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THE CYTOGENETIC EFFECTS OF ORGANOPHOSPHORUS INSECTICIDE DICHLORVOS IN BARLEY (*HORDEUM VULGARE* L.) SEEDLINGS

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Abstract

The cytogenetic effects of dichlorvos on *Hordeum vulgare* L. seeds were investigated. Seeds of *H. vulgare* were treated with different concentrations of dichlorvos and applied for **increasing** concentrations of dichlorvos (0.01, 0.05, 0.1, 0.5 and 1 mg/L) and increased treatment period (10-, 24- and 48-hour) decreased the mitotic index compared with the untreated control. The inhibitory effects of dichlorvos on the mitotic index indicated its genotoxic and mutagenic effects on *H. vulgare* seeds. It can be concluded that DDVP clearly poses a genotoxic risk. The results of the present study are fundamental results and provide direction for future investigations.

Introduction

Pesticides are chemical substances that are widely used against plant pests and diseases. The use of pesticides in commercial agriculture has led to an increase in farm productivity (*Krol et al*., 2000). Pesticides are essential in modern agricultural practices; but, due to their biocidal activity and potential risk to the consumer, the control of pesticide residues in foods is a growing source of concern for the general population (Torres *et al*., 1996). Teratogenic, carcinogenic and toxic properties of pesticides have been reported in the literature. Pesticides acted through a common mechanism of toxicity and conducted cumulative risk assessment (Bernard & Gordon, 2000). Jalilian *et al*. (2000) reported the cytogenetic effect of the pesticides. The presence of their residues in fruits and vegetables can be a significant route to human exposure (Anonymous, 1990).

Organophosphorus (OP) pesticides are widely used in agriculture as insecticides leave residues to varying extents in agricultural produce such as vegetables and fruits (Iram *et al*., 2009). OP compounds exert acute toxic effects that are mainly due to the suppression of neuronal acetylcholinesterase activity (Sachanaa *et al*., 2003). The widespread uses of OP insecticides indicate the extensive availability and potential for accidental and intentional human exposure (El-Behissy *et al*., 2001).

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate -DDVP) is an OP compound used to control household, public health and stored product insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips and white flies in greenhouse, outdoor fruit and vegetable crops (Lotti, 2001). DDVP is used to treat a variety of parasitic worm infections in dogs, livestock and humans. DDVP can be fed to livestock to control botfly larvae in the manure. It acts against insects as both contact and stomach poison (Lotti, 2001). It has been used to make pet collars and pest strips (Murphy, 1980; Roberson & Nolan, 1988). The oral LD_{50} for DDVP is 61–175 mg/Kg in mice, 100–1090 mg/Kg in dogs, 15 mg/Kg in chickens, 25–80 mg/Kg in rats, 157 mg/Kg in pigs, and 11–12.5 mg/Kg in rabbits (Gallo & Lawryk, 1991).

DDVP primarily affects the nervous system through cholinesterase inhibition i.e., the blockage of an enzyme required for proper nerve functioning. In addition, the Environmental profection Agency has classified it as toxicity class I (highly toxic) and a possible human carcinogen because it may cause cancer and there is only a small margin of safety for other effects (Anonymous, 1991).

The aim of the present study was to examine the cytogenetic effects and cytotoxicity of DDVP using as OP insecticide on mitotic index (MI) of *H. vulgare* seeds.

Materials and Methods

Treatment with Dichlorvos: Dry dormant seed samples of *H. vulgare* were treated with 0.01, 0.05, 0.1, 0.5 and 1 mg/L DDVP for 10-, 24- and 48-hour at room temperature.

Germination: Control (untreated) and treated *H. vulgare* seeds were soaked in distilled water for 1 h at room temperature. Then, each group of seeds was transferred to wet Whatman paper in Petri dishes for 24 h at room temperature. After germination, root tips were cut (1.0–1.5 cm length) for determination of the MI.

Fixation and Preparation: Root tips were fixed in pure glacial acetic acid for 30 min before being rinsed and transferred to 70% alcohol, and then stored in a refrigerator until use. For preparation, root tips were hydrolized in 5 mol/L HCl for 20 min at room temperature and then stained in an acetocarmin solution for 1 h (to prepare the acetocarmin solution, 45% glacial acetic acid is melted and cooled to 50°C; then, 1 g acetocarmin is added and the mixture is melted for 10 min). Chromosome spreads were made using the squash technique (Ilbas *et al*., 2005; Mujeeb-Kazı *et al*., 2007).

Examination of Mitotic Index: For the MI, the frequency of mitosis was determined by counting the number of dividing cells of 2000 cells investigated in the untreated and treated groups.

Statistical Analysis: The computer software program SPSS 10.0 was used to analyze the data. Evaluation of the difference in the means of the MI among treated and untreated groups was conducted using ANOVA. The differences between means were compared using least significant differences (LSD) test with $p<0.01$ and $p<0.05$ considered significance.

Results and Discussion

DDVP is a widely used pesticide with high potential for outside organisms of target and human exposure. It has

been showed cytotoxic, genotoxic and mutagenic effects of pesticides with different test systems. Plant test systems among known test systems are more sensitive for determining these effects of pesticides (Anonymous, 1980).

The DNA molecule is a target site of most, if not all, cytotoxic and mutagenic agents. Additionally, a number of agents are acutely cytotoxic to the cell because they damage this DNA molecule. The cytogenetic results of the present study showed that DDVP treatment decreased MI rates compared with the control (untreated) group. Increasing concentrations of DDVP decreased MI at 10-, 24- and 48-hour of treatment time. The differences in MI between dosages of treatment and control were statistically significant for 24- and 48 hour ($p<0.05$ and $p<0.01$). Following exposure to DDVP for 10-hour, the differences in MI between 0.5 and 1 mg/L dosages of treatment and control were statistically significant ($p<0.05$ and $p<0.01$). The highest MI frequency was found to be 8.85% in the control group, whereas the lowest were 6.10% in the group exposed to 0.5 mg/L for 10-hour, 2.50% in the group exposed to 0.5 mg/L for 24-hour and 1.85% in the group exposed to 1 mg/L for 48-hour (Table 1). With respect to MI frequency, the differences between groups exposed to DDVP for 10- and 24-hour were not found to be statistically significant ($p>0.05$), whereas there were significant differences found between 48-hour and other hours (10 and 24) ($p<0.05$). Thus, higher concentrations and longer exposure to DDVP causes a greater inhibition of MI.

Table 1. Mitotic index and percentage of cell division of seeds exposed to different concentrations of dichlorvos.

Treatment period	Dosages of treatmetn (mg/L)	Dividing cells	Percentage of cell division				MI ± SD (%)	Mean MI (%)
			Prophase	Metaphase	Anaphase	Telophase		
10 h	Control	177	48.02	25.42	9.04	17.51	8.85 ± 1.02	7.70 ± 1.40
	0.01	162	56.17	20.37	11.73	11.73	8.10 ± 0.84	
	0.05	164	41.46	16.46	21.95	20.12	8.20 ± 0.87	
	0.1	175	41.71	18.29	21.71	18.29	8.75 ± 1.18	
	0.5	122	35.25	35.25	9.84	19.67	6.10 ± 0.74 **	
	1	124	41.13	18.55	16.94	23.39	6.20 ± 0.54 **	
24 h	Control	177	48.02	25.42	9.04	17.51	8.85 ±1.02	5.25 ± 2.30
	0.01	131	54.96	12.21	22.14	10.69	6.55 ±0.77 **	
	0.05	105	44.76	25.71	10.48	19.05	5.25 ±0.63 **	
	0.1	112	38.39	28.57	18.75	14.29	5.60 ± 0.65 **	
	0.5	50	32.00	28.00	14.00	26.00	2.50 ± 0.25 **	
	1	55	20.00	30.91	40.00	9.09	2.75 ± 0.39 **	
48 h	Control	177	48.02	25.42	9.04	17.51	8.85 ± 1.02	4.60 ± 2.50•
	0.01	104	42.31	18.27	24.04	15.38	5.20 ± 0.62 **	
	0.05	111	41.44	26.13	16.22	16.22	4.70 ± 0.48 **	
	0.1	74	36.49	16.22	10.81	36.49	4.75 ± 0.41 **	
	0.5	54	38.89	25.93	25.93	9.26	2.30 ± 0.20 **	
	1	37	40.54	18.92	16.22	24.32	1.85 ± 0.22 **	

Total numbers of dividing cells were determined by counting among the 2000 cells for each treatment

* - significantly different from control ($p<0.05$)

** - significantly different from control ($p<0.05$ and $p<0.01$)

• - significantly different from 10- and 24-h

MI: Mitotic index

SD: Standard deviation

The percentages (%) of prophase, metaphase, anaphase and telophase for 10-, 24- and 48 hour are given (Fig. 1). After exposure to DDVP for 10-hour, averages of dosages of treatment were found 43.95% for prophase, 22.39% for metaphase, 15.20% for anaphase and 18.45% for telophase. After exposure to DDVP for 24-hour, averages of dosages of treatment were found 39.68% for prophase, 25.13% for metaphase, 19.06% for anaphase and 16.10% for telophase. After exposure to DDVP for 48-hour, averages of dosages of treatment were found 41.28% for prophase, 21.81% for metaphase, 17.04% for anaphase and 19.86% for telophase.

According to this study, increasing concentrations of DDVP decreased the number of dividing cells. The results of several studies are parallel with this study. Amer and Ali (1986) showed that DDVP used at concentrations of 32–250 ppm decreased the MI and increased chromosomal aberrations in *Vicia faba* meristem cells. Kontek *et al*. (2007) reported that after the treatment of *Vicia faba* meristem cells with DDVP at all the tested concentrations, the mitotic activity in all series significantly decreased as compared to control, which indicated a mitodepressive effect. The MI test showed that DDVP was approximately 2-fold more cytostatic in comparison with the control (untreated cells). Sarı (2007) applied different doses of DDVP (2 ml/L, 4 ml/L, 6 ml/L) to *Allium cepa* roots with three different application periods (12-, 24- and 48-h) and observed a decreased MI.

Inhibition of the MI following DDVP treatment indicates that it has cytotoxic and mutagenic effects on *H. vulgare*. Inhibition may be with stopping mitotic cycle in interphase result in inhibition of DNA synthesis. DDVP can be decreasing the MI with inhibition of DNA synthesis and affecting as negative direction of DNA amount.

According to these results, it can be concluded that DDVP clearly poses a genotoxic risk. The results of the present study are fundamental results and provide direction for future investigations.

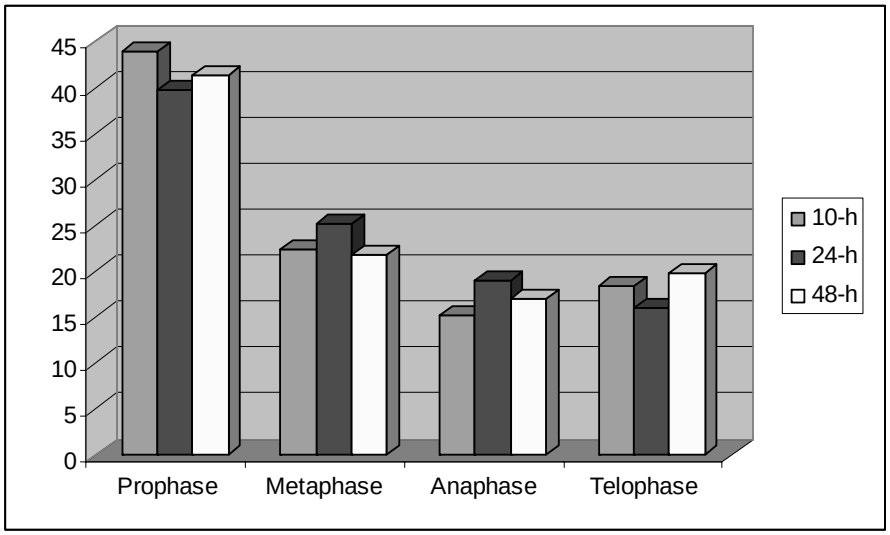

Fig. 1. The percentages (%) of prophase, metaphase, anaphase and telophase for 10-, 24- and 48-hour

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