http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (13): 2508-2511, 2006
ISSN 1028-8880

Cytogenetic Effects of Some Fungicides on Barley Root Tip Meristem Cells

Tülay Aşkin Çelik
Department of Biology, Faculty of Art and Science,
Adnan Menderes University, 09010 Aydın Turkey

Abstract: Two fungicides Derosal 50 WP and Korsikol 18 Dust those widely used in agriculture for seed protection were applied to barley seeds (*Hordeum vulgare* L) in concentrations of 100, 200 and 300 ppm in intervals of 6, 12 and 24 h. In control experiments, distilled water was used instead of fungicides. Effects of Korsikol and Derosal on structure and behaviour of chromosomes were investigated using mitosis analysis on root tip cells of barley seeds which were germinated after the chemical application. In addition, modifying effects of these two fungicides on mitotic index were studied. Cytological findings which were obtained from these experiments showed that the fungicides affect cell division and chromosome structure depending on dose which were used in time intervals.

Key words: Fungicide, Barley root tip cells, cytogenetic effect, chromosome aberrations, mitotic index

INTRODUCTION

Pesticides have some hazardous effects in addition to their benefits. Their undesirable residues in water, food and in environment may cause health problems. Pesticides may also be effective to chance plant genetic system as a result of their mutagenicity (Aokin and Sumer, 1996; Badr, 1988; Bilalolu, 1984; Bilalolu and Ozorgucu, 1988).

A number of studies aiming to explain and to understand effects of fungicides in plant are present in literature. Rayburn *et al*. (1993) stated out that amount of nuclear DNA is decreased by the fungicide, Captan. This fungicide has carcinogenic, mutagenic and teratogenic effects on many organisms. Its highest effects on nucleic acids are due to DNA synthesis inhibition and activity decrease of DNA-polymerase B.

Pusztai (1993) used two fungicides in his experiments, Vitavax and Topsin-Methyl 70 WP. He examined the differences in chlorophyll mutation frequency and cytogenetic effects of fungicides on barley. He found deviations on chromatids and chromosomes types after metaphase observations on barley seed root tip cells. An increase which depends on application time intervals was also found. Similar results were found by Samashekar and Gowda (1984) in *Allium cepa* root tip cells using Vitavax that can cause deviations in mitotic index and mitosis. They stated out that, the most important effects were mito-depressive behaviour in the lowest concentration and total disappearance of mitosis by prophase inhibition in higher concentrations (mitostatic effects). Vitavax caused chromosome breakes, gaps, chromosome and chromatid changes, lagging chromosomes, chromosome bridges and as a result of disappearance of spindle fiber disorders. As a result of inhibited cytoplasmic division, fragmented nuclei and polynucleotide cells were also observed.

It is stated that, two different fungicide (Nimrod and Ribigon-4) causing chromosomal deviations on the root tip cells of *Vicia faba,* had genotoxic effects (Shahin and El Amoodi, 1991). Different application times and different concentrations were used for each fungicides. Chromosomal deviations and numerical chromosomal deviations were found. It was also reported that cells with 2 nucleus, c-metaphases, sticky chromosomes, polyploid cells and lagging chromosomes were formed depending on drastical changes in number of chromosomes. Depending on the increase of application time, there was a remarkable increase in deviations by comprising with control group.

Hemavathy and Krisnamurty (1988) examined cytogenic effects of Cuman L (containing dithiocarbamate) on albino male mice's bone marrow erythrocytes. They found a remarkable increase in univalent and polyploidy, an increase in micronucleus frequency. In their experiment, no translocations in both control and treated groups were observed.

Decreasing effects of fungicides on barley meristem root tip cells in terms of frequency were also reported (Sharma *et al*., 1983). Researchers explained the decrease of chiasma frequency by fungicide use. In another report, chromosomal breakes, lagging chromosomes and micronuclei in *Allium cepa* L. root tip meristematic cells had been shown (Samashekar and Gowda, 1984).

Derosal 50 WP and Korsikol 18 Dust are widely used seed pesticides having fungicide characteristics. In this study, we aimed to examine the effects of these pesticides on mitotic activity and chromosomes in barley root tip meristem cells.

MATERIALS AND METHODS

Barley seeds (*Hordeum vulgare* L.) were purchased from Eskişehir Agricultural Research Institute. These seeds were treated with Korsikol 18 Dust (pentachloronitrobenzene) and Derosal 50 Wp (carbendazim-methylbenzimidazole-2yl-carbamate),having fungicide characteristics. 100, 200 and 300 ppm concentrations of these substances were prepared with distilled water. Distilled water was also used as control. These concentrations were selected according to their uses in agriculture.

Fifity barley seeds were selected for each applications and they were taken into fungicide solutions for 6, 12 and 24 h. At the end of the application period, seeds were washed with tap water for 5-10 min and taken on to the dryer paper. Barley seeds treated with fungicide were transferred to the petri dishes having dryer papers on both inner sides under optimum temperature (18-20°C) for germination. Seed root tips in 1.5-2 cm length were fixed using farmer fixative (3:1 ethyl alcohol: glacial acetic acid). Root tips were squashed in aceto-orcein and examined microscopically for Mitotic Index (MI) and chromosomal aberrations. Chromosomal aberrations were determined by scoring cells with fragments, non-separated chromosomes, mis-polarized chromosomes in randomly picked 3 zones per slide. For each group of concentrations and control, the mean values were calculated. In order to determinate the significance among the means, Independent Samples t-test was applied ($p<0.05$).

RESULTS

Korsikol 18 Dust and Derosal 50 WP, which were applied to the barley seeds, depending on concentration and application time were found to have effects on cell division.

In the Derosal applied seeds, no significant changes in the frequency of dividing cells were occurred (Table 1). Mitotic index values in Table 1, also reflect the same situation. Korsikol 18 Dust application also effected dividing cell ratio depending on concentration and application time. While dividing cell ratio was decreased with concentrations of the fungicide between 100 and 200 ppm, it was increased in 300 ppm (Table 2). Interestingly, application of Derosal decreased chromosomal breakes in comparison of the control. The

Table 1: Effect of Derosal treatment on the mitotic index and chromosome aberrations in the root tip meristem cells in *Hordeum vulgare* L.

Concentrations	Total cells	Dividing Cells	Mitotic İndex (MI) (%)±SD	Fragment	Bridge	Stickiness	Polar deviation	Total chromosome aberrations (%)±SD
Control	13.050	1.831	0.140±0.003	134	30	97	9	14.774±0.031
D-1-1	13.261	1.734	0.127±0.003	136	39	142	45	20.880±0.039
D-1-2	13.958	1.750	0.126±0.003	177	42	112	38	21.088±0.037
D-1-3	13.690	1.771	0.129±0.002	121	35	115	38	17.446±0.036
D-2-1	13.200	1.753	0.135±0.003	126	20	133	63	19.509±0.032
D-2-2	12.490	1.801	0.143±0.002	124	23	43	60	13.881±0.044
D-2-3	12.530	1.762	0.141±0.003	69	16	46	60	10.840±0.039
D-3-1	12.633	1.790	0.143±0.003	94	9	53	68	12.598±0.056
D-3-2	10.594	1.396	0.130±0.003	78	11	61	70	15.758±0.035
D-3-3	12.468	1.768	0.141±0.003	152	28	140	84	23.069±0.038

D-1-1: Derosal-100 ppm-6 h, D-1-2: Derosal-100 ppm-12 h, D-1-3: Derosal-100 ppm-24 h, D-2-1: Derosal-200 ppm-6 h, D-2-2: Derosal-200 ppm-12 h, D-2-3: Derosal-200 ppm-24 h, D-3-1: Derosal-300 ppm-6 h, D-3-2: Derosal-300 ppm-12 h, D-3-3: Derosal-300 ppm-24 h

Table 2: Effect of Korsikol treatment on the mitotic index and chromosome aberrations in the root tip meristem cells in *Hordeum vulgare* L.

Concentrations	Total cells	Dividing cells	Mitotic İndex (MI) (%)±SD	Fragment	Bridge	Stickiness	Polar deviation	Total chromosome aberrations (%)±SD
Control	13.058	1.831	0.140±0.003	134	30	97	9	14.774±0.031
K-1-1	11.022	1.494	0.134±0.003	132	74	262	134	40.295±0.055
K-1-2	11.559	1.431	0.124±0.003	194	73	261	145	47.050±0.062
K-1-3	12.200	1.480	0.125±0.029	277	91	209	178	51.013±0.045
K-2-1	10.834	1.396	0.130±0.004	254	57	138	280	52.140±0.063
K-2-2	11.696	1.553	0.130±0.002	274	69	242	160	48.615±0.046
K-2-3	13.455	1.384	0.123±0.003	253	76	160	132	44.870±0.054
K-3-1	10.285	1.377	0.125±0.003	250	40	106	115	37.109±0.046
K-3-2	10.116	1.505	0.153±0.004	264	46	73	108	32.623±0.050
K-3-3	9.561	1.506	0.165±0.003	379	60	121	125	45.485±0.053

K-1-1: Korsikol-100 ppm-6 h, K-1-2: Korsikol-100 ppm-12 h, K-1-3: Korsikol-100 ppm-24 h, K-2-1: Korsikol-200 ppm-6 h, K-2-2: Korsikol-200 ppm-12 h, K-2-3: Korsikol-200 ppm-24 h, K-3-1: Korsikol-300 ppm-6 h, K-3-2: Korsikol-300 ppm-12 h, K-3-3: Korsikol-300 ppm-24 h

highest increase in chromosomal breakes were obtained from the application of 300 ppm for 24 h. Korsikol had an increasing effect on chromosomal breakes in every dosage and time. Korsikol was more effective in bridge formation than Derosal.

In stickness of chromosomes, no correlation was observed between different doses and time intervals of Derosal. For example, 300 ppm for 24 h was the most. effective dose. Korsikol application increased chromosomal stickness independently from dose and time.

Mispolarization was observed in Derosal 50 WP and Korsikol 18 Dust application. It was not depended on application doses but it seems to be depended on application time

In other doses, there were no correlation. In all Korsikol applications, when compared with the control group except 100 ppm application, an increase of mis-polarization was investigated. It can be stated out that both Derosal 50 WP and Korsikol 18 Dust applications are effective on chromosomal behaviour. This effect is significant in $p<0.05$.

DISCUSSION

Fungicides used in these experiments, have different effects on cell division and chromosomes changes.

When Derosal 50 WP applied to barley seeds in different doses and times, no effect was obtained on cell division when compared with control group. In Korsikol 18 Dust applications, similar results were also observed, except 300 ppm application for 24 h, which increased the rate of dividing cells. Mitotic index values also supported these results.

Many researchers, reported that different pesticides effect mitotic activity and cause mitotic deviations. For example, Samashekar and Gowda (1984), stated that Vitavax causes mito-depressive behaviour in *Allium cepa* L. root tip cells treated with lowest concentration and prophase inhibition in the highest concentration, it causes inhibition of mitosis (mitotic effect).

Derosal and Korsikol changed chromosome structure and chromosomal behaviour depending on applied concentrations and time. Structural and behavioral changes observed in chromosomes were; fragments, chromosome bridges, mis-polarizations and sticky chromosomes.

Pusztai and Vegh (1978) observed that two fungicides called Topsin-methyl 70 SWP and Vitavax cause chromatid and chromosome aberrations in barley's root tip cells. He also found that Vitavax cause chromosome breakes, gaps, chromatidal deviations, lagging chromosomes and chromosome bridges depending on both disappearance of spindle fiber and some irregularities during cell division (Hemavathy and Krishnamurthy, 1988). Also, many other researchers reported similar results, regarding with fungicide application (Rayburn *et al*., 1993; Shahin and El Amoodi, 1991; Sharma *et al*., 1983).

Derosal and Korsikol also caused chromosome stickiness. Although chromosome stickiness were observed in Derosal treatment, these were not related with application time and dose.

Although, chromosomal stickness in Korsikol application was depended on application time, it was not correlated with doses which were applied. Different researchers also reported chromosome stickiness and accumulations as a result of pesticide applications (Hemavathy and Krishnamurthy, 1988; Sharma *et al*., 1983; Chauhan *et al*., 1986; Yuzbaoiolu, 2003; Zakia *et al*., 1990).

Furthermore, Derosal and Korsikol caused mis-polarization. Different researchers reported that, Afolon (Bilalolu, 1985) and Diuron (Badr, 1988) cause multipolar divisions.

Although, applied fungicides have different effects on cell division mechanism depending on concentration and time, according to our findings, they are acting like a mutagen. They change chromosome structure and behaviour. Our results are concordance with the results of other researchers.

REFERENCES

Aokin, T. and O. Sumer, 1996. Basudin 20 EM in arpa mitotik kromozomlari uzerine etkileri. Tr. J. Biol., 20: 21-28.

Badr, A., 1988. Cytogenetic activities of some fungicides. Cytologia (Tokyo), 53: 635-640.

Bilalolu, R. and B. Ozorgucu, 1988. Stomp`un *Allium cepa* L`da sitolojik etkileri. Cum. Univ. Fen-Ed. Fak. Fen Bilim. Derg., 7: 129-138.

Bilalolu, R., 1984. Stomp ve hyvar xin *Allium cepa* L'de olupturduklari mitotik etkiler ve kromozomal deipmeler. Cum. Univ. Fen-Ed. Fak. Fen Bilim. Derg., 2: 53-70.

Bilalolu, R., 1985. Gromoxone, afolon ve korthion`un hucre bolunmesi Ve kromozomlar uzerine etkileri. Univ. Fen-Ed. Fak. Fen Bilim. Derg., 3: 191-204.

Chauhan, L.K.S., T.S.S. Dikshith and V. Sundararaman, 1986. Effects of Deltamethrin on plant cells. I. Cytological effect on the root meristems of *Allium cepa*. Mut. Res., 171: 25-30.

Hemavathy, K.C. and N.B. Krushnamurthy, 1988. Cytogenetic effects of Cuman L. A dithiocarbamate fungicide, Mut. Res., 288: 57-60.

Pusztai, T. and A. Vegh, 1978. Mutagenic effect of pesticides. I. Cytological effects on some substituted urea herbicides in barley. Acta Bot. Acad. Sci. Hungaricase Tomus, 24: 327-342.

Pusztai, T., 1993. Chromosomal aberrations and chlorophyll mutations induced by some pesticides in barley. Acta Bot. Hungarica, 29: 55-66.

Rayburn, L.A., L. Wayne and L. Pedersen, 1993. Mcmurphy, M. The fungicide Captan reduce nuclear DNA content in maize seedling. Pestic. Sci., 37: 79-82.

Samashekar, R.K. and M.T.G. Gowda, 1984. Effects of a fungicide Vitavax on *Allium cepa*. Cytologia, 49: 177-181.

Shahin, S.A. and K.H. El Amoodi, 1991. Introduction of numerical chromosomal aberrations during DNA synthesis using the fungicides Nimrod and Rubigan-4 in root tips of *Vicia faba* L. Mut. Res., 261: 169-176.

Sharma, C.B.S.R., B.N. Behera, D.S.S. Raju and B.G.S. Rao, 1983. Effects of some systemic fungicides on chiasma frequencies in of *Hordeum vulgare*. Cytologia, 48: 749-752.

Yuzbaoiolu, D., 2003. Cytogenetic effects of fungicide Afugan on the meristematic cells of *Allium cepa* L. Cytologia, 68: 237-243.

Zakia, M.A., A.E. Fawzia, A.A.E.K. Zakia and A.E.S. Iman, 1990. Alterations in nucleic acids, protein content and mitotic division of *Vicia faba* root tip cells as affected by malathion and tamaron insecticides. Cytologia, 55: 349-355.