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Genotoxic Effects of the Insecticide Cypermethrin on the Root Meristem Cells of Sunflowers (*Helianthus annuus* L.)

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Abstract In this study, the genotoxic effects of the insecticide cypermethrin on the root meristem cells of sunflowers (*Helianthus annuus* L.) were investigated. The roots were treated with 10- 25- 50- and 100-ppm concentrations of cypermethrin for 6, 12 and 24 h. The mitotic index and mitotic abnormalities were determined in both control and test groups. The cypermethrin showed a marked mitodepressive action on mitosis. The types of mitotic abnormalities included disturbed metaphase, c-mitosis, stickiness, laggards and chromatid bridges. A pronounced toxic effect was observed at the 50-ppm concentration. Cypermethrin may have genotoxic effects on sunflowers.

Keywords *Helianthus annuus* · Cypermethrin · Cytotoxicity · Genotoxicity · Sunflower

The use of pesticides in current agricultural practices is steadily increasing. The efficiency of the pesticides for the better exploitation of plant species of economic importance is well known (Ajay and Sarbhoy 1987; Inceer et al. 2004). However, the potential side effects of the pesticides, which are mutagenic and/or carcinogenic agents to non-targeted organisms (Wuu and Grant 1966; Fishbein 1972; Grover and Tyagi 1980; Pavlica et al. 1998), are worthy of extended studies in greater depth.

Cypermethrin is one of the synthetic pyrethroid insecticides; it has traditionally been used as a chemical pesticide to control several agriculturally important insect pests (Chauhan et al. 1999; Saxena et al. 2005). However, it has detrimental effects on the environment, especially plants, animals and human beings. Moreover, it is harmful to natural suppressing factors of the insect pests, such as predators and parasitoids. Because the chemical pesticide question is a social issue, the objectives of nutrition, health and environmental quality can be addressed more completely by considering the effects of chemical pesticides on non-target organisms.

The sunflower is an important plant that belongs to the Asteraceae family (McGregor 1976). Cypermethrin is used to control insect pests such as the American bollworm (*Heliothis armigera* Hb.) in sunflower fields at concentrations in the range of 100–125 ppm (MARA 1996).

Recent concern about the hazardous effects of chemical pesticides in the environment has led scientists to consider studying the effects on non-target organisms. Although there are several studies on the effects of cypermethrin on non-target organisms (Bhunya and Pati 1988; Kara et al. 1994; Chauhan et al. 1999), to our knowledge no studies are available on the genotoxic effects of cypermethrin on sunflowers. In the present study we report the effects of cypermethrin on the mitotic cell division and somatic chromosomes of sunflower for the first time.

Materials and Methods

Four different concentrations (10, 25, 50 and 100 ppm) of the cypermethrin (±) alpha-cyano-(3 phenoxyphenyl) methyl (+)-*cis*, *trans*-3-(2,2-dichloroethenyl) 2,2 dimethylcyclo-propane carboxylate were used for cytogenetic assays (Cox 1996, Fig. 1). Test solutions were prepared using tap water. Seeds were placed directly in the test

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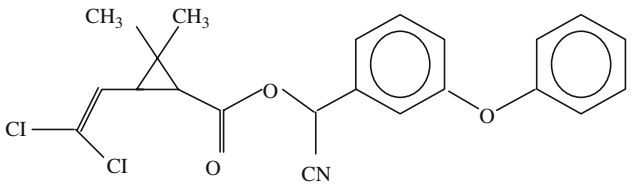

Fig. 1 Structure of cypermethrin. (±) alpha-cyano-(3 phenoxyphenyl) methyl (+)-*cis*, *trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate

solutions, and controls were placed in only tap water. The seeds were treated with the test solutions for 6, 12 and 24 h and were then allowed to recover for 10–15 min in tap water. The seeds were then placed on wet filter paper in petri dishes and left in the dark at 22–23°C (Inceer and Beyazoglu 2000). After germinating, the root tip meristems of both the experimental and control sets were excised and fixed in ethanol-acetic acid (3:1) for cytogenetic analysis following Feulgen's schedule (Darlington and La Cour 1976). For each variable, 5–6 root tip squashes were prepared, and a minimum of 500 mitotic cells were counted from each slides under a light microscope (Leica DM 4000).

Analysis of variance (One-Way ANOVA) of the data was done with the SPSS computer program. The Dunnet t (2-sided) Multiple Range Test was employed to determine the statistical significance of differences among the means. The statistical analysis presented in Table 1 indicates significant variation ($p = 0.05$) in mitotic cells when comparing the number of normal and abnormal cells at each concentration with the control.

Results and Discussion

The genotoxic effects of the different concentrations of cypermethrin on the mitotic cell division of the root tip cells of sunflowers are given in Table 1 and Fig. 2. As shown in Table 1, the insecticide significantly decreased the mitotic index (MI) at 50- and 100-ppm concentrations compared to their controls but not at 10 and 25 ppm, depending on the time (Fig. 2). The reduction in MI at the 100-ppm concentration of cypermethrin after 24 h was 55% compared to the control. Rank and Nielsen (1997) reported that if the LD_{50} value is considered the highest concentration and the others are below the LD_{50} for the genotoxicity test, the MI will not decrease well below 50% of the control.

In addition to the percentage of MI, the results of mitotic abnormalities are shown in Table 1 and Fig. 3. The insecticide treatments resulted in five types of common abnormalities: disturbed metaphase, c-mitosis, stickiness, laggards and chromatid bridges (Fig. 4). Our results reveal that as the concentration of the insecticide increased, the percentage of total abnormalities gradually increased. Similar findings have also been reported by others in other systems (Nandi 1985; Bhunya and Pati 1988; Chauhan et al. 1999). Furthermore, the various pesticides acted

Table 1 Type and percentage of mitotic abnormalities in the root tips of sunflowers exposed to cypermethrin

Time (h)	Concentration (ppm)	MI ± SD	Percent of abnormalities					Total abnormalities (%)
			Disturbed metaphase	C-mitosis	Stickiness	Laggards	Chromatid bridge	
6	Control	27.01 ± 0.82	0.35 ± 0.61	–	–	–	–	0.35
	10	26.07 ± 0.22	1.40 ± 0.79	0.60 ± 0.36	–	0.36 ± 0.24	0.36 ± 0.27	2.72
	25	26.81 ± 0.45	1.30 ± 0.25	0.65 ± 0.33	3.01 ± 0.56*	0.65 ± 0.35	0.65 ± 0.45	6.26
	50	21.13 ± 0.65*	2.73 ± 0.13*	0.92 ± 0.38*	3.53 ± 0.57*	0.80 ± 0.50	0.80 ± 0.60	8.78
	100	20.30 ± 0.52*	2.80 ± 0.15*	1.10 ± 0.20*	2.50 ± 0.60*	0.90 ± 0.50*	0.90 ± 0.51	8.20
12	Control	26.26 ± 0.35	0.42 ± 0.51	–	0.35 ± 0.35	–	0.25 ± 0.25	1.02
	10	25.44 ± 0.22	1.46 ± 0.50	0.20 ± 0.20	2.37 ± 0.41*	0.41 ± 0.40	0.90 ± 0.56	5.34
	25	25.20 ± 0.22	2.07 ± 0.38*	0.41 ± 0.40	2.39 ± 0.48*	0.90 ± 0.40	0.41 ± 0.42	6.18
	50	20.28 ± 0.15*	3.00 ± 0.50*	1.74 ± 0.37*	2.86 ± 0.59*	1.15 ± 0.49*	1.15 ± 0.49	9.90
	100	18.95 ± 0.47*	4.52 ± 0.53*	2.25 ± 0.38*	3.58 ± 0.58*	1.50 ± 0.50*	2.30 ± 0.50*	14.15
24	Control	24.30 ± 0.20	–	–	0.37 ± 0.35	0.23 ± 0.15	0.43 ± 0.25	1.03
	10	23.82 ± 0.24	1.84 ± 0.41	1.05 ± 0.80	2.16 ± 0.56*	0.74 ± 0.46	0.74 ± 0.46	6.53
	25	20.58 ± 0.37	2.25 ± 0.35*	1.31 ± 0.57*	4.39 ± 0.55*	1.52 ± 0.60*	1.02 ± 0.32	10.49
	50	15.50 ± 0.55*	3.99 ± 0.49*	1.63 ± 0.35*	5.90 ± 0.46*	1.40 ± 0.50*	1.40 ± 0.40*	14.32
	100	10.96 ± 0.75*	6.50 ± 0.60*	3.60 ± 0.50*	7.32 ± 0.67*	4.56 ± 0.58*	3.60 ± 0.50*	25.58

* Significant from the control $p = 0.05$

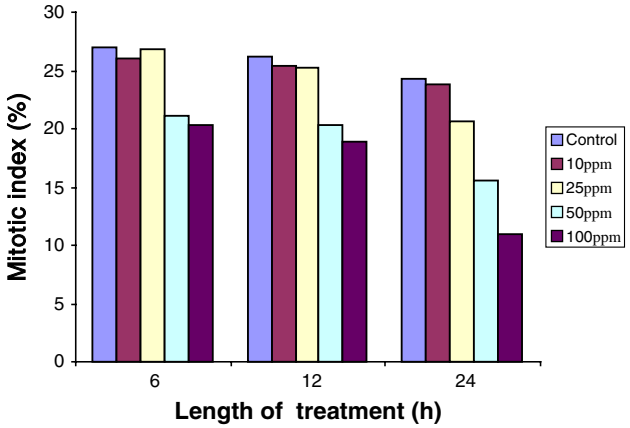

Fig. 2 Frequency of mitotic index (MI) in the root tips of sunflowers exposed to cypermethrin

differently in disturbing the spindle apparatus. The inhibition of spindle formation has been shown to lead to severe abnormalities such as c-mitosis, stickiness, unequal distribution, chromatid bridges and laggards (Inceer et al. 2004).

It is known that some insecticides inhibit mitosis in different plants. These include some synthetic pyrethroids (Chauhan et al. 1986) and organophorus (Rao et al. 1987) insecticides in *Allium cepa*, methyl parathion, dimethioate, oxydemeton methyl, azinphos methyl and phoxim (Gòmez-Arroyo et al. 1988), malathion and tamaron (Zakia et al. 1990) in *Vicia faba*; and basudin in *Hordeum vulgare* (Çelik and Sümer 1996). Such a significant decreasing in the MI indicates that cypermethrin interferes with the normal sequence of the cell cycle to reduce the number of cells starting to divide at interphase (Badr 1986). The reduction in the mitotic activity could be due to the inhibition of DNA synthesis, which is one of the major prerequisites for a cell to divide (Sadia and Vahidy 1994; Yuzbasıoglu et al. 2003; Inceer et al. 2004). Saxena et al. (2005) also showed that exposing the root tips of *Allium sativum* to cypermethrin led to the destabilization of the DNA structure and unwinding of the DNA helix, thereby

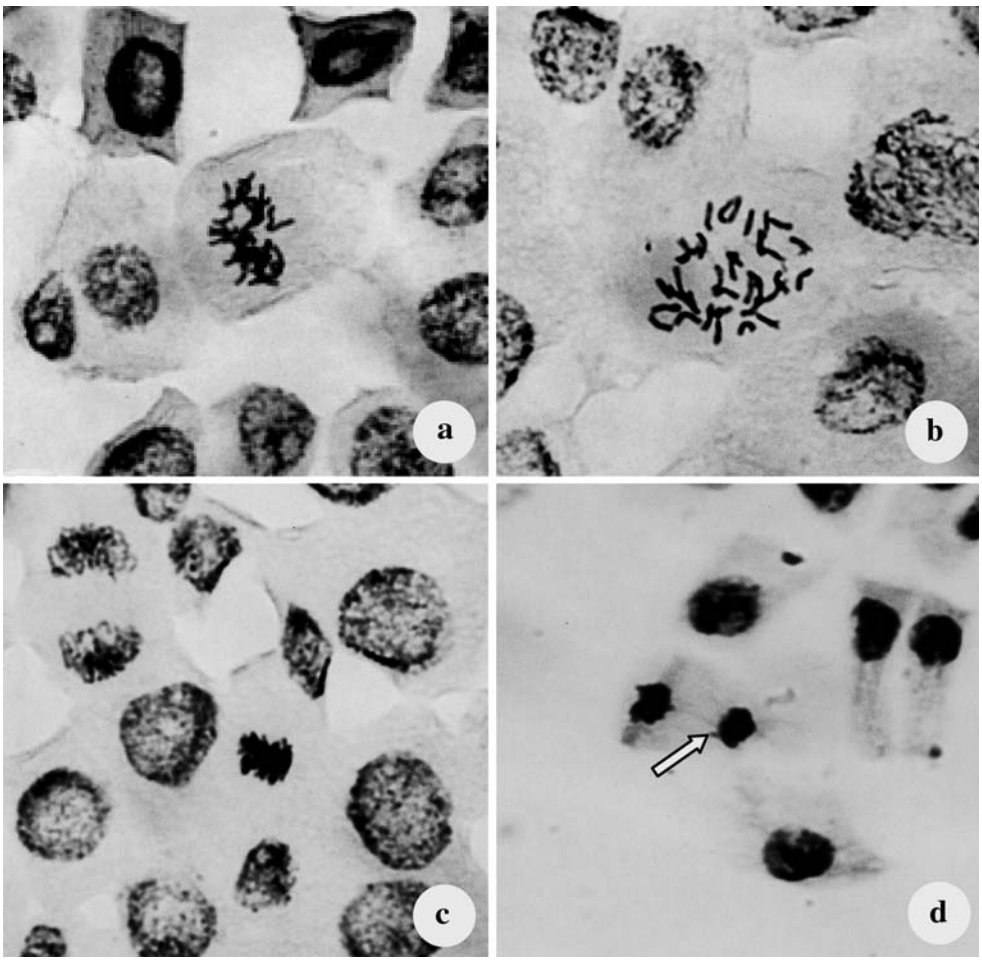

Fig. 3 Mitotic abnormalities induced by cypermethrin in root meristem cells of sunflowers: **a** distributed metaphase, **b** c-mitosis, **c** stickiness, **d** laggards (*arrow*). 500×

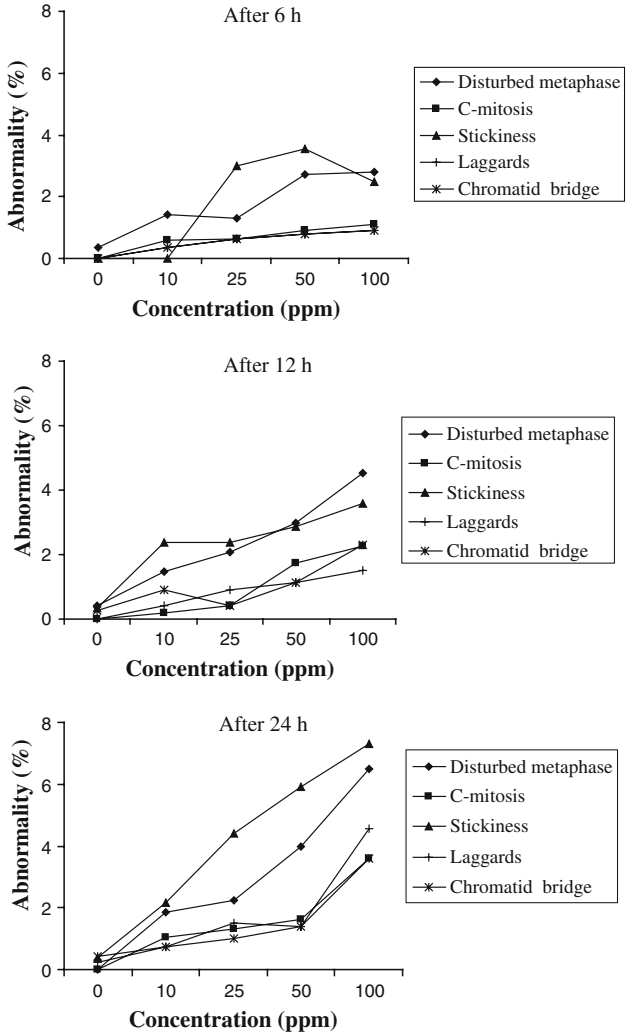

Fig. 4 Percentage of abnormalities with distributed metaphase, c-mitosis, stickiness, laggards and chromatid bridges in sunflowers after cypermethrin treatments

inducing chromosomal damage. These citations suggest that cypermethrin could have the same effect on mitotic cell division in sunflowers.

Rank and Nielsen (1997) reported that if a chemical is able to cause damage to the chromosomes in a reliable plant assay, then the chemical should be considered as having the potential to damage the chromosomes of other organisms in the environment. In the present study, clastogenic types of the insecticide cypermethrin should be regarded as an agent that shows mutational activity in sunflowers.

In conclusion, the present results indicate that cypermethrin, like other pesticides in the environment, can be absorbed by higher plants and may adversely affect their genomes, thus cause damage to plants. Wide application of insecticides for the control of insects in agricultural practices is a potential threat to the genetic constitution of economically important plants like sunflowers. Hence, it is necessary to test the genotoxic effects of insecticides on plants and other systems before considering their applications for agricultural purposes.

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