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ESTIMATION OF GENOTOXIC POTENTIAL OF CARBENDAZIM IN FENUGREEK

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ABSTRACT. Because the studies on pesticide effects evidenced a diversity of chromosomal aberrations, in relation to the analysed class of compounds, we considered necessary to test the influence of carbendazim fungicide on genetic material of fenugreek (*Trigonella foenum graecum* L.), which can constitute, as other plant species, monitoring systems in the evaluation of possible genetic risk of the pesticide use. Mitotic index, the frequency of cell division phases, the types and incidence of ana-telophase aberrations, and the types of metaphase abnormalities were studied. The mitotic index was lower in all carbendazim treated variants; the most numerous ana-telophase aberrations and metaphase abnormalities were noted in 0.5% and 1.0% carbendazim treated variants. The effect of carbendazim on height growth of plantlets in early ontogenetic phases was also investigated – an inverse relation between concentration and plantlets' height was registered.

Keywords: carbendazim, chromosome aberrations, mitotic index, *Trigonella foenum graecum* L.

INTRODUCTION

The studies on genetic effects of pesticides evidenced a diversity of chromosomal aberrations, in relation to the respective class of substances. The first such observations, published in 1931 by KOSTOFF (cited by Grant, 1978), refer to the nicotine sulphate effect on tobacco plants, seed number and on meiotic irregularities, considered as cause of partial plant sterility. Carbendazim is a fungicide of major concern due to its suspected hormone disrupting effects. It plays a very important role in plant disease control, being also used in post-harvest food storage, and as a seed pre-planting treatment (Quian, 1996; Hicks, 1998).

Plants are valuable genetic assay systems for screening and monitoring environmental pollutants (Singh et al., 2008), they constituting indicators of cytotoxic, cytogenetic, and mutagen effects of chemical polluters by the detection of lesions induced at genetic level by the respective compounds. *Trigonella foenum graecum* L. (Fabaceae) can represent a monitoring system in the evaluation of possible genetic risk of carbendazim and other pesticides, because it is a species of great pharmaceutical, industrial, and culinary interest and its chromosome complement has a relatively reduced number of chromosomes of large sizes (Căpraru et al., 2006). The trigonelline alkaloid has shown potential for use in cancer therapy (http://www.pfaf.org/database/plants.php?Trigonella+foenum-graecum).

The aim of present study was to determine the amplitude of carbendazim induced effects at the level of fenugreek genetic material and on mitosis intensity in root meristems of this plant, after carbendazim treatment.

MATERIALS AND METHODS

Carbendazim ($C_9H_9N_3O_2$) (methyl 2-benzimidazolecarbamate) (Fig. 1) is a broad spectrum benzimidazole carbamate fungicide with systemic activity (ACP, 1992), with molecular weight=191.187 g/mol. We used a preparation produced in Romania, by Oltchim S.A. Râmnicu Vâlcea, furnished by Agricultural Station from Podu Iloaiei Iaşi. Four carbendazim concentrations were tested: 0.1%, 0.2%, 0.5%, and 1.0%. The solutions were applied for 3 hours to fenugreek seeds.

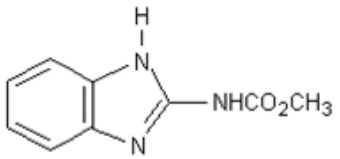

Fig. 1 Carbendazim structure

The fixation of root tips (10-15 mm) was done for about 24 hours in ethylic alcohol/acetic acid, 3:1 mixture, at room temperature. After 10 min of hydrolysis in 50% HCl, the plant material was stained in modified charbol fuchsin solution (Gamborg and Wetter, 1975). For each variant, five slides were prepared according squash method, in 45% glacial acetic acid, and 10 microscopic fields were microscopically analyzed on every slide. A Nikon Eclipse 600 light microscope was used for this analysis. Photos were taken with a Nikon Cool Pix 950 digital camera, at 1600x1200 dpi resolution.

The different phases of mitosis were counted to calculate the mitotic index (MI) and phase indices, respectively prophase index (PI%), metaphase index

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(MI%), anaphase index (AI%), and telophase index (TI%).

Mitotic Index (MI) = TDC x100/TC
PI% = prophase cells x 100/TDC
MI% = metaphase cells x 100/TDC
AI% = anaphase cells x 100/TDC
TC% = telophase cells x 100/TDC, where:
TDC = total dividing cells, and TC = total analyzed (dividing and non-dividing) cells

Also, the percentage of aberrations and abnormalities in metaphase and ana-telophase was calculated:

$T_{A\text{-}Tabr} = T_A\text{-}T_{abr}/TDC$
$T_{M\ abn} = M_{abn}/TDC$, where:

$T_{A\text{-}T\ abr}$ = total percentage of ana-telophase aberrations
$T_{M\ abn}$ = total percentage of metaphase abnormalities
TDC = total dividing cells

RESULTS AND DISCUSSIONS

Effects of carbendazim on plantlets length

Stimulatory effect of carbendazim on 10 days old fenugreek plantlets is low at 0.1% concentration (x±Sx=47.30±1.98, comparatively to 46.70±2.38, for control). The other variants have smaller values than control, an inverse relation existing between concentration and length of plantlets obtained from treated seeds (at higher concentrations, the average length is more reduced) (Table 1, Fig. 2).

Table 1

Influence of carbendazim on height (mm) of 10 days old fenugreek plantlets

Variant	Height (x±Sx)
Control	46.70±2.38
0.1%	47.30±1.98
0.2%	45.17±1.75
0.5%	43.10±1.91
1.0%	40.12±1.78

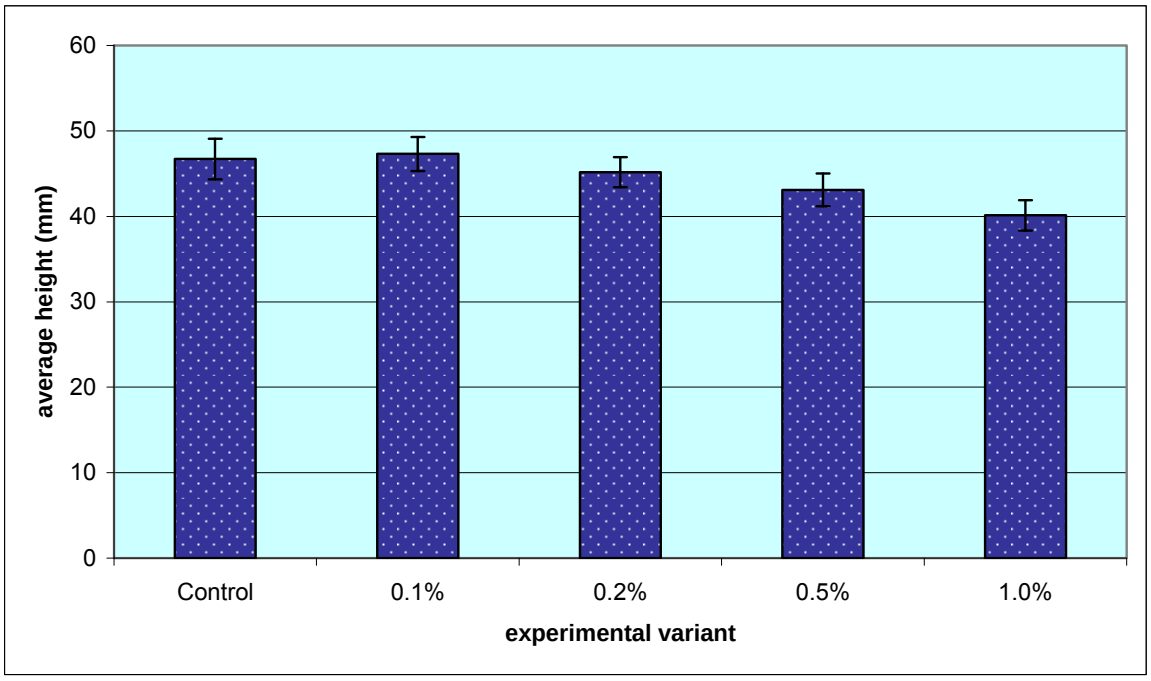

Fig. 2 Graphic representation of carbendazim effect on height of 10 days old fenugreek plantlets, depending on pesticide concentration

Evolution of mitotic index after the treatment with carbendazim

Data of the present study revealed that all carbendazim concentrations diminished the mitotic index, although the decline of this division parameter was not very high (Table 2, Fig. 3). Mitotic index registered smaller values especially for 0.5% and 1.0% concentrations where the inhibitory effect was marked (the values are 1.3 – 1.4 times higher than control, namely 13.7±0.74% and 13.1±1.1%, comparatively to 18.4±1.09% for control). An indirect relation was noted between concentration increase and value of mitotic

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 2, 2010, pp. 39-44

index. Carbendazim may contain cytotoxic compounds causing the cell death, resulting in the decline of mitotic index. The reduction of this parameter may be either due to the inhibition of DNA synthesis at S-phase (Sudhakar et al., 2001), to the blocking of G1 suppressing DNA synthesis (Schneidermann, 1971) or to the blocking in G2 preventing the cells from entering mitosis (El-Ghamery et al., 2000).

Table 2 – part 1

Influence of carbendazim on cytogenetic parameters of fenugreek

Nr.	Variant	Total cells*	Dividing cells*	Mitotic index* (%)	Indices of mitotic phases* PI%	MI%	AI%	TI%
1.	Control	1238.4±79.6	229.2±22.6	18.4±1.09	71.6±2.1	10.5±0.66	6.0±1.09	12.1±1.5
2.	0.1%	944.6±135	159.8±25.4	16.8±0.7	55.8±3.8	16.7±0.9	7.0±0.6	20.4±2.9
3.	0.2%	1086.4±176	153.6±8.5	15.7±1.4	51.2±1.7	15.7±2.0	9.8±0.8	23.0±2.4
4.	0.5%	978.8±17.7	134.6±7.5	13.7±0.74	55.8±3.9	16.2±1.1	12.1±2.2	15.7±1.8
5.	1%	1093.6±90.4	140.6±4.7	13.1±1.1	49.06±1.7	18.52±1.9	10.21±0.5	22.18±1.9

Table 2 - part 2

Influence of carbendazim on cytogenetic parameters of fenugreek

Nr.	$A\text{-}T_{abr}$ (%) Total	types					M_{abn} (%) Total	types		
		bridges	expulsed	laggards	multipolar	complex		C met	exp	sticky
1.	3.49	1.3	0.08	1.83	0.17	0.08	0.87	0.00	0.20	0.67
2.	4.38	1.75	1.00	0.62	0.37	0.62	1.00	0.13	0.87	0.00
3.	6.38	3.90	0.26	1.30	0.13	0.78	1.04	0.52	0.52	0.00
4.	8.61	1.33	0.44	2.82	0.00	2.20	8.46	0.00	3.11	4.30
5.	8.10	2.04	0.70	2.80	0.99	0.99	5.12	0.71	0.85	3.40

Relative to the incidence of division phases, both in control and in treated variants, the prophase index registered the highest level, generally followed by telophase, metaphase and anaphase indices. The average values of the prophase index were smaller in all carbendazim treated variants, comparatively to control, but the other three indices presented average values higher than those of control.

Evaluation of the number and types of chromosomal aberrations induced by carbendazim

Various chromosomal aberrations were observed in carbendazim treated variants (Table 2, Fig. 4). The ana-telophases with chromosome aberrations registered in carbendazim variants surpassed the control: 0.2% carbendazim concentration induced 1.8 times more ana-telophase aberrations, while 0.5% and 1.0% carbendazim treated variants showed 2.4, respectively 2.3 times more aberrations than untreated control. The most encountered ana-telophase aberrations were the bridges and the laggards but also other types of ana-telophase abnormalities were noted: multipolar formations, expulsed chromosomes, unusual arrangements and disorganized chromosome formations. The most numerous bridges are in 0.2% variant, but more bridges than control appeared too in the other three carbendazim variants. An important number of lagging chromosomes were noted for 0.5% and 1.0% carbendazim treated variants. The 0.5% variant presented also an enough increased number of complex aberrations (multipolar ana-telophases with bridges, ana-telophases with lagging and expulsed chromosomes etc.) – 2.20%, comparatively to 0.08% for control. The significant incidence of bridges and lagging chromosomes can be an evidence of the alteration at the level of chromosome attachment and sliding on spindle fibres. The presence of lagging chromosomes may be attributed to the delayed terminalization, stickiness of chromosome ends or failure of chromosomal movement (Permjit and Grover, 1985), while the chromosomal bridges observed in the present study may be the result of dicentric chromosomes formation due to the breaking and reunion of chromosomes (Tomkins and Grants, 1972). The two maximum tested concentrations of carbendazim induced lysis zones of chromatin material.

Metaphase abnormalities

The effect of carbendazim is also visible on the number of abnormal metaphases (with expulsed chromosomes, with unusual configurations of chromosome in metaphase plate, C-like metaphases) (Table 2, Fig. 5). In human and mammals, carbendazim induces mitotic spindle abnormalities and reduces the metaphase inter-centromere distance of sister chromatids, indicating reduction of tension on kinetochores and determining the metaphase arrest (Yenjerla et al., 2009).

In our experiment, all treated variants registered values higher than control, but in 0.1% and 0.2% carbendazim treated variants the increase was non-significant. Unlike these, 0.5% concentration induced almost 10 times more metaphase abnormalities, while

the frequency of abnormal metaphases in 1% treated variant was approximately 6 times higher than control.

The presence of C-metaphases confirms the literature data on colchicine-like effect of carbamate pesticides. C-mitoses are the result of division spindle inactivation, followed by random scattering of chromosomes in cell. Delayed centromere division can induce chromosome configurations of C-type (Storey et al., 1968). Because of their effectiveness as C-mitotic chemicals, they are used to induce artificial polyploidy (Levan, 1968). For example, with carbamate propham a 16x ploidy level was obtained.

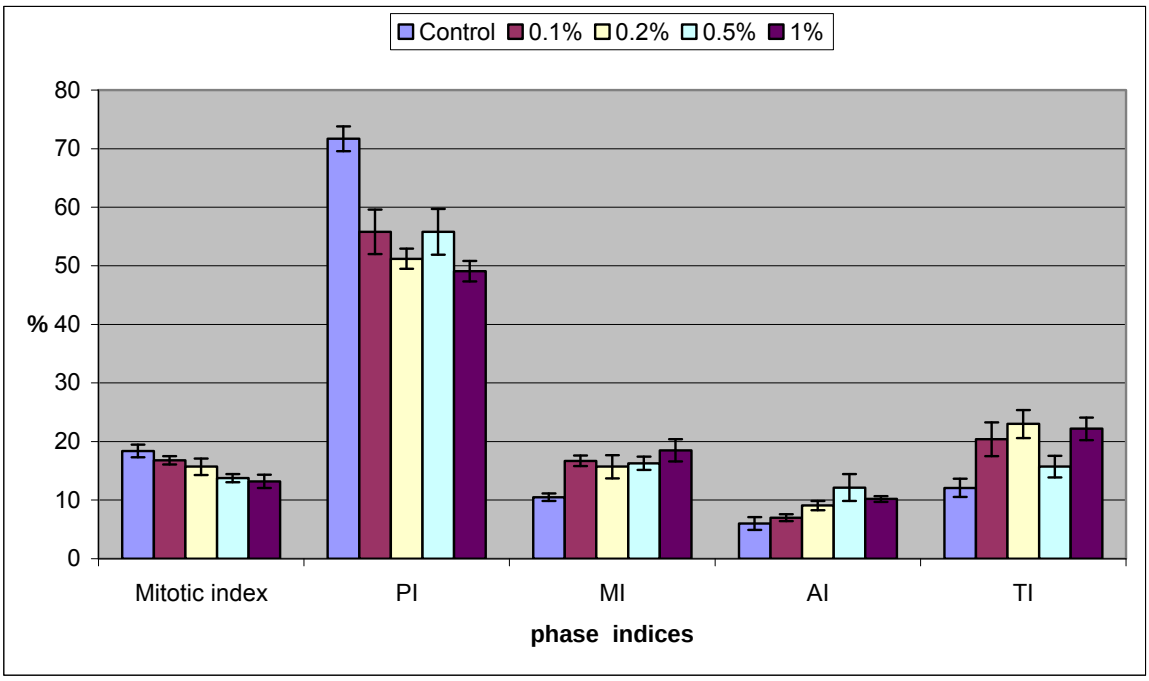

Fig. 3 Graphic representation of the evolution of mitotic index and phase indices in fenugreek root tip meristems, after carbendazim treatment, depending on pesticide concentration

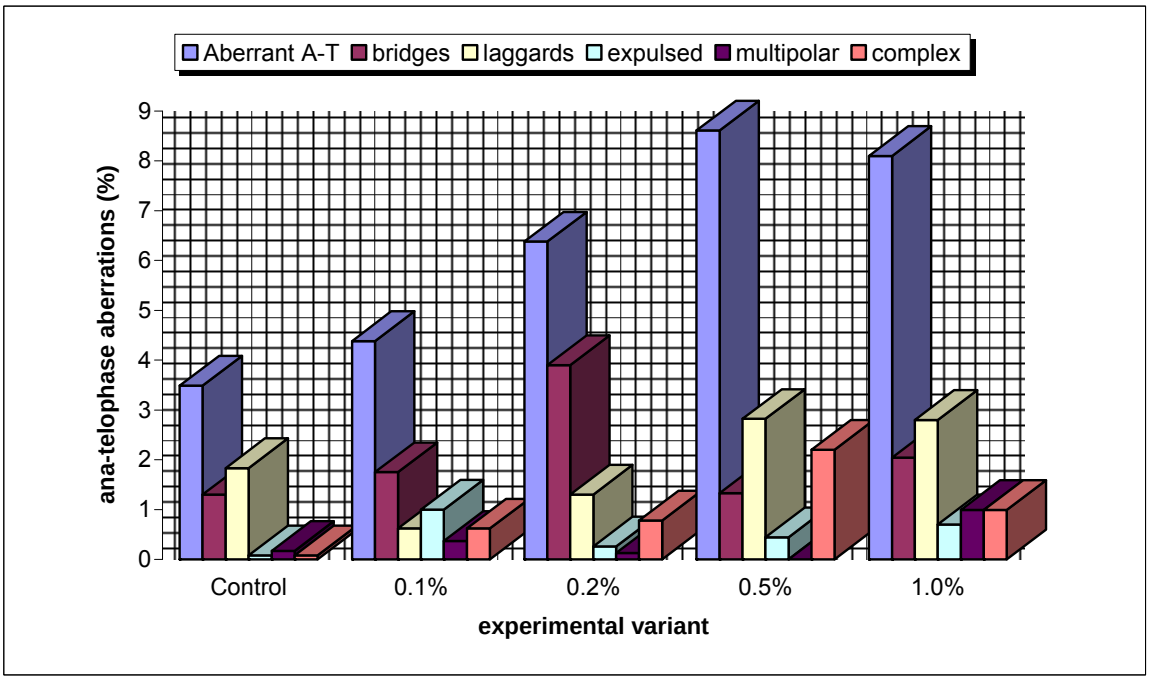

Fig. 4 Graphic representation of frequency of aberrations in ana-telophases of fenugreek root tip meristems, after carbendazim treatment, depending on pesticide concentration

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 20, issue 2, 2010, pp. 39-44

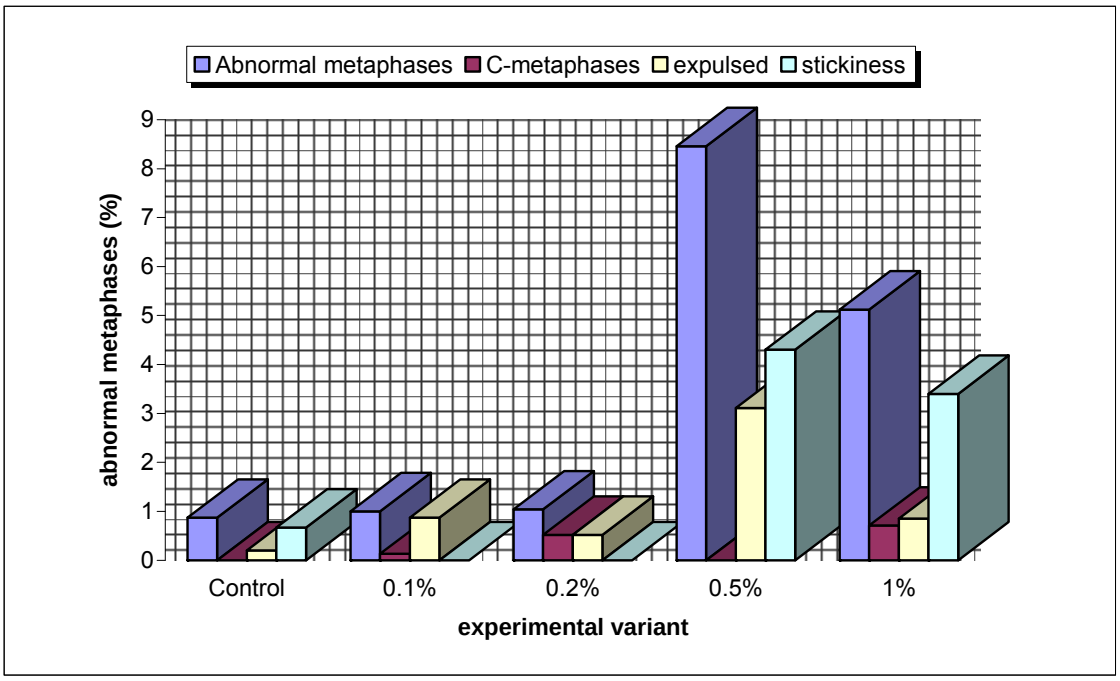

Fig. 5 Graphic representation of frequency of metaphase abnormalities in fenugreek root tip meristems, after carbendazim treatment, depending on pesticide concentration

The 0.5% variant showed significant increases of the frequency of expulsed chromosome from equatorial plate (3.11%, comparatively to control – 0.20%) and stickiness phenomenon – 4.30% of metaphases presented sticky chromosomes, comparatively to 0.67% in control. The stickiness was also important in 1.0% carbendazim treated variant (3.40%). Chromosome stickiness, a very frequent chromosomal abnormality observed in somatic cells of *Trigonella foenum graecum* L., is presumably due to intermingling of chromatin fibres, fact leading to subchromatid connections between chromosomes (Klasterska et al., 1976).

The results of this study are generally in accordance with the data published for other species concerning the genotoxic effects of carbendazim (Singh et al., 2008 in *Hordeum vulgare* L., for example). As the chromosome aberrations are indicators of genotoxicity, it can be concluded that carbendazim have, by the high number of induced aberrations, genotoxic effects in root meristem cells of *Trigonella foenum graecum* L.

CONCLUSIONS

Carbendazim had a slight inhibitory effect on length growth of plantlets. Mitotic index registered smaller values especially for 0.5% and 1.0% concentrations where the inhibitory effect was marked. The most numerous ana-telophase aberrations were noted in 0.5% and 1.0% carbendazim treated variant with more than 2 times higher levels than control. The most frequent types of aberrations were the bridges and the lagging chromosomes. The abnormal metaphases were at the highest percentage in 0.5% carbendazim treated variant, followed by 1.0% concentration.

REFERENCES

Advisory Committee on Pesticides, Evaluation on carbendazim, evaluation of fully approved or provisionally approved products, No. 58. Ministry of Agriculture, Fisheries and Food, 1992.

Căpraru G, Băra IC, Băra II, Cîmpeanu M, Maxim VE, Numerical and morphostructural characteristics of *Trigonella foenum graecum* (2n=16) mitotic chromosomes. Proceedings of 4th Conference on Medicinal and Aromatic Plants of South-East European Countries, Iaşi, Romania, 28th – 31 May 2006, Alma Mater Publ. House, 53–57, 2006.

El-Ghamery AA, El-Nahas AI, Mansour MM, The action of atrazine herbicide as an inhibitor of cell division on chromosomes and nucleic acid content in root meristems of Allium cepa and Vicia faba. Cytologia, 65, 277–287, 2000.

Gamborg OL, Wetter LR, Plant tissue culture methods. National Research Council, Saskatoon, Canada, 1975.

Grant WF, Chromosome aberrations in plants as a monitoring system. Environmental Health Perspectives, 27, 37–43, 1978.

Hicks B, Generic pesticides – the products and markets. Agrow reports, PJB publications, 1998.

Klasterska I, Natrajan AT, Ramel C, An interpretation of the origin of subchromatid aberrations and

chromosome aberrations and chromosome stickiness as a category of chromatid aberrations. Hereditas, 83:153–162, 1976.
Levan A, The effect of colchicine on root mitoses in Allium. Hereditas, 24, 147, 1968.
Permjit K, Grover IS, Cytological effects of some organophosphorus pesticides. Meiotic effects. Cytologia, 50, 199–211, 1985.
Quian Y, Transformation and expression of the resistance gene to carbendazim into Trichoderma harzianum. Resistant Pest Management, 6, 8–12, 1996.
Schneidermann MH, Dewey WC, Highfield DP, Inhibition of DNA synthesis in synchronized Chinese hamster cell treated in G1 with cycloheximide. Experimental Cell Research 67:147–155, 1971.
Singh P, Srivastava AK, Singh AK, Cell cycle stage specific application of cypermethrin and carbendazim to assess the genotoxicity in somatic cells of Hordeum vulgare L. Bulletin of Environmental Contamination and toxicology (Bull Environ Contam Toxicol), 81, 258–261, 2008.
Storey WB, Jordan LS, Mann JD, Carbamate herbicides – new tools for cytological studies. California Agriculture, 22(8), 12–13, 1968.
Sudhakar R, Ninge Gowda KN, Venu G, Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa. Cytologia, 66, 235–239, 2001.
Tomkins DJ, Grants WF, Comparative cytological effects of pesticides: menazon metrobromuron and tetrachloroisophthalonitrile in Hordeum vulgare and Tradescantia. Canadian Journal of Genetics and Cytology, 14, 245–256, 1972.
Yenjerla M, Cox C, Wilson L, Jordan MA, Carbendazim inhibits cancer cell proliferation by suppressing microtubule dynamics, The Journal of Pharmacology and Experimental Therapeutics (JPET), 328(2), 390-398, 2009.
http://www.pfaf.org/database/plants.php?trigonella+foenum-graecum