	100000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	16	22	19	28	0	0	0	3	3	0	6	0	0	0	3	0	0	0	0
	100	30	20	17	11	0	0	0	11	0	2	2	0	2	0	0	0	0	0	5
	500	33	13	31	0	0	0	0	2	4	0	17	0	0	0	0	0	0	0	0
	1000	43	27	14	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	10
	5000	18	8	18	3	1	0	0	0	1	0	2	0	1	0	2	1	0	0	45
	10000	9	10	2	3	0	0	0	1	0	1	1	0	1	0	0	11	0	0	61
	50000	0	0	0	0	0	0	0	5	5	25	18	2	7	2	3	12	5	0	16
	100000	0	32	5	3	1	0	0	1	0	1	1	0	0	0	0	5	6	0	45
12 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	47	8	17	0	0	0	10	4	0	2	10	0	0	2	0	0	0	0	0
	100	23	6	27	2	0	2	23	0	0	4	8	0	5	0	0	0	0	0	0
	500	22	13	39	0	1	0	8	2	0	0	11	1	3	0	0	0	0	0	0
	1000	19	11	6	14	0	2	3	0	0	0	13	0	0	0	0	0	0	0	32
	5000	25	10	40	5	0	0	0	3	0	0	8	0	0	0	0	0	0	0	9
	10000	0	0	0	0	0	0	8	38	0	0	46	0	8	0	0	0	0	0	0
	50000	0	46	26	3	1	1	0	3	0	1	2	0	0	0	0	10	6	1	0
	100000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	14	10	36	11	0	0	10	8	0	0	11	0	0	0	0	0	0	0	0
	100	13	15	7	7	4	0	5	5	0	2	8	1	2	0	0	0	2	0	29
	500	16	7	34	11	2	0	7	4	1	3	6	1	1	0	0	0	1	1	5
	1000	16	19	17	16	5	0	5	1		2	12	0	2	0	1	0	1	0	3
	5000	30	13	42	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	9
	10000	3	0	0	0	0	0	2	1	0	0	2	0	0	0	0	1	2	0	89
	50000	19	12	19	4	2	0	8	11	0	0	6	0	2	0	0	6	9	2	0
	100000	25	6	10	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51
48 hrs	control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	12	8	11	14	0	4	2	2	0	1	21	0	2	0	1	1	0	1	20
	100	33	6	23	2	0	4	1	8	1	3	3	0	1	0	2	0	3	1	9
	500	31	16	25	3	0	0	0	3	4	0	1	0	1	1	0	0	2	4	8
	1000	30	45	35	3	0	0	0	11	2	2	41	0	0	0	2	1	0	0	7
	5000	0	3	0	13	0	0	3	11	0	0	16	0	3	0	3	0	0	0	48
	10000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figs. 1–8. Cytological abnormalities in the root tip meristems of *A. cepa* treated with PMB and PN; 1) nucleolar vacuolation, 2a) late prophase (PMB), 2b) late prophase (PN), 3a) c-metaphase (PMB), 3b) c-metaphase (PN), 4a) Sticky metaphase (PMB), 4b) Sticky metaphase (PN), 5a) disturbed metaphase (PMB), 5b) disturbed metaphase (PN), 6a) metaphase with forward chromosome (PMB), 6b) metaphase with forward chromosome (PN), 7a) anaphase bridge (PMB), 7b) anaphase bridge (PN), 8a) disturbed anaphase (PMB), 8b) disturbed anaphase (PN).

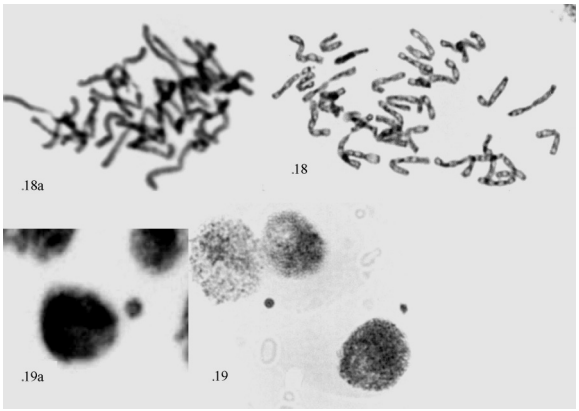
ment was type of c-metaphase (Fig. 3) and sticky metaphase (Fig. 4). C-mitosis was observed at all treated roots at PN concentrations and most of the PMB concentrations. C-mitosis was first described by Levan (1938) in root tips of *A.cepa* L. as an in activated of the spindle followed by the random scattering of the condensed chromosomes. C-metaphase abnormality was produced as a result of inhibition of spindle fiber formation. In this case it causes an arrest mainly at metaphase. Such an arrest may be one of the causes for MI inhibition. These results are in an agreement with the results obtained for those chemicals, which show an effect like colchicines, such as mercury compounds (Ramel 1969) and Igran (El-Khodary *et al*. 1987).

Klasterska *et al*. (1976) and McGill *et al*. (1974) suggested that chromosome stickiness arises from improper folding of the chromosome fiber into single chromatids and the chromosomes become attached to each other by subchromatid bridges Chromosome stickiness reflects highly toxic effects, usually of an irreversible type, probably leading to cell death. Stickiness is regarded as a physiological effect exerted by PMB and PN in plants, which has been considered to affect the proteins of the chromosomes. Stickiness of the chromosomes observed in this study can be interpreted as the intercalation of the food preservative with DNA leading to entanglement of chromatin treads

Figs. 9–17. Cytological abnormalities in the root tip meristems of *A. cepa* treated with PMB and PN; 9) fragment formation (PN), 10a) early anaphase (PMB), 10b) early anaphase (PN), 11a) anaphase with lagging chromosomes (PMB), 11b) anaphase with lagging chromosomes (PN), 12a) anaphase with forward chromosomes (PMB), 12b) anaphase with forward chromosomes (PN), 13) anaphase bridges with forward chromosomes (PN), 14a) tetrapolar anaphase (PMB), 14b) tetrapolar anaphase (PN), 15) telophase with forward chromosome (PN), 16) telophase with laggard chromosome (PN), 17a) telophase with bridge (PMB), 17b) telophase with bridges (PN).

(McGill *et al*. 1974). Similar results with the chromosomal stickiness of metaphase stage were obtained after treatment with the insecticide (Amer and Farah 1985). The other metaphase abnormalities were observed as disturbed (Fig. 5) and forward metaphase (Fig. 6).

Nine anaphase abnormalities in root tips of *A. cepa* which treated with PN (Table 4) and seven anaphase abnormalities on PMB (Table 3) were observed. In the present study most common anaphase abnormalities were anaphase bridges (Fig. 7) and disturbed anaphase (Fig. 8) at both of the food preservatives. Chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosome segments. This type of abnormally was observed also in the mitosis of *Vicia faba* after treatment with insecticide (Amer and Farah 1985). Disturbed anaphase observed at all time periods and all concentrations on the root tip cells of *A. cepa*, which treated with PN. While this kind of abnormalities rarely observed at the cells of treated with PMB (Tables 3 and 4). Disturbed metaphase and anaphases may be due to the disturbance of the spindle apparatus. Fragment forma-



Figs. 18–19. Cytological abnormalities in the root tip meristems of *A. cepa* treated with PMB and PN; 18a) tetraploid c-metaphase (PMB), 18b) tetraploid c-metaphase (PN), 19a) micronucleus (PMB), 19a) micronuclei (PN).

tion was observed rarely according to the anaphase bridges (Fig. 9). The other anaphase abnormalities are early anaphase (Fig. 10), laggings (Fig. 11) and forwards (Fig.12), anaphase bridges with forward chromosomes (Fig. 13), tripolar tetrapolar and multipolar anaphases (Fig. 14). These irregular spreading of chromosomes may be attributed to the disturbance of spindle apparatus. Lagging and forward chromosomes induced. These food preservatives are acting only on spindle apparatus resulting in scattered anaphases and laggard and forward chromosomes.

Three types of telophase abnormalities were recorded. These are chromosome forwards (Fig. 15) and laggards (Fig. 16), bridges (Fig. 17) and tetraploid c-metaphase (Fig. 18).

In addition to the above-mentioned type of abnormalities, interphase cells with micronuclei (Fig. 19) were observed; such micronuclei may originate from lagging chromosomes, which were observed in the mitotic stages (Tables 3 and 4). As a rule, micronuclei formation is the result of acentric fragments or laggards being excluded from the nucleus proper during mitosis (Ma *et al*.1995).

Potassium metabisulphite and potassium nitrate has been found to be mitotoxic as the mitotic index decreases with the increase in the concentration of the food pereservative and the treatment period. In higher doses it was found to have more deleterious effects. Both food preservatives induced a number of chromosome aberrations especially PN. The most pronounced effect was the colchicines type action resulting in the formation of c-metaphase figures, which indication of spindle fibers. A number of other abnormalities are the result of this action in the spindle fiber. These are chromosome lagging and forwards, anaphase and telophase bridges and micronucleated interphase cells. Chromosomal aberrations induced by PMB and PN are similar to aberrations induced by other food preservatives (Meng and Zhang 1992, Rencüzoğulları, 2001). Chromosomal aberrations have been considered as reliable indicators of mutagenic activity by Mohandas and Grant (1972). So we can state that such chromosomal irregularities may have clastogenic effect of their inducers. For this reasons it is necessary to be careful when using PMB and PN as a food preservatives.

References

Abe, S. and Sasaki, M. 1977. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J. Natl. Cancer Inst. **58**: 1635–1643.

Akin, A. and Sümer, S. 1991. The mutagenic effect of sodium nitrite and monosodium glutamate used as food additives demonstrated by Salmonella microsome test system. Microbiol. Bul. **25**: 94–107.

Amer, S. and Farah, O. R. 1985. Cytological effects of pesticides XV Effects of the insecticide “Methamidos” on root mitosis of *Vicia faba*. Cytologia **50**: 521–526.

Anonymous 1990. The Ministry of Health of Turkey Food Codex Instructions, 20541: 2–41.

Beu, S. L., Schwarz, O. J. and Hughes, K. W. 1976. Studies of the herbicide “Paraquate” I. Effects on cell cycle and DNA synthesis in *Vicia faba*. Can. J. Genet. Cytol. **18**(1): 93–99.

Binkerd, E. F. and Kolari, O. E. 1975. The history and use of nitrate and nitrite in the curing of meat Food Cosmet. Toxicol. **13**: 655–661.

Bushra, A., Farah, M. A., Ali, M. N. and Ahmad, W. 2002. Clastogenicity of pentachlorophenol, 2,4-D and Butachlor evaluated by Allium root tip test. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, **514**: 105–113.

Deysson, G. 1968. Amitotic substances. Int. Rev. Cytol. **24**: 99–148.

El-Khodary, S., Habib, A. and Haliem, A. 1987. Effect of the Herbicide Igran on root mitosis of *Allium cepa* 12th International Congress for Statistics, Computer science, Social and Demographic Research, Cario, Egypt. 28 March–2 April. pp. 133–150.

Gömürgen, A.N. 2000. Cytological effect of the herbicide 2,4-D isooctylester 48% on root Mitosis of *Allium cepa.* Cytologia **65**: 383–388.

Hasegawa, M. M., Nishi, Y., Ohkawa, Y. and Inui, N. 1984. Effects of sorbic acid and its salts on chromosome aberrations, sister chromatid exchanges and gene mutations in cultured Chinese hamster cells. Food Chem. Toxicol. **22**: 501–507.

Ishidate, M. Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada, M. and Matsuoka, A. 1984. Primary mutagenicity screening of food additives currently used in Japan. Food. Chem. Toxicol. **22**: 623–636.

Klasterska, I., Natarajan, A. T. and Ramel, C. 1976. An interpretation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromatid aberrations. Hereditas **83**: 153.

Levan, A. 1938. The effect of colchicine on root tip mitosis in Allium. Hereditas **24**: 471–486.

Luca. D., Luca, V., Cotor, Fl. and Railenau, L. 1987. *In vivo* and *in vitro* cytogenetic damage induced by sodium nitrite. Mutation Research/Genetic Toxicology **189**(3): 333–339.

Ma, T. H., Xu, Z., Zu, C., McConnell, E. V. Rabago, Arreola, G. A. and Zhang, H. 1995. The improved Allium/Vicia root tip micronucleus assay for clastogenecity of the environmental pollutants. Environmental Mutagenesis **334**: 185–195.

Matsuoka, A., Hayashi, M. and Ishidate, M. Jr. 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*. Mutation Research/Genetic Toxicology **66**(3): 277–290.

McGill, M., Pathak, S. and Hsu, T. C. 1974. Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness. Chromosoma **47**: 157–167.

Meng, Z. and Zhang, L. 1992. Cytogenetic damage induced in human lymphocytes by sodium bisulphide. Mutation Res: **298**: 63–69.

Mohandas, T. and Grant, W. F. T. 1972. Cytogenetics effects of 2,4-D and amitrol in relation to nuclear volume and DNA content in some higher plants. Can. J. Genet. Cytol. **14**: 245–256.

Mukherjee A., Giri, A. K., Talukder, G. and Sharma, A. 1982. Sister chromatid exchanges and micronuclei formations induced by sorbic acid and acid-nitrite *in vivo* in mice. Toxicol. Let. **42**: 47–53.

Njagi, G. D. E. and Gopalan, H. N. B. 1982. Cytogenetic effects of the food Preservatives—Sodium benzoate and sodium sulphite on *Vicia faba* root meristems. Mutation Research/Genetic Toxicology **102**(3): 213–219.

Ramel, C. 1969. Genetic effect of organic compounds I. Cytological investigation of Allium roots. Hereditas **61**(1/2): 208–230.

Rencüzoğulları, E., İla, H. B., Kayraldız, A. and Topaktaş, M. 2001a. Chromosome aberrations and sister chromatid exchanges in cultured human lymphocytes treated with sodium metabisulfit a food preservative. Mutation research/Genetic Toxicology, and Environmental Mutagenesis **490**(2): 107–112

—, Kayraldız, A., İla, H. B., Çakmak, T. and Topaktaş, M. 2001b. The Cytogenetical Effects of Sodium Metabisulfite, a Food Preservative in Root Tip Cells of *Allium cepa* L. Turk J. Biol. **25**: 361–370.

Saldamli, İ. 1985. Gida katki maddeleri ve ingredientler, Hacettepe Üniversitesi Mühendislik Fakültesi Gida Mühendisliği Bölümü 197pg.

Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Osshita, M., Kabasawa, K., Iwama, S., Taniguchi, K. and Tsuda, S. 2002. The comet assay with 8 mouse organs: result with 39 currently used food additives. Genetic Toxicology and Environmental Mutagenesis **519**(1–2) : 103–119.

Schneiderman, M. H., Dewey, W. C. and Highfield, D. P 1971. Inhibition of DNA synthesis in synchronized Chinise hamster cell treated in G_1 with cycloheleximide. Exp. Cell Res. **67**: 147–155.