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Effects of the Insecticide Pyrethroid II in the Ames Test, and on *Hordeum vulgare* and *Vicia faba*

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Abstract. The insecticide pyrethroid II, representing synthetic pyrethroids of the second generation, was not found to be mutagenic in *Salmonella typhimurium* strains TA1535, TA100, TA1538, TA98 and TA97. High concentrations of the tested insecticide inhibited the germination and length of roots of germinating seeds, the height of plants cultivated *in vitro*, and slightly increased the frequency of aberrant anaphases and telophase in root-tips of *Vicia faba* and *Hordeum vulgare*.

The synthesis of biologically active and photostable pyrethroids promoted the research aimed at their applicability as insecticides (Elliot 1978, Casida *et el.* 1983, Scott *et al.* 1978).

Pyrethroids of the first generation, derived from natural pyrethrins are effective insecticides, but their utilization in agriculture is limited owing to their low photostability. But the synthetic pyrethroids of the second generation *e. g.* permethrin, resmethrin, cypermethrin, bioresmethrin, fenvalerate, deltamethrin, cismethrin and the third generation are photostable (Casida *et al.* 1983) and relatively non-toxic to man (Elliot 1978). Because of the great agricultural potential of pyrethroids (Harris and Turnbull 1978) we aimed our work at the evaluation of the genotoxic and some biological effects of the developing insecticide pyrethroid II in different test systems.

MATERIALS AND METHODS

Test Compound

Liquid form of the synthetic pyrethroid II was kindly provided by the Research Institute of Chemical Technology in Bratislava (Czechoslovakia) in the form of

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so-called technical pyrethroid II. The emulsion concentrate contains 15 % of the effective compound (supercypermethrin). The test compound belongs to the developing series of synthetic insecticide preparations and is very similar to the synthetic pyrethroids alfa-cypermethrin and cypermethrin. For its dilution dimethyl sulfoxide (DMSO) was used.

1. Bacterial Assay (The Ames test)

Five *Salmonella typhimurium* strains were used (TA1535, TA100, TA1538, TA98 and TA97) as were recommended by Maron and Ames (1983), Claxton *et al.* (1987). Media were prepared according to Ames *et al.* (1975) in agreement with the revised method of Maron and Ames (1983).

Diagnostic Mutagens

2-aminofluorence (2AF), 9-aminoacridine (9AC) and 4-nitroquinoline (4NONO) were used as positive control. S9 mix was prepared according to the standard method described by Maron and Ames (1983). The S9 fraction was prepared from Delor 103 pretreated male rats (strain Wistar SPF).

Testing Procedure

The plate incorporation test (Ames *et al.* 1975, Maron and Ames 1983, Claxton *et al.* 1987) was used as a testing assay.

2. Plant Assays

Hordeum vulgare, cv. Fatran and a new cultivar SK2611 and *Vicia faba* cv. Inovec and a new cultivar HS3168–75 were used.

Pyrethroid II was tested on both plant species after a 24 h exposition of seeds in three concentrations. The effect of pyrethroid II was evaluated after seed treatment:

- on early stadia of ontogenesis
- in culture of embryos *in vitro*
- in root meristems as based on frequency of chromosome aberrations

Affecting Early Stadia of Ontogenesis

The seeds were soaked in 0.1 %, 0.05 % and 0,01 % pyrethroid II at room temperature. The concentrations were selected according to their toxicity. In each experimental variant 300 seeds, and 100 seeds in the control, were treated. The seeds germinated (following their washing for 2 minutes with distilled water) on wet filter paper and gauze in an incubator at the temperature of 25 ± 1 °C. After 96 h, the total germination and the length of roots were evaluated.

Embryogenesis *in vitro*

The culture *in vitro* was established after 24 h of seed treatment and the sterile isolation of embryos. For a primoculture medium : Murashige-Skoog + 1 % saccharose + 2,4-dichlorophenoxyacetic acid (2,4-D) 2 $mg.l^{-1}$ was used. Embryos were cultivated at temperature of 22 °C and 16 h photoperiod. In each variant, 240 embryos were planted on the medium without growth factors and 60 embryos on the medium with 2,4-D. After 10 days of cultivation, the height of plants in the culture and possible differentiating effect of pyrethroid II in comparison with 2,4-D were evaluated.

Chromosome Aberrations

The frequency of chromosome aberrations were studied on squash preparations prepared from root tips after treatment with pyrethroid II. The roots were fixed in

Table 1.

Evaluation of the plate incorporation assay with pyrethroid II using *Salmonella typhimurium* strains with or without activation by liver S9 from delor 103-treated rats.

Compound tested	Concentration [μg per plate]		Mean number of His^+ revertants per plate				
			TA 1535	TA 100	TA 1538	TA 98	TA 97
Control (DMSO)		− S9	13 ± 3	87 ± 2	15 ± 1	14 ± 1	75 ± 1
		+ S9	16 ± 1	122 ± 4	23 ± 2	24 ± 1	116 ± 3
Pyrethroid II	750	− S9	7 ± 1	70 ± 4	12 ± 1	8 ± 1	62 ± 4
		+ S9	12 ± 2	79 ± 1	17 ± 1	23 ± 1	120 ± 5
Pyrethroid II	150	− S9	13 ± 1	81 ± 4	14 ± 0	12 ± 0	68 ± 3
		+ S9	15 ± 4	110 ± 3	24 ± 1	25 ± 1	130 ± 6
Pyrethroid II	75	− S9	10 ± 2	92 ± 4	13 ± 1	14 ± 1	74 ± 6
		+ S9	14 ± 2	120 ± 2	22 ± 0	26 ± 3	120 ± 3
Pyrethroid II	15	− S9	12 ± 2	84 ± 2	15 ± 1	14 ± 3	77 ± 4
		+ S9	14 ± 1	123 ± 3	26 ± 4	23 ± 1	112 ± 6
4NQNO	0.2	− S9	27 ± 2	409 ± 26	16 ± 1	40 ± 3	93 ± 8
2AF	100	+ S9	460 ± 37	674 ± 33	503 ± 21	637 ± 31	490 ± 20
9AC	100	− S9	12 ± 1	80 ± 3	13 ± 1	15 ± 1	508 ± 35

The numbers given are mean values (mean ± S.D.) of three experiments with three plates per each concentration in each experiment

ethanol – acetic acid (3 : 1) after 48–72 h when they were about 0.5–1 cm long and stained according to the Feulgen method, chromosome aberrations found in anaphase (A) and telophase (T) were divided into three categories: fragments, bridges and other changes (lagging chromosomes, bridges with fragments, more fragments). The significance of differences between treatments and respective controls were evaluated by Student's t -test.

RESULTS

1. Bacterial Assay

Pyrethroid II showed a toxic effect at the two highest concentrations used (1500 μg and 750 μg per plate) in all bacterial strains. Its toxicity was reduced in the presence of S9 mix.

Table 1 shows the frequencies of His^+ revertants after application of pyrethroid II in a dose range of from 15 to 750 μg per plate in the absence and the presence of mammalian metabolic activation system. At the highest concentration used in the

Table 2

Evaluation of the effect of pyrethroid II on early stadia of ontogenesis (mean values of germination and mean values of root length).

Plant tested	Concentration [%]	Germination [%]	Length of roots [cm]	[%]
Hordeum vulgare				
cv. Fatran	0.1	31.0	2.5	43.9
	0.05	33.3	2.8	49.1
	0.01	73.0	3.7	64.9
	Control – DMSO	100.0	5.7	100.0
cv. SK 2611	0.1	14.5	2.2	38.2
	0.05	24.7	2.3	41.8
	0.01	57.2	3.8	69.1
	Control – DMSO	100.0	5.5	100.0
Vicia faba				
cv. Inovec	0.1	100.0	1.9	109.8
	0.05	96.7	2.0	117.6
	0.01	93.2	2.0	117.6
	Control – DMSO	100.0	1.7	100.0
cv. HS 3168-75	0.1	99.2	2.1	78.1
	0.05	100.6	2.2	84.6
	0.01	100.0	2.0	76.9
	Control – DMSO	100.0	2.6	100.0

absence of S9 mix the frequency of revertants with respect to the control due to the toxicity of test compound was reduced. From the data presented in Table 1 it follows that pyrethroid II did not induce mutations in any bacterial strain used.

2. **Plant Assays**

Evaluation of the effect of pyrethroid II on early stadia of ontogenesis shows that in barley the insecticide reduces the % of germination and the length of roots. This reduction depends on the dose used and is more pronounced in the cultivar SK 2611. In both cultivars of bean, the germination was less influenced and the values of root length were even slightly increased after treatment in the cultivar Inovec (Table 2).

In experiments with cultivation of embryos after the treatment *in vivo* it was found that pyrethroid II did not induce calluses or any morphological variations. But in the early stadia of embryo development *in vitro*, the treatment of seeds before the

Table 3

Evaluation of the effect of pyrethroid II on embryocultures after 10 days of cultivation *in vitro*

plant tested	Concentration [%]	% of survival	Mean value of plant height [cm]	% of control	t	*p*
Hordeum vulgare						
cv. Fatran	0.1	70.0	2.6	49.5	12.22	**
	0.05	81.6	3.3	61.5	10.41	**
	0.01	85.0	4.3	80.1	5.01	**
	Control – DMSO	87.9	5.4	100.0		
cv. SK 2611	0.1	70.4	2.5	50.0	11.95	**
	0.05	82.3	3.5	69.4	9.87	**
	0.01	84.5	4.2	82.3	5.11	**
	Control – DMSO	86.2	5.1	100.0		
Vicia faba						
cv. Inovec	0.1	57.1	1.1	34.4	4.94	**
	0.05	56.1	1.7	53.1	3.26	**
	0.01	58.4	1.8	56.2	2.90	**
	Control – DMSO	64.2	3.2	100.0		
cv. HS 3168-75	0.1	52.1	1.0	30.9	6.72	**
	0.05	57.5	1.3	38.2	4.94	**
	0.01	56.0	1.3	39.7	3.53	**
	Control – DMSO	71.5	3.4	100.0		

**Differences are significant to the respective control ($p < 0.01$ in Student's t test)

isolation of embryos reduced their growth (Table 3). The decrease in the % of survival and in the height of plants is also statistically significant.

The cytogenetic analysis of meristematic cells in root tips of both plant species revealed a slight increase in the frequency of chromosome aberrations after pyrethroid II treatment (Table 4). Bridges were the most numerous category in allmost all experimental variants.

DISCUSSION

Technical pyrethroid II did not display mutagenic activity on five *Salmonella* strains in the Ames test. The results are in accordance with those obtained by testing pyrethroids of the second generation (*e. g.* cypermethrin, permethrin, deltamethrin, bioresmethrin, resmethrin, cismethrin, fenvalerate) with *Salmonella typhimurium*, *Escherichia coli*, *Bacilus subtilis*, *Saccharomyces cerevisiae* human fibroblast and V79 Chinese hamster cells (Miyamoto 1976, Kavlock *et al.* 1979, Simmon *et al.* 1979, Pluijmen *et al.* 1984).

Table 4

Frequency of chromosome aberrations in anaphase (A) and telophase (T) of mitosis after the treatment with pyrethroid II

Plant tested	Concentration [%]	Number of A + T evaluated	Frequency of aberrant A + T [%]	Frequency of chromosome aberrations		
				Number of fragments	Number of bridges	Number of others
Hordeum vulgare						
cv. Fatran	0.1	519	3.27 ± 0.78	5	11	2
	0.05	512	2.19 ± 0.63	1	10	0
	0.01	507	3.55 ± 0.82	5	13	0
	Control – DMSO	519	0.38 ± 0.27	0	2	0
cv. SK 2611	0.1	501	2.79 ± 0.79	2	11	1
	0.05	504	2.75 ± 0.73	3	11	0
	0.01	502	2.39 ± 0.68	3	9	0
	Control – DMSO	502	0.39 ± 0.27	0	2	0
Vicia faba						
cv. Inovec	0.1	503	10.93 ± 1.39	24	21	10
	0.05	501	6.58 ± 1.10	7	21	5
	0.01	507	5.71 ± 1.03	4	20	5
	Control – DMSO	514	0.39 ± 0.27	0	1	1
cv. HS 3168-75	0.1	500	5.00 ± 0.97	8	15	2
	0.05	521	4.03 ± 0.86	6	14	1
	0.01	526	5.89 ± 0.86	14	13	4
	Control – DMSO	548	0.36 ± 0.26	0	1	1

Pluijmen *et al.* (1984) found out that in the absence of an exogenous metabolic system, bioresmethrin, resmethrin and cismethrin were at certain concentrations very toxic to Chinese hamster cells, but in the presence of rat hepatocytes no toxicity was observed. In our experiments on bacteria, pyrethroid II was toxic at the two highest concentrations in the absence of mammalian metabolic activation, and in the presence of S9 mix the toxicity was reduced. This protective effect is similar to that of rat hepatocytes (Pluijmen *et al.* 1984) and may be attributable to the effective metabolic detoxication of pyrethroids.

Nowadays it is recommended to evaluate the potential mutagenic effect of pesticides on plant test systems. Testing the toxic and morphogenic effects and its cytogenetic activity can be supposed to be one of the first steps of testing the biological activity of pesticides.

In spite of the fact that almost all pesticides applied at higher consentrations usually cause the growth inhibition of plants and the physiological damage of a cell that can be manifested by damaged mitosis, and our concentrations of pyrethroid II used in plant assays were higher than those that were planned to be used in practice, its producer, taking into consideration our results and its relatively high toxicity (Sandal 1990, personal communication) decided to replace pyrethroid II with another preparation of the same series.

REFERENCES

Ames, B. N., Mc. Cann, J., Yamasaki, E.: Methods for detecting carcinogens and mutagens with *Salmonella* (mammalian-microsome mutagenicity test). – Mutat. Res. **31** : 347–364, 1975.

Casida, J. E., Gammon, D. W., Glickman, A. H., Lawrence, L. J.: Mechanism of selective action of pyrethroid insecticides. – Annu. Rev. Pharmacol. Toxicol. **23** : 413–438, 1983.

Claxton, L. D., Allen, J., Auletta, A., Mortelmans, K., Nestman, E., Zeiger, E.: Guide for *Salmonella typhimurium* (mammalian-microsome mutagenicity test). – Mutat. Res. **113** : 173–212, 1987.

Elliot, M.: Future use of natural and synthetic pyrethroids. – Gen. Pharmacol. **9** : 387–398, 1978.

Harris, L. R., Turnbull, L. A.: Laboratory studies on the contact toxicity and activity in soil of four pyrethroid insecticides. – Can. Entomol. **110** : 285–288, 1978.

Kavlock, R., Chernoff, N., Baron, R., Linder, R., Rogers, E., Caver, B., Dilley, J., Simmon, V.: Toxicity studies with decamethrin, a synthetic pyrethroid insecticide. – J. environ. Pathol. Toxicol. **2** : 751–765, 1979.

Maron, D. M., Ames, B. M.: Revised methods for *Salmonella* mutagenicity test. – Mutat. Res. **113** : 173–215, 1983.

Miyamoto, J.: Degradation, metabolism and toxicity of synthetic pyrethroids. – Environ. Health Perspect. **14** : 15–18, 1976.

Pluimen, M., Drevon, C., Montesano, R., Malaveille, C., Hautefeuille, A., Bartsch, H.: Lack of mutagenicity of synthetic pyrethroids in *Salmonella typhimurium* strains and V79 Chinese hamster cells. – Mutat. Res. **137** : 7–15, 1984.

Scot, B. R., Kasica, V. A., Watus, M. D., Evans, E. L., Mitchell, A. D., Mortelmans, K. E.: Mutagenicity of diallate and triallate herbicides in a battery of a short-term tests. – Environ. Mutag. **3** : 203–212, 1978.

Simmon, V. F., Riccio, E. S., Robinson, D. E., Mitchell, A. D.: *In vitro* Microbiological Mutagenicity and Unscheduled DNA Synthesis Studies of Fifteen Pesticides. Final Report – Phase III. – SRI Project LSU–3493, 170, 1979.