

# CYTOLOGICAL EFFECTS OF FUNGICIDE BAVISTIN ON THE ROOT MERISTEM OF COFFEE

R. K. Somashekar, Siddaramaiah, K. P. Srinath and P. Venkatasubbaiah

Department of Botany, Bangalore University, Jhana Bharathi Campus, Bangalore, India.

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## ABSTRACT

Bavistin, a systemic fungicide, was tested for its mitodepressive and antimitotic effect on coffee (*Coffea arabica* L.). A drastic reduction in the mitotic index occurred as the concentration and duration of treatment increased. Bavistin also influenced the frequency of mitotic phases and behaved as a potential clastogenic and turbagenic agent. The fungicide induced spindle abnormalities leading to the occurrence of C-metaphase, binucleate cells and cells with bridges, breaks, laggards and micronuclei. The recommended dosage of 0.05% was found to be quite unsafe in the present study, since this fungicide produced various forms of cell abnormalities.

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**KEY WORDS:** Bavistin. Mitotic effect. Coffee. Spindle abnormalities. Chromosome aberrations.

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## INTRODUCTION

Bavistin (methyl 1,2-benzimidazole carbamate) is a systemic fungicide used extensively for controlling a wide range of pathogens. It is also a chemical used as seed dressing against *Rhizoctonia solani* (Venkatasubbaiah and Muthappa, 1981) and as foliar spray to control leaf spot of coffee caused by *Cercospora coffeicola*. Although fungicides are helping man to boost agricultural production, fungicides as well as other pesticides have profound secondary consequences. Studies in different plant systems using various

fungicides have shown that they act as potential mutagens and carcinogens (Corden, 1969; Sathaiah et al., 1975; Zutshi and Kaul, 1975; Taromohan, 1975; Burnett et al., 1980; Pandita et al., 1981; Sathaiah and Reddy, 1981).

This study is a report on the cellular damage induced by the fungicide Bavistin on coffee.

## MATERIALS AND METHODS

Bavistin (50% WP) at the rate of 0.05, 0.1 and 0.125% was mixed with coffee seeds in a dry flask for 6, 12,

24 and 48 hr and shaken for 10 min. The seeds were then plated on standard blotters and incubated at 26°C for 12 days. The root tips were excised on the 13th day, treated with 0.03M hydroxyquinoline and fixed in acetic acid-alcohol solution. Recovery experiments were also carried out. The control was maintained without any treatment. The root tips were prepared for microscopic examination using the haematoxylin squash method (Darlington and La-Cour, 1976). About 2000 cells were screened to determine mitotic index and aberrant mitosis.

## RESULTS AND DISCUSSION

Root tips of control plants showed normal division. In treated plants, the mitotic index gradually decreased with increase in concentration and duration of Bavistin treatment (Fig. 1). Chromosomal aberrations, on the other hand, tended to increase with increase in Bavistin concentration and prolonged duration of treatment (Fig. 2). The fungicide seemed to have maximum effect on the metaphase and anaphase stages (Fig. 3). As many as 56% metaphase cells and 35% anaphase cells were found to be affected at 0.125% concentration after a 48-hr treatment. Bavistin mainly acted on the spindle apparatus in many of the abnormalities. The other abnormalities observed included gaps, breaks, fragments, stickiness, bridges and extreme fragmentation (Fig. 4).

Pandita et al. (1981) observed an increase in mitotic index in *Allium cepa* treated with Bavistin above

0.2% concentration and after 24-hr treatment. Nevertheless, they found that metaphase cells present at the time of chemical treatment completed their division normally. However, this was not true in the case of *Coffea arabica* where damage occurred during metaphase. This indicates that Bavistin affected the already formed karyokinetic spindle in addition to the achronic spindle resulting in the accumulation of cells at metaphase with decrease in number of cells at anaphase stages. Similar disturbances in the mitotic cycle, such as decrease in mitotic index and spindle abnormalities, have been observed in different plant systems after treatment with fungicides (Herichova, 1972; Ahmed and Grant, 1972; Sahu et al., 1981; Sapre and Hallale, 1981).

Bavistin inhibited spindle formation leading to the accumulation of C-metaphase cells (Fig. 4b). C-metaphase induced by Bavistin resulted in restitution of nuclear endopolyploid cells as observed by Pandita et al. (1982). Spindle disfunction also led to the unequal distribution of chromatin, scattering of chromosomes, laggards and the formation of binucleate cells (Fig. 4). Purine derivatives are known to suppress the cell plate formation which is an essential step for the formation of binucleate cells (Kihlman and Levan, 1949). The error of spindle organization led to split or multipolar spindle. Fungicides such as Dexon, Carbofuran and Bavistin are also known to induce binucleate and multinucleate conditions (Pandita et al., 1981; Sahu et al., 1981 and Panda et al., 1977).

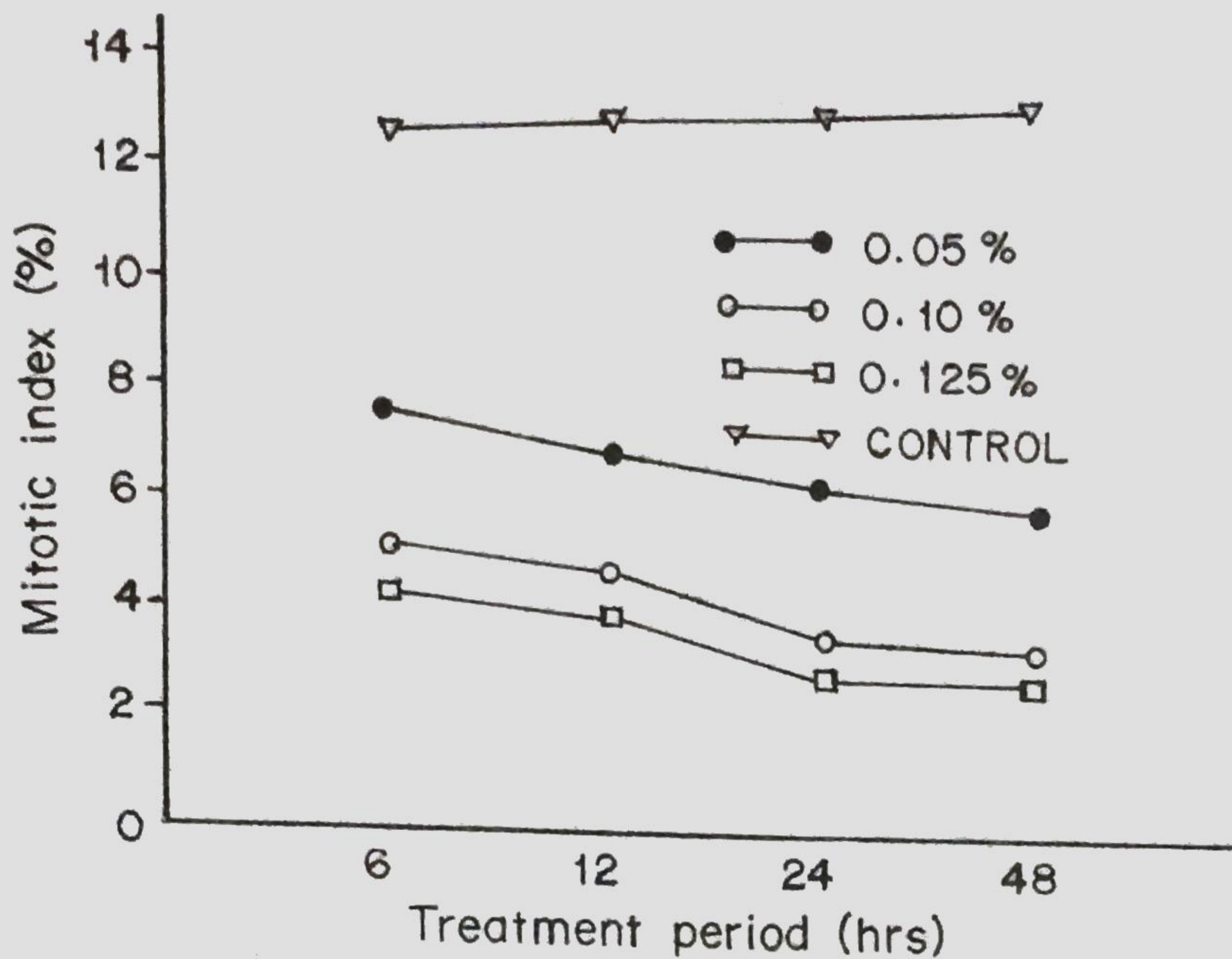


Fig. 1. Percent mitotic index observed in cells treated with various concentration of fungicide at different treatment periods.

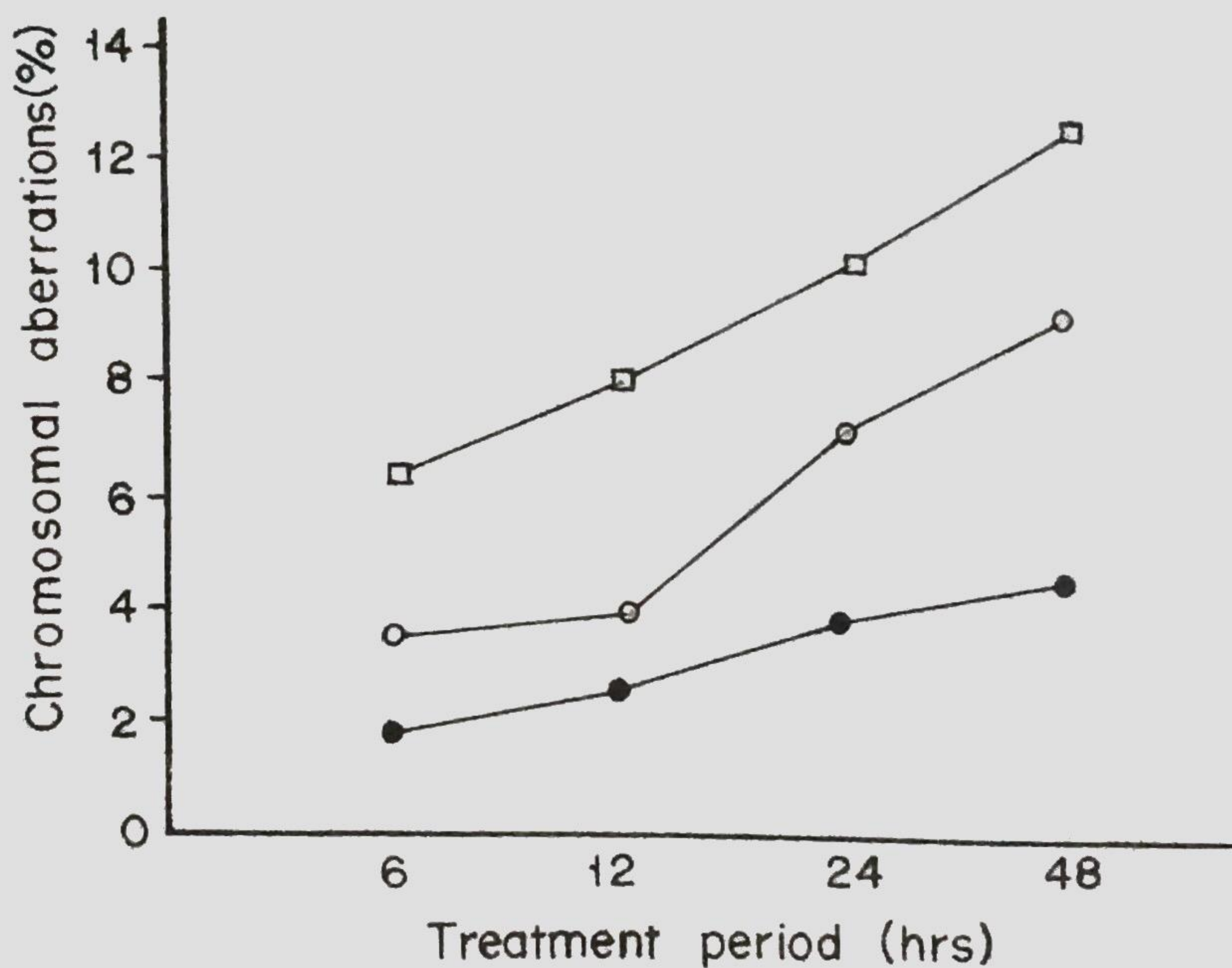


Fig. 2. Percent gross abnormalities of cells treated with various concentrations of fungicide at different periods.

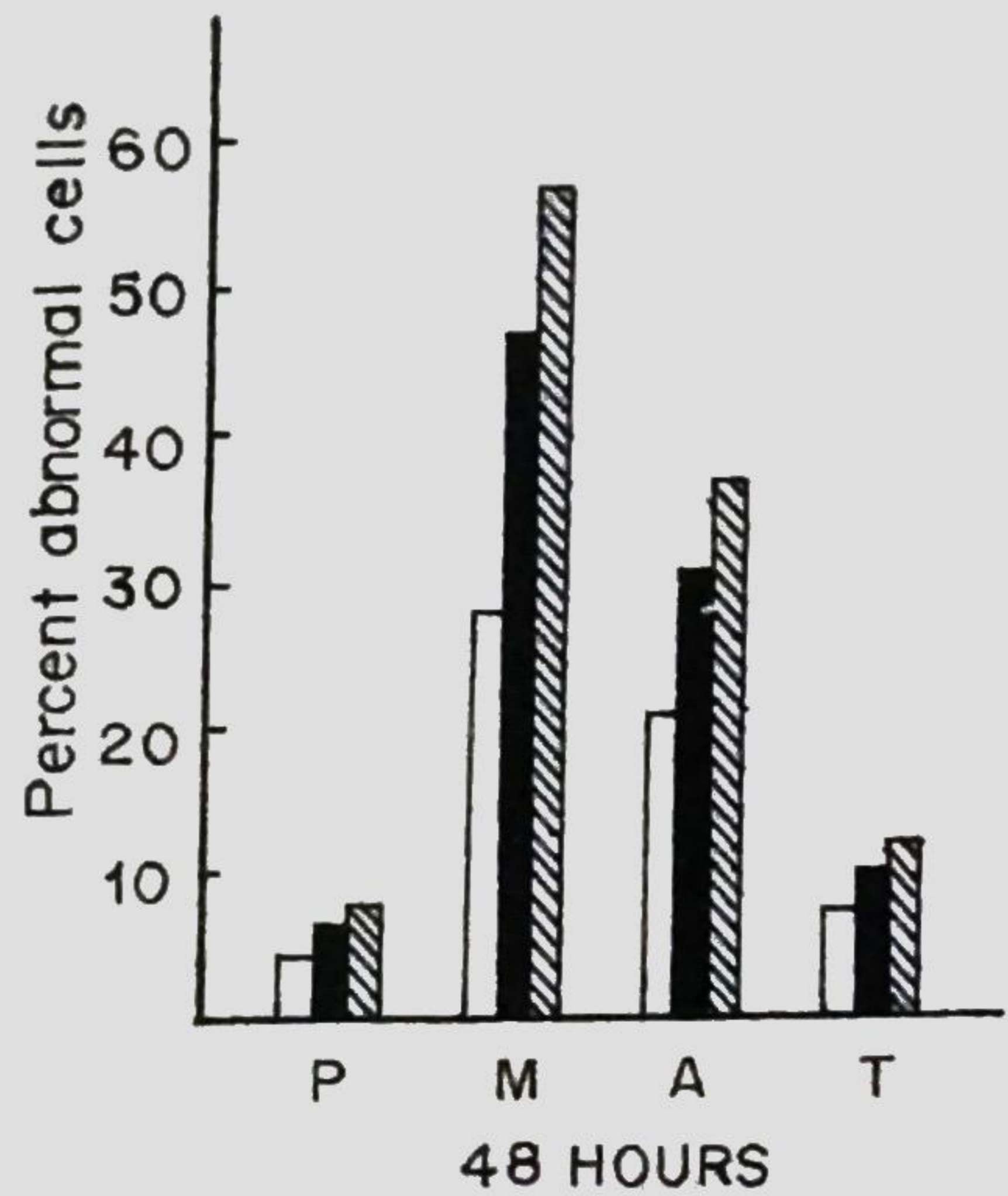
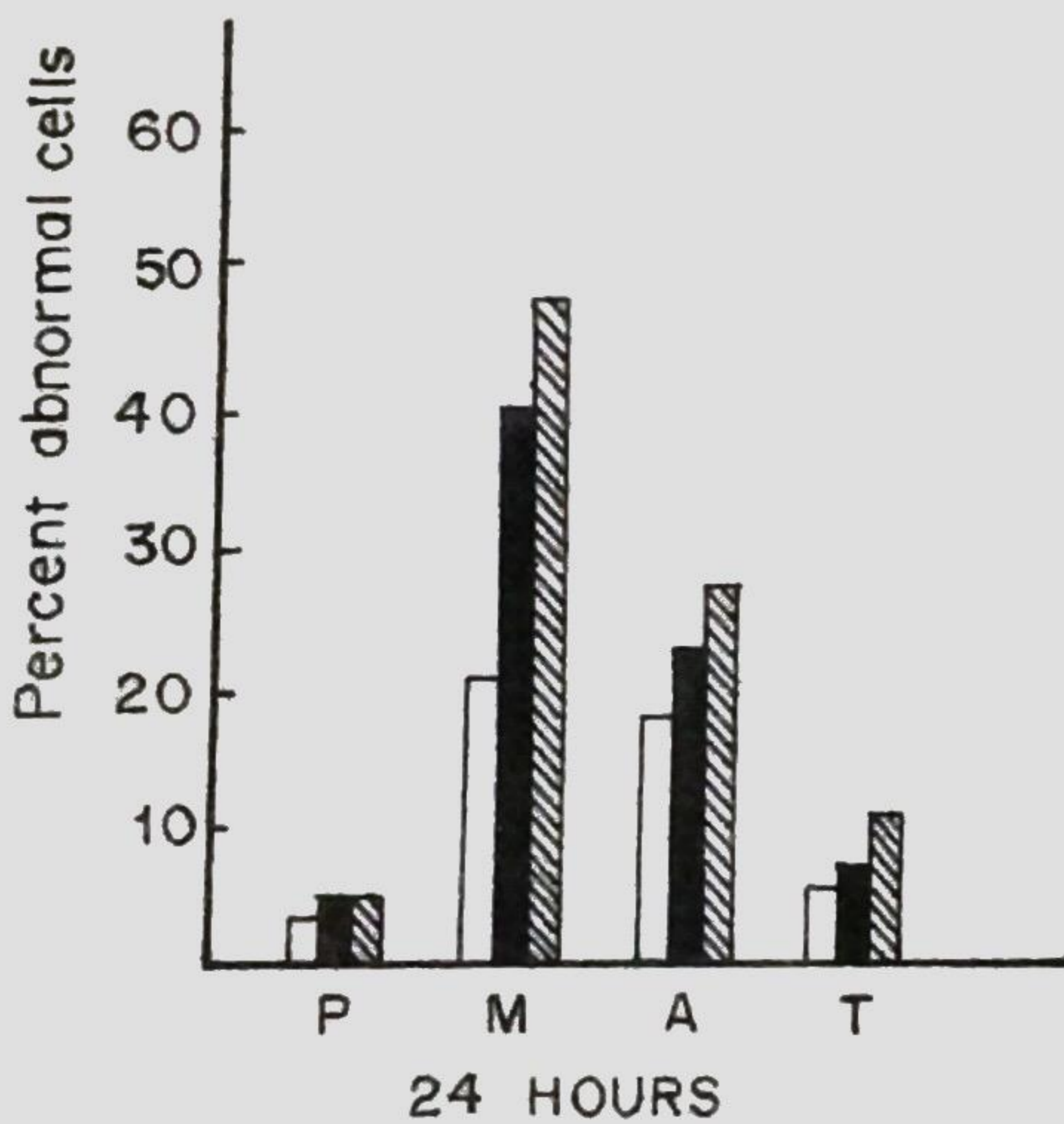
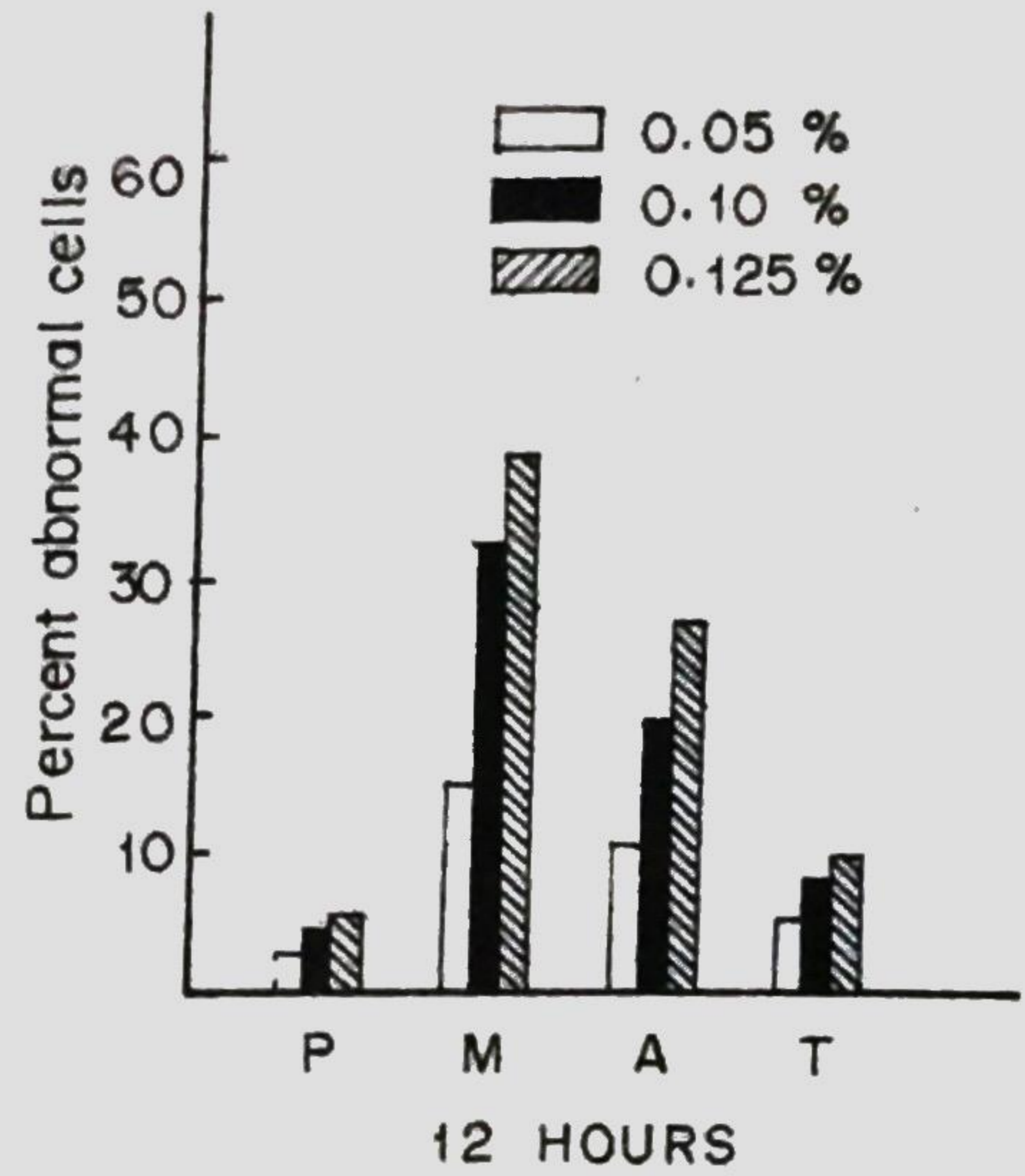
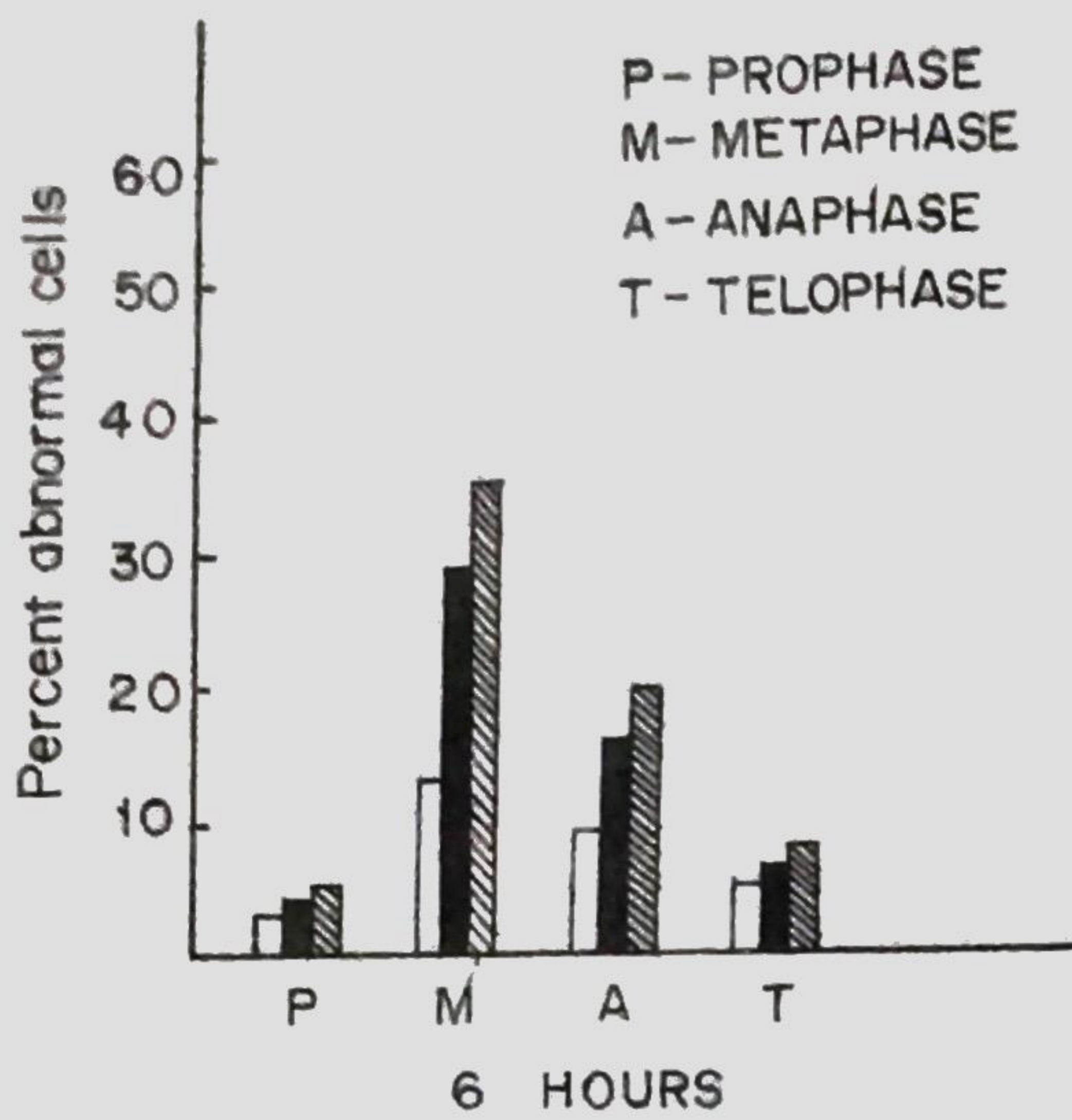


Fig. 3. Percentage of cells with abnormalities observed at different stages of mitosis — exposed to: A. 6-hr treatment, B. 12-hr. C. 24-hr and D. 48-hr treatment.

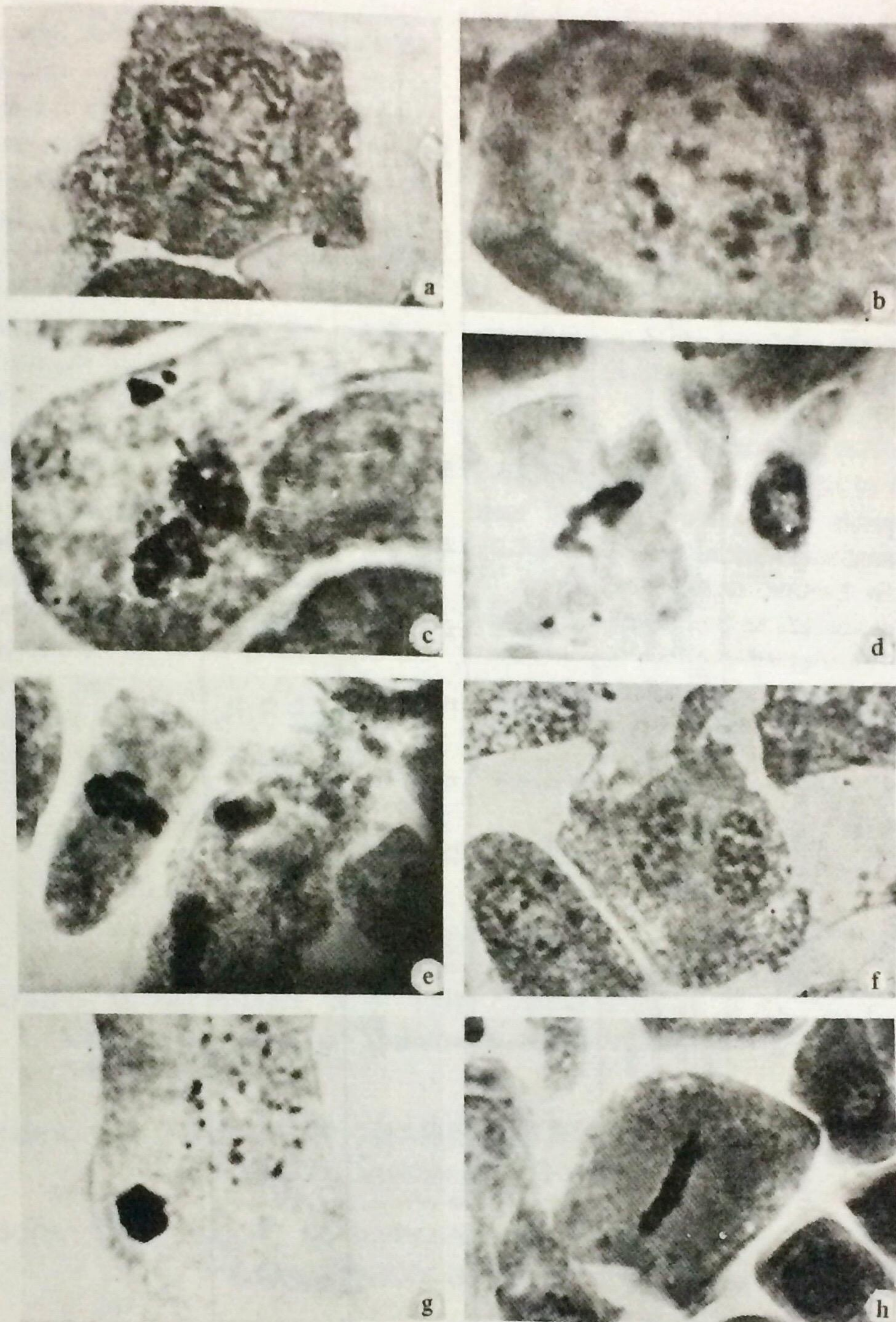


Fig. 4. Various cell abnormalities observed upon treatment with Bavistin: a) dispersion of chromosome within the cytoplasm, 1600x; b) C-metaphase, 1800x; c) telophase chromosomes sticking together with bits of chromatin material, 1700x; d) sticky irregular shaped metaphase with laggards, 450x; e) sticky metaphase with laggard. Also note telophase with chromosomes sticking together and irregular separation towards the pole, 1300x; f) binucleate cell, 1250x; g) cell showing micronuclei, 1600x; h) sticky metaphase, 1400x.

**Table 1.** Mitotic abnormalities observed in *Coffea arabica* treated for 6, 12, 24 and 48 hr with various concentrations of Bavistin.

Conc. (%) and Duration (hr)	No. of Cells Observed	Structural Abnormalities			% Aber- rant Cells	Spindle Abnormalities			% Aber- rant Cells	Others	% Total Aber- rant Cells	Average				
		Gaps	Breaks	Frag- ments		Extreme fragmen- tation	C-meta- phase	Poly- ploids					Lag- gards	Binu- cleate Cells	Group- ings	Stick- iness
0.05 + 6	1900	0	0	3	2	9	0	6	3	0.94	6	6	0	6.63	1.83	
0.05 + 12	2100	0	0	5	2	13	0	7	7	1.28	9	11	0	0.95	2.56	3.20
0.05 + 24	2175	1	1	8	3	18	1	7	13	1.79	14	17	1	1.47	3.85	
0.05 + 48	2301	1	2	10	3	23	1	9	16	2.12	17	23	1	1.78	4.59	
0.1 + 6	2482	0	0	9	2	17	0	7	15	1.49	16	21	0	1.49	3.42	
0.1 + 12	2334	1	3	13	3	21	1	10	17	2.09	19	27	1	2.01	4.95	6.21
0.1 + 24	2077	2	2	16	5	28	2	13	21	3.08	23	36	3	2.98	7.26	
0.1 + 48	9848	2	3	20	6	33	2	16	26	3.95	26	41	5	3.69	9.23	
0.125 + 6	2064	0	2	13	5	29	0	15	20	3.10	21	26	2	2.37	6.43	
0.125 + 12	2130	1	3	17	7	33	2	17	26	3.66	29	33	4	3.09	8.06	
0.125 + 24	1968	2	5	21	8	36	2	19	31	4.47	31	41	6	3.96	10.21	9.30
0.125 + 48	1930	3	7	26	9	43	3	23	37	5.49	36	47	8	4.71	12.53	

Bavistin induced structural abnormalities leading to the accumulation of cells with gaps, breaks, fragments and extreme fragmentation. However, their occurrence was not significant (Table 1). Stickiness of the chromatids was another common feature observed. A similar effect of some pesticides including fungicides in different plant systems has been recorded (Sathaiah and Reddy, 1981; Sahu et al., 1981; Prasad and Pramer, 1968; Amer and Ali, 1969; Tomkins and Grant, 1972).

Bridges formed by chromatin fibers which join sister chromatids at metaphase and hold the chromatids together until late anaphase and sometimes even up to telophase, were observed less frequently. A similar observation was noted by Ahmed and Grant (1972) in *Tradescantia* and

*Vicia faba* after treatments with mercurial fungicides. Spindle abnormalities, breaks and laggards contributed to the formation of cells with micronuclei (Fig. 4g).

The recommended dosage of 0.025 to 0.05% Bavistin for controlling collar rot of coffee has been found to be unsafe in the present study, since this concentration of the fungicide induced various kinds of abnormalities (Venkatasubbaiah and Muthappa, 1981). Hence, there is a necessity for further evaluation of the safe dosage to be used for controlling the disease. Furthermore, a detailed investigation of other crop plants treated with various concentrations of Bavistin will throw more light on the nature of cellular damage induced and its implication to environmental pollution.

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