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Cell Cycle Stage Specific Application of Cypermethrin and Carbendazim to Assess the Genotoxicity in Somatic Cells of *Hordeum Vulgare* L

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Abstract In this study, the genotoxicity of commonly used insecticide cypermethrin and fungicide carbendazim were examined on root meristem cells of *Hordeum vulgare* L. Cypermethrin and carbendazim were applied with concentrations of 0.05%, 0.1% and 0.5% at different presoaking durations of 7 h, 17 h and 27 h which falls into G_1, S and G_2 phases of cell cycle, respectively. The cytogenetic studies of the root meristem cells of *Hordeum vulgare* L. showed that S phase of the cell cycle was more sensitive in comparison to other phases of cell cycle.

Keywords Cypermethrin · Carbendazim · Cell cycle · Chromosomal aberration

Pesticides are designed to kill pests and synthetic pyrethroids are among the most potent and effective pesticides available. Cypermethrin, one of the important insecticides, is widely used in fields of cotton, cereals, vegetables and fruits as well as for seed storage and public health. Its structure is based on pyrethrum, a natural insecticide contained in chrysanthemum flowers but it has a higher biological activity and is more stable than its natural model. It rapidly paralyzes the nervous system of insects (Reigart 1999). Carbendazim is a fungicide of major concern due to its suspected hormone disrupting effects. It is a systemic benzimidazole fungicide (ACP 1992) that plays a very important role in plant disease control (Quian 1996). It is also used in post-harvest food storage, and as a seed pre-planting treatment (Hicks 1998).

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Generally the Screening tests need sensitive indicators which provide fast and reliable information. Plant assays are unique in the sense that they provide valuable genetic assay systems for screening and monitoring environmental pollutants. Now, plants are recognized as excellent indicators of cytogenetic and mutagenic effects of environmental chemicals and are applicable for the detection of environmental mutagens both indoor and outdoor (Grant 1982; Srivastava and Singh 2007). Therefore, the present study was to determine the genotoxicity of cypermethrin and carbendazim in specific stages of cell cycle on root meristem cells of *Hordeum vulgare* L.

Materials and Methods

The selection of the cypermethrin and carbendazim were on the basis of their frequent use in this region. Technical grade of cypermethrin [(RS) – alpha-cyano-3-phenoxy benzyl-2, 2-dimethyl (1R, 1S)-cis, trans-3-(2, 2-dichlorovinyl) cyclopropanecarboxylate), 25% EC, B. No. A1-B-A110] and carbendazim [(Methyl (1H-benzimidazol-2-yl) carbamate), 75%, WPE-0045)] were procured from BSF India Ltd., India and Nagarjun Agrichem Ltd., Hyderabad, India, respectively. Ethyl Methane Sulphonate (EMS) was brought from Sigma Aldrich, USA. Fixatives/stains used in the study were brought from Loba Chemie and Merck Ltd., Mumbai India. Three different concentrations (0.05%, 0.1% and 0.5%) of cypermethrin, carbendazim and positive control ethyl methane sulphonate were used in three replications. The used concentrations were determined on the basis of their doses applied in agricultural fields by the farmers.

The seeds of *Hordeum vulgare* L. variety karan-4 were obtained from the Institute of Agricultural science, Banaras Hindu University, Varanasi, India. A total of 150 seeds were

taken for each treatment. They were soaked in water for 1 h to complete hydration and left for different presoaking durations (7 h, 17 h and 27 h) which fall in three different stages of cell cycle i.e., G_1, S and G_2 phases, respectively (Singh et al. 2007). All the presoaked seeds were treated with each concentration of cypermethrin carbendazim and ethyl methane sulphonate for 6 h. The equal numbers of seeds were also treated with distilled water to be used as a negative control. The treated seeds were washed under the running tap water for duration of 4 h. All the seeds were placed on moist filter paper in petri plates to germinate at 20 ± 1°C. The appropriate root tips were randomly collected from each petri plate for cytological studies. The collected root tips were washed thoroughly and fixed in Carnoy's solution (6 ethyl alcohol:3 chloroform:1 acetic acid) for overnight and preserved in 70% alcohol at 4°C for cytological studies. These root tips were hydrolyzed in 1 N HCl and stained with 0.5% hematoxylin. All the observations were made from temporarily prepared slides. In each replication, a minimum of 4,000 to 4,500 cells were observed. The data recorded for mitotic index and chromosomal aberrations were statistically analyzed by using New Duncan's Multiple test.

Results and Discussion

Effects of cypermethrin and carbendazim along with the positive control ethyl methane sulphonate and negative control on mitotic index are presented in Table 1. At 17 h presoaking duration the significant inhibition in mitotic index was observed at 0.1% and 0.5% concentrations of cypermethrin and carbendazim treated root meristem cells of *Hordeum vulgare* L. A significant decrease in the mitotic index was also observed at 27 h. presoaking duration in 0.1% and 0.5%.

The various chromosomal aberrations like stickiness, chromosomal bridges, laggards, chromosomal breaks and binucleated cells were observed in cypermethrin, carbendazim and ethyl methane sulphonate treated root meristem cells. Stickiness and chromosomal breaks were the most frequent chromosomal abnormalities. The maximum percentage (15.48%) of chromosomal aberration was encountered at 17 h presoaking duration with 0.5% concentration of cypermethrin, carbendazim and ethyl methane sulphonate (Tables 2, 3, 4).

Data of the present study revealed that each concentration of cypermethrin and carbendazim diminished the mitotic index and induced the chromosomal aberrations at all presoaking durations which confirm their mitodepressive, cytotoxic and genotoxic effects (Saxena et al. 2005). However, the significant reduction in mitotic index and induction of chromosomal aberrations at 17 h presoaking duration confirm the higher sensitivity of S phase of the cell cycle.

Table 1 Mitotic index at different concentration of carbendazim and cypermethrin

Exp. time and concentration	Mitotic index at different concentration (Mean ± SD)		
	0.05%	0.1%	0.5%
Control			
7 h	9.95 ± 0.79		
17 h	9.82 ± 0.26		
27 h	9.90 ± 0.52		
EMS			
7 h	8.89 ± 0.65	8.65 ± 0.32	7.85 ± 0.90
17 h	7.32 ± 0.54	7.15 ± 0.45	6.32 ± 0.84*
27 h	7.48 ± 0.36	7.30 ± 0.98	6.85 ± 0.32
Cypermethrin			
7 h	8.43 ± 0.84	8.01 ± 0.41	7.17 ± 1.09
17 h	7.36 ± 0.72	5.80 ± 0.59**	4.53 ± 0.31**
27 h	7.07 ± 1.10	5.12 ± 0.31**	4.86 ± 0.99**
Carbendazim			
7 h	8.68 ± 0.47	7.80 ± 1.12	6.81 ± 0.32
17 h	7.91 ± 0.33	6.49 ± 0.83**	5.67 ± 1.06**
27 h	8.58 ± 0.67	7.11 ± 1.19	6.2 ± 0.88*

Significance level calculated by student's *t* test

Data obtained from 3,000 to 4,000 cells and expressed as Mean ± SD

* $p < 0.05$; ** $p < 0.01$

Table 2 Types and percentage of chromosomal abnormalities induced by cypermethrin in the root tip cells of *Hordeum vulgare* L

Exp. times & concentrations	Chromosomal abnormalities (%)					Total chromosomal aberrations (%)
	ST	BRI	LAG	CB	BN	
Control						
7 h	0.21	ND	ND	ND	ND	0.21
17 h	0.26	ND	ND	ND	ND	0.26
27 h	0.24	ND	ND	ND	ND	0.24
0.05%						
7 h	0.63	ND	ND	ND	ND	0.63
17 h	1.29	0.22	0.21	0.55	ND	2.27
27 h	1.15	0.32	ND	0.57	ND	2.04
0.1%						
7 h	1.16	ND	0.22	0.62	0.06	2.06
17 h	1.92	0.68	1.46	1.75	0.38	6.19**
27 h	1.64	0.48	0.91	0.96	0.24	4.23
0.5%						
7 h	1.73	0.91	0.51	1.25	0.16	4.56
17 h	3.85	3.85	1.24	4.62	1.92	15.48***
27 h	1.43	1.46	2.22	2.38	0.42	7.91**

Significantly different from the control at ** $p < 0.01$, *** $p < 0.001$

ST = Stickiness, BRI = Bridges, LAG = Laggards, CB = Chromosomal breaks, BN = Bi Nucleated cells

Table 3 Types and percentage of chromosomal abnormalities induced by carbendazim in the root tip cells of *Hordeum vulgare* L

Exp. times and concentrations	Chromosomal abnormalities (%)					Total chromosomal aberrations (%)
	ST	BRI	LAG	CB	BN	
Control						
7 h	0.21	ND	ND	ND	ND	0.21
17 h	0.26	ND	ND	ND	ND	0.26
27 h	0.24	ND	ND	ND	ND	0.24
0.05%						
7 h	0.38	ND	ND	ND	ND	0.38
17 h	0.96	0.52	ND	0.38	ND	1.86
27 h	0.56	0.12	ND	0.12	ND	0.8
0.1%						
7 h	0.65	0.65	ND	0.2	0.02	1.52
17 h	2.26	1.75	1.32	2.26	0.61	8.2**
27 h	1.02	1.53	ND	1.42	0.36	4.33
0.5%						
7 h	1.32	1.06	ND	0.94	0.61	3.93
17 h	2.06	2.22	1.46	3.40	0.5	9.64**
27 h	1.68	0.84	2.25	2.38	0.94	8.09**

Significantly different from the control at ** $p < 0.01$

ST = Stickiness, BRI = Bridges, LAG = Laggards, CB = Chromosomal breaks, BN = Bi Nucleated cells

Table 4 Types and percentage of chromosomal abnormalities induced by ethyl methane sulphonate in the root tip cells of *Hordeum vulgare*

Exp. times and concentrations	Chromosomal aberrations (%)					Total chromosomal aberrations (%)
	ST	BRI	LAG	CB	BN	
Control						
7 h	0.21	ND	ND	ND	ND	0.21
17 h	0.26	ND	ND	ND	ND	0.26
27 h	0.24	ND	ND	ND	ND	0.24
0.05%						
7 h	0.54	ND	ND	ND	ND	0.54
17 h	1.21	0.52	ND	0.38	ND	2.11
27 h	0.84	0.12	ND	0.12	ND	1.08
0.1%						
7 h	0.95	0.38	0.32	0.24	0.02	1.91
17 h	1.47	1.06	1.74	0.56	0.35	5.18
27 h	1.25	0.85	0.85	0.35	0.18	3.48
0.5%						
7 h	1.01	1.65	1.20	1.20	0.46	5.52
17 h	2.92	2.75	2.70	1.85	0.69	10.91**
27 h	2.53	1.62	2.53	0.64	0.27	7.59**

Significantly different from the control at ** $p < 0.01$

ST = Stickiness, BRI = Bridges, LAG = Laggards, CB = Chromosomal breaks, BN = Bi Nucleated cells

The significant decline of mitotic index with higher concentrations at 17 h presoaking duration is the outcome of inhibition of cell division which reflects the cytotoxic potential of cypermethrin and carbendazim in *Hordeum vulgare*. Cypermethrin and carbendazim may contain undesirable cytotoxic compounds causing the cell death resulting in the decline of mitotic index. The reduction of mitotic index may be either due to the inhibition of DNA synthesis at S-phase (Sudhakar et al. 2001) or blocking of G_1 suppressing DNA synthesis (Schneidermann 1971) or blocking in G_2 preventing the cells from entering mitosis (El-Ghamery et al. 2000). The findings of the present study are in line of our previous observation showing the mitotic inhibition in the root meristem cells of *Hordeum vulgare* L. by insecticide and fungicide (Singh et al. 2007).

Chromosome stickiness was a very frequent chromosomal abnormality observed in somatic cells of *Hordeum vulgare* L. This stickiness is presumably due to intermingling of chromatin fibers which leads to subchromatid connections between chromosomes (Klasterska et al. 1976). Chromosomal break was another most frequent chromosomal aberration induced which indicates the clastogenic potential of cypermethrin and carbendazim. 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). 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).

As the chromosome aberrations are adjunct indicators of genotoxicity, it can be concluded that cypermethrin and carbendazim have the potential to cause genotoxic effects in root meristem cells of *Hordeum vulgare* L. However, the treatments during the synthesis (S) phase of cell cycle may cause more damage to cells and affect the viability of seeds and plants as well.

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