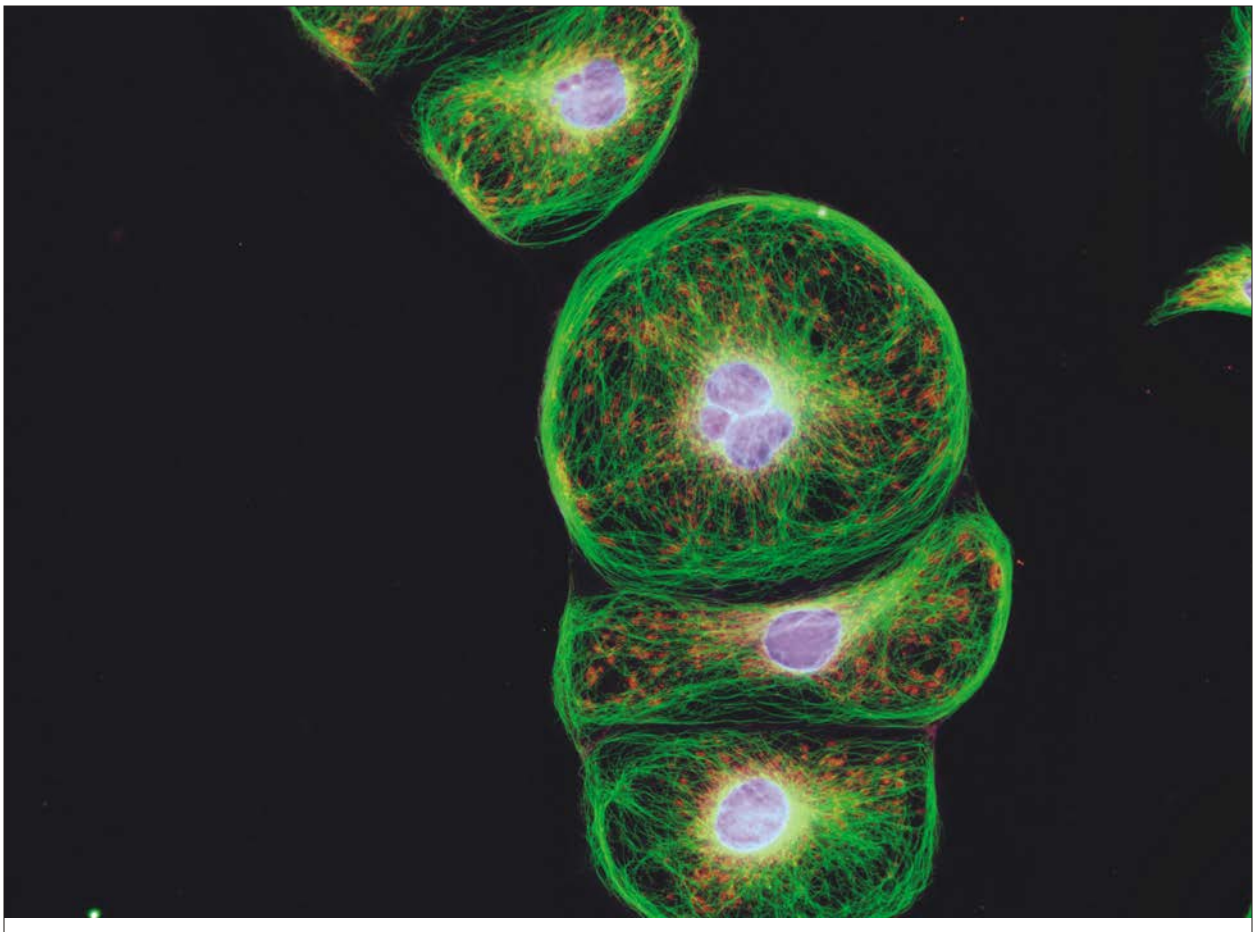

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Cytotoxicity of the Fungicides Azoxystrobin and Difenoconazole in Root Tips of *Allium cepa* L.

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Abstract: Non-target effects of two broad spectrum, foliar spray, systemic fungicides, Amistar (azoxystrobin 25% EC) and Score (difenoconazole 25% EC) in terms of cytotoxicity were investigated at concentrations ranging from 0.44 - 2200 μg (a.i.) mL^{-1}. The test material used was the root meristems of *Allium cepa*. At the recommended dose for field application (2.2 μg (a.i.) mL^{-1}), Difenoconazole depressed mitotic index by 4.305% while Azoxystrobin showed a decrease of 1.282% over untreated control at 12 h exposure period. The extent of chromosomal abnormalities has direct relationship with the concentration of the active ingredients and treatment time. The fungicide treated root meristems tended to recover from the cytotoxic effects when they were transferred to distilled water. The rate of recovery as indicated by increasing mitotic index and decreasing incidence of cytological abnormalities was highly pronounced in Azoxystrobin treated roots when compared with those treated with Difenoconazole.

Key words: Amistar, score, chromosomal abnormalities, mitotic index, cytotoxicity

Introduction

Increased use of pesticides for insect, weed and disease control in the past decade has proved the fact that certain agricultural chemicals may cause changes, which include inhibition of cell division, induction of chromosomal abnormalities and chromosomal damage. Chromosomal aberrations induced by agrochemicals in crop plants is widely used as an indicator of genetic damage. Grant (1978) selected root meristems as experimental systems as they are very sensitive to environmental changes and they represent normal plant-cell populations. It is a short-term assay and can be used with minimum space requirements. A number of workers have demonstrated the cytotoxic effects of different agrochemicals on plant species (Mousa, 1982; Ahmad and Yasmin, 1992; Mosuro *et al*., 1999; Chandra *et al*., 2002).

As new fungicides may induce many mitotic anomalies, they should undergo a rigorous testing for cytotoxic/mutagenic activity before their release due to the seriousness of the consequences.

The cytological effects of different agrochemicals on plant species have been studied by many workers (Grover and Tyagi, 1980; Njagi and Gopalan, 1981; Mousa, 1982; Amer and Ali, 1983; Soriano, 1984; Amer and Farah, 1985; Amer and Ali, 1986; Kumar and Sinha, 1989; Adam *et al*., 1990). Fungicides are known to induce mutation and are proved to be potential mutagens (Sahu *et al*., 1981). Many of the fungicides and their metabolic derivatives have been reported to be both carcinogenic and mutagenic (Kumar and Banerjee, 2001; Chandra *et al*., 2002). Higher plants have been used as testorganisms for studying the effects of genotoxic substances in the environment.

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Devi *et al*. (1991) analyzed the long-term effects of fungicides on both mitotic and meiotic systems in *Allium cepa* and concluded that they can induce chromosomal aberrations. With increasing concentrations of the pesticides lindane, pirimiphos methyl, glyphosate and 2,1- metachlor: atrazine in *A. cepa* root cells (Mosuro *et al*., 1999), endocel and monocil in *Vicia faba* root tip cells (Singh, 2001) and trifluralin in *Vicia faba* root cells (Chandra *et al*., 2002), a decline in mitotic index was observed. These are in accordance with the result obtained with the root tip cells of *Allium cepa* treated with aluminium sulphate (Sreedevi and Bindu, 2004). Davids (1973) reported a reduction in mitotic index accompanied by inhibition of DNA synthesis by diethyl sulphate. Contradictory to this, malathion tended to increase the mitotic index of root tips of *V. faba* (Zakia *et al*., 1990).

Mitotoxicity and clastogenicity effects were induced in onion by a variety of insecticides and pesticides like quinalphos, monocrotophos, thriam, parathion and malathion (Bhanja *et al*., 1988; Devi *et al*., 1991; Kiranmani *et al*., 1994).

Different cytological aberrations viz., chromosome fragments at mitotic metaphase and chromatin bridges, fragments, laggards and bridges with fragments and/or laggards at mitotic anaphase were observed in the pesticide treated *V. faba* seeds in frequencies significantly higher than those in the control (Singh, 2001).

Many type of mitotic aberrations induced by pesticides such as binucleate cells, c-metaphase, polyploidy cell, tripolar anaphase, chromatin bridges and lagging chromosomes were observed by Chandra *et al*. (2002).

Amistar (azoxystrobin 25% EC) and Score (difenoconazole 25% EC) are the two broad spectrum, foliar, systemic fungicides, yet to be released to the farmers and planters in India by Syngenta India Limited, Mumbai. The objective of the present study was to investigate their non-target effects in terms of cytotoxicity at concentrations ranging from 0.44 - 2200 μg (a.i.) mL^{-1}. For both the fungicides, manufacturer's recommended dose for foliar spray is 2.2 μg (a.i.) mL^{-1}.

Materials and Methods

Test Material

Bulbs of *Allium cepa* L. var. Co.15 were used as the test material. They were obtained from the Horticulture Division, Tamil Nadu Agricultural University, Coimbatore, India. All sets of measurement were repeated by conducting a separate set of measurements on a separately executed experiment. Means of two sets of experiments are statistically analyzed.

Fungicide Treatment

Healthy and uniform bulbs of *Allium cepa* were selected from the same collection. The outer scales were removed from the bulbs and apices of the root primordia exposed. Bulbs were then allowed to sprout in wet sand for 72 h at 25±1°C in dark. When 10-15 roots emerged upto 0.3 to 0.5 cm long, the roots were excised and transferred to the fungicide solutions of concentrations ranging from 0.44 to 2200 μg (a.i.). mL^{-1} of Azoxystrobin and Difenoconazole and incubated at 2 different time intervals i.e., 12 and 24 h. A set retained in distilled water served as control. The experiment was conducted at room temperature (25±1°C) and three bulbs were used for each treatment.

Recovery Treatments

The fungicide treated root tips were recovered by transferring to distilled water and incubating for different time intervals viz., 0 (without any recovery period), 12, 24, 48 and 72 h.

Cytological Observations

Treated root tips were transferred to the fixative (3:1 alcohol: acetic acid) for a minimum period of 24 h. Root tips were hydrolyzed in 1N HCl at 60°C for 5 min and squashes were made in 2% acetocarmine. Mitotic index was computed (Mosuro *et al*., 1999) by determining the mitotic cell frequency at the root tip cells as:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells observed}} \times 100$$

Percentage of cells showing chromosomal abnormalities such as chromosomal non-orientation, star metaphase, stickiness, clumps, rings, univalents, breaks, bridges, laggards, chromosome fragments, multipolarity, micronuclei, binucleate cells, giant cells, trinucleate cells, nuclear vacuole and chromatin elongation were recorded at the appropriate mitotic stages.

Results

Mitostatic Effect

Table 1 gives the mitotic indices in *Allium cepa* root mersitems treated with Azoxystrobin and Difenoconazole. Generally, Difenoconazole was more cytotoxic over Azoxystrobin. The mitotic index decreased significantly with increasing concentrations of the fungicides and the duration of the exposure. At the recommended dose for field application (2.2 μg (a.i.) mL^{-1}), Difenoconazole depressed mitotic index by 4.305% while Azoxystrobin showed a decrease of 1.282% over untreated control at 12 h exposure period. At the highest concentration of 2200 μg (a.i.) mL^{-1}, the 24 h treatment with Difenoconazole totally blocked mitosis. But in the case of Azoxystrobin, a very low percentage (2.098%) of mitosis was observed. The mitotic index recovered slowly when the treated root tips were incubated in distilled water over varying periods viz., 12, 24, 48 and 72 h.

Chromosomal Abnormalities

Data on chromosomal aberrations induced by the fungicides Azoxystrobin and Difenoconazole in the root tip cells of *Allium cepa* are presented in Table 2. The extent of chromosomal abnormalities is directly related to the concentration of the active ingredients and treatment time. Both the fungicides induced maximum percentage of abnormalities during ana-telophase stage (Table 2). Of the two fungicides, Difenoconazole induced the highest number of abnormalities in the dividing cells.

Non-orientation of chromatids, star metaphase, clumping, ring formation, univalents and breaks (gaps) were the abnormalities noted during metaphase stage (Table 2). Breaks, with a per cent frequency of 4.60 and clumping with 4.31 were the highest abnormalities observed in the cells treated with Azoxystrobin and Difenoconazole respectively. Univalents were the infrequently observed abnormality at and above 44 μg (a.i.) mL^{-1} in Azoxystrobin and 22 μg (a.i.) mL^{-1} in Difenoconazole.

During ana-telophase stage (Table 2), bridges, laggards, fragments, multipolar cells and micronuclei cells were commonly observed. Cells treated with the highest concentrations of Difenoconazole and Azoxystrobin showed respectively 6.53 and 3.69% of micronuclei closely followed by multipolar cells.

The abnormalities noted during interphase stage (Table 2) were giant cells, nuclear vacuolation, chromatin elongation, binucleate cells and trinucleate cells, of which chromatin elongation was more frequent with a frequency of 4.414% in Difenoconazole and 2.357% in Azoxystrobin. The frequency

Table 1: Impact of the fungicides Amistar and Score on the mitotic index in *Allium cepa*

Conc. (μg (a.i.) mL^{-1})	Treatment period (h)	Recovery period (h)	Cells observed	Mitosis cells	Mitotic index (%)
Amistar					
0	12	0	586	79	13.481
		12	585	80	13.675
		24	560	78	13.928
		48	562	79	14.056
		72	542	77	14.206
	24	0	584	80	13.698
		12	589	81	13.752
		24	564	80	14.184
		48	563	80	14.209
		72	584	84	14.385
0.44	12	0	562	71	12.633
		12	555	71	12.792
		24	519	69	13.294
		48	541	74	13.678
		72	567	79	13.932
	24	0	589	77	13.073
		12	606	81	13.366
		24	615	83	13.495
		48	618	87	14.077
		72	670	96	14.328
1.10	12	0	525	65	12.380
		12	560	70	12.500
		24	564	72	12.765
		48	524	68	12.977
		72	515	70	13.592
	24	0	540	68	12.592
		12	498	64	12.851
		24	477	62	12.997
		48	582	77	13.230
		72	487	67	13.757
1.46	12	0	624	76	12.179
		12	512	63	12.304
		24	544	69	12.683
		48	545	70	12.844
		72	540	72	13.330
	24	0	715	89	12.447
		12	612	78	12.745
		24	674	89	13.204
		48	700	94	13.428
		72	699	96	13.733
2.20	12	0	450	54	12.000
		12	472	58	12.288
		24	515	64	12.427
		48	523	67	12.810
		72	475	61	12.842
	24	0	623	76	12.199
		12	672	83	12.351
		24	614	79	12.866
		48	507	66	13.017
		72	572	75	13.111
4.40	12	0	598	65	10.869
		12	524	52	10.992
		24	420	47	11.190
		48	428	49	11.448
		72	515	61	11.844

Table 1: Continued

Conc. (μg.a.i.mL^{-1})	Treatment period (h)	Recovery period (h)	Cells observed	Mitosis cells	Mitotic index (%)
	24	0	521	54	10.364
		12	552	60	10.869
		24	564	62	10.992
		48	584	65	11.130
		72	592	69	11.655
22.00	12	0	587	60	10.221
		12	563	59	10.479
		24	571	62	10.858
		48	619	68	10.985
		72	657	73	11.111
	24	0	672	62	9.226
		12	684	64	9.356
		24	545	53	9.724
		48	704	68	9.659
		72	418	42	10.047
44.00	12	0	675	62	9.185
		12	664	62	9.337
		24	572	54	9.440
		48	542	53	9.778
		72	570	57	10.000
	24	0	705	64	9.078
		12	621	57	9.178
		24	648	62	9.567
		48	654	64	9.785
		72	607	60	9.884
220.00	12	0	601	46	7.653
		12	577	45	7.798
		24	612	48	7.843
		48	615	50	8.130
		72	567	48	8.465
	24	0	521	39	7.485
		12	587	48	7.666
		24	474	37	7.805
		48	619	49	7.915
		72	724	59	8.149
440.00	12	0	625	32	5.120
		12	631	33	5.229
		24	669	37	5.530
		48	654	38	5.810
		72	606	36	5.940
	24	0	654	33	5.045
		12	621	32	5.152
		24	640	35	5.468
		48	687	38	5.531
		72	509	30	5.893
2200.00	12	0	653	20	3.062
		12	659	22	3.338
		24	623	22	3.531
		48	661	24	3.630
		72	625	24	3.840
	24	0	572	12	2.097
		12	660	16	2.424
		24	646	17	2.631
		48	587	16	2.725
		72	707	21	2.970

Table 1: Continued

Conc. (μg (a.i.) mL^{-1})	Treatment period (h)	Recovery period (h)	Cells observed	Mitosis cells	Mitotic index (%)
Score					
0	12	0	586	79	13.481
		12	585	80	13.675
		24	560	78	13.928
		48	562	79	14.056
		72	542	77	14.206
	24	0	584	80	13.698
		12	589	81	13.752
		24	564	80	14.184
		48	563	80	14.209
		72	584	84	14.385
0.44	12	0	786	105	13.358
		12	450	61	13.555
		24	532	73	13.721
		48	612	86	14.052
		72	693	98	14.141
	24	0	418	53	12.679
		12	534	69	12.921
		24	598	78	13.043
		48	612	81	13.235
		72	657	90	13.698
1.10	12	0	446	51	11.434
		12	473	55	11.627
		24	481	57	11.850
		48	499	60	12.024
		72	515	63	12.233
	24	0	452	49	10.840
		12	412	45	10.922
		24	517	57	11.025
		48	546	62	11.355
		72	580	67	11.551
1.46	12	0	411	43	10.462
		12	428	46	10.747
		24	576	63	10.937
		48	612	68	11.111
		72	687	78	11.353
	24	0	422	43	10.189
		12	479	49	10.229
		24	509	55	10.805
		48	569	62	10.896
		72	430	47	10.930
2.20	12	0	745	76	10.201
		12	633	65	10.268
		24	598	62	10.402
		48	696	74	10.632
		72	669	72	10.762
	24	0	425	39	9.176
		12	531	50	9.416
		24	554	54	9.747
		48	460	46	10.000
		72	612	62	10.130
4.40	12	0	690	67	9.710
		12	721	71	9.847
		24	712	71	9.971

Table 1: Continued

Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Recovery period (h)	Cells observed	Mitosis cells	Mitotic index (%)
		48	725	73	10.068
		72	708	73	10.310
	24	0	473	42	8.879
		12	488	44	9.016
		24	509	48	9.430
		48	536	52	9.701
		72	568	56	9.859
22.00	12	0	446	32	7.174
		12	455	33	7.252
		24	482	37	7.676
		48	529	41	7.750
		72	571	48	8.406
	24	0	622	44	7.073
		12	679	49	7.216
		24	699	52	7.439
		48	409	31	7.579
		72	720	55	7.638
44.00	12	0	498	21	4.216
		12	528	23	4.356
		24	549	25	4.553
		48	606	30	4.950
		72	631	35	5.546
	24	0	477	20	4.192
		12	561	24	4.278
		24	584	25	4.280
		48	490	22	4.489
		72	624	29	4.647
220.00	12	0	677	16	2.363
		12	537	14	2.607
		24	598	18	3.010
		48	652	22	3.374
		72	468	17	3.632
	24	0	440	9	2.045
		12	511	11	2.152
		24	678	16	2.359
		48	488	12	2.459
		72	657	18	2.739
440.00	12	0	499	4	0.801
		12	463	4	0.863
		24	502	5	0.996
		48	487	5	1.026
		72	515	6	1.165
	24	0	635	2	0.314
		12	660	3	0.454
		24	625	3	0.480
		48	583	4	0.686
		72	527	4	0.759
2200.00	12	0	417	2	0.479
		12	579	1	0.172
		24	472	2	0.423
		48	541	2	0.369
		72	445	2	0.449
	24	0	620	-	-
		12	619	-	-
		24	628	-	-
		48	654	-	-
		72	1080	1	0.092

Note: '-' indicates the absence of mitosis

Analysis of variance for mitotic index

SV	DF	SS	MS	F
Replicate	2	0.00432	0.00216	<1
Treatment	199	10730.29414	53.92108	2230.64**
Concentration ©	9	9004.23850	1000.47094	41388.06**
Recovery (R)	4	58.45474	14.61368	604.55**
Treatment time (T)	1	17.81996	17.81996	737.19**
Fungicides (F)	1	1199.94304	1199.94304	49639.94**
CXR	36	4.57359	0.12704	5.26**
CXT	9	6.82187	0.75799	31.36**
CXF	9	406.82844	45.20316	1869.99**
RXT	4	0.25144	0.06286	2.60*
RXF	4	1.27629	0.31907	13.20**
TXF	1	5.57115	5.57115	230.47**
CXRXT	36	3.58605	0.09961	4.12**
CXRXF	36	3.96384	0.11011	4.55**
CXTXF	9	14.30183	1.58909	65.74**
RXTXF	4	0.26226	0.06556	2.71*
CXRXTXF	36	2.40115	0.6670	2.76**
Error	398	9.62083	0.02417	
Total	599	10739.91929		

cv = 1.88%, ** = Significant at 1% level, * = Significant at 5% level

Table 2: The extent of chromosomal aberration in the root meristem of *Allium ceps* treated with the fungicides Amistar and Score

			Aberrant cells (%)			
Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Reco very period (h)	Meta phase	Ana-telphase	Inter phase	Total aberrant cells (%)
Amistar						
0	12	0	-	-	-	-
		12	-	-	-	-
		24	-	-	-	-
		48	-	0.160	-	0.160
		72	0.348	0.200	-	0.548
	24	0	-	-	-	-
		12	-	-	-	-
		24	-	-	-	-
		48	-	0.167	-	0.167
		72	0.613	0.334	-	0.947
0.44	12	0	1.200	2.914	0.427	4.541
		12	0.905	2.464	0.512	3.620
		24	0.459	1.505	0.242	2.206
		48	-	1.470	-	1.470
		72	-	0.189	-	1.189
	24	0	1.438	3.287	0.742	5.467
		12	1.428	2.826	0.634	4.888
		24	1.405	2.256	0.207	3.868
		48	0.884	1.720	0.174	2.782
		72	0.666	1.020	-	1.686
1.10	12	0	1.300	2.958	0.603	4.861
		12	1.298	2.941	0.546	4.708
		24	0.666	2.343	0.289	3.298
		48	-	1.689	0.242	1.931
		72	-	1.492	-	1.492
	24	0	1.535	3.821	0.750	6.106
		12	1.515	3.298	0.649	5.462
		24	1.431	2.600	0.602	4.633

Table 2: Continued

Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Reco very period (h)	Aberrant cells (%) Meta phase	Ana-telphase	Inter phase	Total aberrant cells (%)
		48	0.915	1.834	0.176	2.925
		72	0.705	1.047	0.169	2.281
1.46	12	0	1.858	3.339	0.945	6.142
		12	1.680	3.159	0.910	5.749
		24	1.226	2.862	0.401	4.489
		48	0.571	2.352	0.250	3.173
		72	-	1.913	0.229	2.142
	24	0	2.985	4.964	1.428	9.377
		12	2.540	3.361	1.228	7.129
		24	2.225	2.739	0.817	5.781
		48	1.873	1.900	0.784	4.557
		72	1.089	1.190	0.537	2.816
2.20	12	0	2.189	3.517	1.462	7.168
		12	1.890	2.818	1.376	6.084
		24	1.437	2.773	1.035	5.245
		48	0.884	2.138	0.550	3.572
		72	0.598	1.875	0.444	2.917
	24	0	3.130	5.165	1.764	10.029
		12	3.048	3.621	1.557	8.226
		24	2.874	2.788	1.127	6.789
		48	1.923	1.986	0.823	4.732
		72	1.219	1.871	0.544	3.634
4.40	12	0	3.252	4.319	2.355	9.986
		12	3.184	3.910	1.769	8.863
		24	2.000	3.339	1.075	6.414
		48	1.323	2.604	0.602	4.529
		72	1.037	1.880	0.550	3.467
	24	0	4.306	5.194	2.721	12.221
		12	4.242	4.914	2.276	11.432
		24	3.481	4.194	1.197	8.872
		48	2.304	2.285	1.146	5.735
		72	1.701	2.133	0.639	4.473
22.00	12	0	5.122	5.391	3.050	13.563
		12	4.408	4.687	2.895	11.990
		24	3.625	3.716	2.127	9.468
		48	2.514	2.896	1.016	6.426
		72	1.807	2.310	0.616	4.733
	24	0	5.775	5.483	3.201	14.459
		12	5.588	4.507	2.910	13.005
		24	4.572	4.225	2.600	11.397
		48	3.906	2.554	1.174	7.634
		72	2.486	2.233	1.707	6.426
44.00	12	0	5.339	6.317	3.653	15.309
		12	4.705	6.210	3.245	14.160
		24	3.827	5.335	3.095	12.251
		48	3.063	2.842	1.873	7.778
		72	1.914	2.439	1.384	5.737
	24	0	7.573	6.853	3.861	18.287
		12	7.434	6.359	3.441	17.234
		24	6.557	5.190	3.434	15.181
		48	5.734	3.151	2.030	10.915
		72	5.094	2.503	1.824	9.421
220.00	12	0	8.333	9.644	6.382	24.359
		12	7.833	9.138	5.040	22.011

Table 2: Continued

Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Reco very period (h)	Aberrant cells (%) Meta phase	Ana-telphase	Inter phase	Total aberrant cells (%)
		24	6.925	6.940	4.238	18.103
		48	6.440	6.349	3.118	15.907
		72	5.295	5.116	2.075	12.486
	24	0	9.888	10.130	6.483	26.501
		12	8.522	9.942	5.846	24.310
		24	8.216	7.167	4.370	19.753
		48	7.115	6.613	3.345	17.073
		72	6.981	4.373	2.895	14.249
440.00	12	0	10.493	10.490	6.967	27.950
		12	10.339	9.948	5.646	25.933
		24	9.422	8.042	5.566	23.030
		48	8.823	7.283	3.762	19.868
		72	7.363	5.158	2.443	14.964
	24	0	10.693	10.668	8.597	29.958
		12	10.567	10.178	7.865	28.610
		24	10.153	8.170	6.352	24.675
		48	9.830	7.344	5.443	22.617
		72	8.998	5.281	4.878	19.157
2200.00	12	0	14.482	11.550	7.784	33.816
		12	13.879	10.355	7.392	31.626
		24	13.015	10.104	6.054	29.173
		48	12.500	8.244	4.743	25.487
		72	11.928	6.867	4.572	23.367
	24	0	15.533	11.571	9.090	36.194
		12	14.358	11.216	8.857	34.431
		24	13.822	10.320	8.139	32.281
		48	13.494	8.468	6.250	28.212
		72	12.588	8.965	4.954	26.507

Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Reco very period (h)	Aberrant cells (%) Meta phase	Ana-telphase	Inter phase	Total aberrant cells (%)
Score						
0	12	0	-	-	-	-
		12	-	-	-	-
		24	-	-	-	-
		48	-	0.160	-	0.160
		72	0.348	0.200	-	0.548
	24	0	-	-	-	-
		12	-	-	-	-
		24	-	-	-	-
		48	-	0.167	-	0.167
		72	0.613	0.334	-	0.947
0.44	12	0	1.864	4.838	3.344	10.046
		12	1.851	4.172	3.066	9.089
		24	0.956	3.812	2.742	7.51
		48	0.875	3.264	2.108	6.247
		72	0.713	2.419	1.182	4.314
	24	0	3.153	4.956	3.597	11.706
		12	2.608	4.261	3. 459	6.869
		24	2.285	4.245	3.225	9.755
		48	1.840	3.824	3.073	8.737
		72	1.553	3.802	2.272	7.627

Table 2: Continued

Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Reco very period (h)	Aberrant cells (%) Meta phase	Ana-telphase	Inter phase	Total aberrant cells (%)
1.10	12	0	2.173	8.024	4.826	15.023
		12	1.848	7.922	4.606	14.376
		24	1.639	7.363	4.032	13.034
		48	1.250	5.958	3.051	10.259
		72	0.956	4.379	2.292	7.627
	24	0	4.301	8.253	5.597	18.151
		12	3.913	8.126	4.936	16.975
		24	3.245	7.633	4.487	15.365
		48	2.409	6.336	4.248	12.993
		72	2.281	5.829	3.030	11.14
1.46	12	0	6.109	9.533	5.513	21.155
		12	5.282	8.829	4.981	19.092
		24	5.219	7.589	4.385	17.193
		48	4.460	7.038	3.114	14.612
		72	4.578	5.909	2.469	12.956
	24	0	6.200	12.056	5.623	23.879
		12	6.197	11.475	5.150	22.822
		24	5.989	11.147	5.122	22.258
		48	5.067	8.724	4.656	18.447
		72	4.599	6.637	3.322	14.558
2.20	12	0	7.066	10.588	6.613	24.267
		12	6.919	9.815	5.160	21.894
		24	5.928	9.112	4.408	19.448
		48	5.008	8.009	4.025	17.042
		72	4.789	6.986	3.151	14.926
	24	0	7.710	14.215	6.739	28.664
		12	7.042	13.265	6.257	26.564
		24	6.054	11.168	5.077	22.299
		48	5.353	10.526	4.829	20.708
		72	4.618	9.452	3.385	17.455
4.40	12	0	7.942	12.476	9.621	30.039
		12	6.486	11.660	8.931	27.077
		24	6.021	11.389	8.207	25.617
		48	5.502	10.434	7.929	23.865
		72	5.273	10.162	6.250	21.685
	24	0	7.753	14.455	10.243	32.451
		12	7.500	14.018	9.121	30.639
		24	6.932	13.231	8.456	28.619
		48	5.882	12.997	8.067	26.946
		72	4.918	11.111	6.373	22.402
22.00	12	0	9.262	14.312	10.139	33.713
		12	8.566	13.867	9.906	32.339
		24	7.508	13.452	8.464	29.424
		48	7.246	13.082	8.179	28.507
		72	7.065	12.152	7.610	26.827
	24	0	9.965	14.513	10.491	34.969
		12	8.808	14.062	10. 299	22.87
		24	8.075	13.852	9.725	31.652
		48	7.760	13.184	8.874	29.818
		72	6.944	12.758	8.260	27.962
44.00	12	0	10.499	16.298	11.015	37.812
		12	10.309	15.672	10.535	36.516
		24	9.863	15.313	9.962	35.138
		48	9.764	14.428	9.210	33.402

Table 2: Continued

Conc. (µg (a.i.) mL^{-1})	Treatment period (h)	Recovery period (h)	Aberrant cells (%) Meta phase	Ana-telophase	Inter phase	Total aberrant cells (%)
		72	9.409	14.223	9.090	32.722
	24	0	10.245	16.830	11.185	38.26
		12	9.893	15.939	10.839	36.671
		24	9.554	15.510	10.035	35.099
		48	8.888	15.212	9.586	33.686
		72	8.116	15.192	9.433	32.741
220.00	12	0	12.565	17.973	11.463	42.001
		12	12.244	17.069	10.839	40.152
		24	11.985	16.719	10.211	38.915
		48	11.700	15.930	9.466	37.096
		72	11.131	15.654	9.219	36.004
	24	0	13.365	18.312	11.564	43.241
		12	12.581	18.048	11.433	42.062
		24	12.186	17.927	10.498	40.611
		48	11.725	17.420	9.810	38.955
		72	11.224	17.413	9.568	38.205
440.00	12	0	15.855	22.262	11.599	49.716
		12	15.068	22.118	11.054	48.24
		24	14.828	21.601	10.851	47.28
		48	14.056	21.497	10.305	45.858
		72	13.438	20.255	9.841	43.534
	24	0	16.369	23.260	11.892	51.521
		12	16.047	23.135	11.774	50.956
		24	15.834	23.017	11.623	50.474
		48	15.077	23.000	11.245	49.322
		72	14.310	22.997	11.229	48.536
2200.00	12	0	17.697	23.679	11.892	53.268
		12	17.406	23.021	11.658	52.085
		24	17.247	22.638	11.433	51.318
		48	16.928	21.654	11.269	49.851
		72	16.267	21.080	11.183	48.53
	24	0	17.719	25.911	12.437	56.067
		12	17.030	25.818	12.355	55.203
		24	16.300	25.787	12.353	54.44
		48	15.950	25.697	12.248	53.895
		72	14.740	25.301	12.203	52.244

Note: '-' indicates absence of any abnormality

Analysis of variance for chromosomal abnormalities

SV	DF	SS	MS	F
Replicate	2	0.1445	0.0722	3.35*
Treatment	119	133279.3394	669.7454	31014.49**
Concentration ©	9	75880.6171	8431.1797	390430.05**
Recovery (R)	4	4064.3157	1016.0789	47052.46**
Treatment time (T)	1	744.0852	744.0852	34457.01**
Fungicides (F)	1	46295.3341	46295.3341	2143838.74**
CXR	36	154.5242	4.2923	198.77**
CXT	9	40.2674	4.4742	207.19**
CXF	9	5442.5012	604.7224	28003.41**
RXT	4	2.3755	0.5939	27.50**
RXF	4	41.8246	10.4562	484.20**
TXF	1	16.9391	16.9391	784.41**
CXRXT	36	47.3399	1.3150	60.89**

Analysis of variance for chromosomal abnormalities

SV	DF	SS	MS	F
CXRXF	36	436.7338	12.1315	561.78**
CXTXF	9	88.5057	9.8340	455.39**
RXTXF	4	1.3784	0.3446	15.96**
CXRXTXF	36	22.5972	0.6277	29.07**
Error	398	8.5946	0.0216	
Total	599	133288.0785		

cv = 0.7%, ** = Significant at 1% level, * = Significant at 5% level

of giant cells was the lowest and were observed at and above the concentration of 44 μg (a.i.) mL^{-1} in Azoxystrobin and 4.4 μg (a.i.) mL^{-1} in Difenoconazole.

Recovery of the fungicide treated cells in distilled water significantly decreased the number of cytological abnormalities. Further, a negative relationship was observed with the frequency of abnormal cells and the recovery period. The recovery from cytological abnormalities is more pronounced in Azoxystrobin treated cells when compared with those of Difenoconazole treatment.

Discussion

It is obvious that many of the agrochemicals have cytotoxic and mutagenic properties and are environmentally hazardous (Burnett *et al*., 1980). The present investigation examines mitodepressive and cytotoxic activities of the two foliar sprays, Azoxystrobin and Difenoconazole in the root tip meristems of *Allium cepa*. This plant was selected as the test material in the present study because of its low chromosome number and larger chromosomal size. *Allium* species are favourable cytological materials as they also have the advantage of being available round the year and can be easily handled and cultivated (Kihlman, 1971). The cytological effects of Azoxystrobin and Difenoconazole on the root cells were examined on the basis of changes in mitotic index and other induced abnormalities. A strong, dose dependant impact is obvious in terms of decline in mitotic index with increasing concentration and duration of exposure (Table 1).

Mitotic inhibition and reduction in mitotic index by fungicides Vitavax-200 and Dithane S-60 were reported by Al-Najjar and Soliman (1980) on wheat. Similar results were obtained in *Vicia faba* with Triflurain (Chandra *et al*., 2002). Such a reduction in mitotic activity could be due to the inhibition of DNA synthesis which is considered as one of the major prerequisites for a cell to divide (Zakia *et al*., 1990).

Cytological abnormalities induced by the two fungicides, Azoxystrobin and Difenoconazole were similar to aberrations induced by other pesticides and chemical mutagens. The common abnormalities encountered during metaphase were non-orientation, star metaphase, clumping, ring formation, univalents and breaks (gaps). The ana-telophase abnormalities observed were bridges, laggards, fragments, multipolar cells and micronuclei cells. Giant cells, nuclear vacuolation, chromatin elongation, binucleate cells and trinucleate cells were observed during interphase. The frequencies of the different types of abnormalities were significantly influenced by the fungicides, their concentration and the exposure period (Table 2). The anomalies observed in the present study were also recorded by several workers in the pesticide treated root tip meristems (Adam *et al*., 1990; Chand *et al*., 1991; Ahmad and Yasmin, 1992; Kumar and Kumar, 2000; Singh, 2001; Chandra *et al*., 2002).

Inhibition of spindle formation might have led to disturbed meta- and anaphase. The error of spindle organization may even lead to split or multipolar spindle. Many workers have reported the role of certain pesticides in spindle formation (Amer and Mikhael, 1986;

El-Khodary *et al*., 1987, 1989). Stickiness and clumping of metaphase and bridge of anaphase have been attributed to the formation of dicentric chromosomes as a result of breakage and reunion (Sinha, 1989). Ring chromosomes in low frequency have been observed following treatments with Azoxystrobin and Difenoconazole. Kaur and Grover (1985) also reported low frequency ring chromosomes in root tip cells of barley treated with Anthio, Ekalux, Phendal and Rogar.

One of the frequent abnormality noticed in the present study was the appearance of ana-telophase bridges involving one or more chromosomes. They may be due to the general stickiness of chromosomes at metaphase (Al-Najjar and Soliman, 1980). Not all the bridges, especially those that appear at low concentration of the pesticides, are due to chromosome stickiness but may be due to breakage and reunion of chromosomes (El-Khodary *et al*., 1990).

The occurrence of micronuclei has been regarded as reliable parameter for the clastogeneticity/mutageneticity of an agent (Auerbach, 1976). Micronuclei may originate from lagging chromosome or from acentric fragments, which were observed in the mitotic stages (El-Khodary *et al*., 1989; Ahmad and Yasmin, 1992). The occurrence of binucleate cells in the interphase indicates that the fungicides inhibited the cell plate formation. Binucleate cells were also noticed in organophosphorous pesticides treated root meristems of *Allium* and *Hordeum* (Grover and Malhi, 1988).

When a set of fungicide treated root tip meristems were transferred to distilled water and incubated for varying periods, the cells tended to recover from the fungitoxic effect (Table 1-2). The rate of recovery as indicated by increasing mitotic index and decreasing incidence of cytological abnormalities was highly pronounced in Azoxystrobin treated roots when compared with those treated with Difenoconazole. Similar recovery in *A. cepa* root meristems treated with Asulum, MSMA, Chlorpyriphos and Endosulfan (Rao and Rao, 1980) is already reported.

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