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Metolachlor and 2,4-Dichlorophenoxyacetic Acid Sensitivity of *Salvinia natans*

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The free-floating freshwater fern *Salvinia natans* grows abundantly in ponds and lakes of India and is able to remove mercury (II) and copper (II) from waste water (Sen and Mondal 1987, 1990). In central and east Europe it is very rare. In Slovenia it is on the red list of threatened plants and is distributed in lakes, surrounded by fields, most frequently treated with herbicides metolachlor and 2,4-dichlorophenoxyacetic acid (2,4-D). We report the effects of these two herbicides on growth, chlorophyll production and morphological changes of *Salvinia natans* in chemically defined and sterile conditions.

MATERIALS AND METHODS

The spores of *Salvinia natans* were collected from the Petišovci lake, east Slovenia (46°31'18"N, 16°27'10"E) and stored at 4°C during the winter. In spring (May), the mixture of microspores and macrospores were sterilized by pre-washing with tap water, dipping into 70% ethanol for 5 seconds, followed in 1% NaClO for 5 min and rinsed 3 times with sterile tap water (Pierik, 1987). Sterilized spores were suspended in sterile Knop's medium (Von Denffer et al. 1978) supplemented with 6.2 mg/L of Na-Fe-EDTA (Knop's Na-Fe-EDTA medium). Germination was performed at room temperature (22 ± 2°C) and day-light illumination (14.8 ± 0.8 hr light/9.2 ± 0.8 hr dark photoperiod). After 70 days, young freshwater ferns with 8 - 22 leaves were obtained.

Three plants with 14 ± 1.3 leaves were transferred into each of three glasses containing 1, 0.1 and 0.01 mg/L of metolachlor (commercially available Dual® 500 EC; T.D. Ruše, Slovenia) and into five glasses containing 100, 10, 1, 0.1 and 0.01 mg/L of 2,4-D in the form of dimethylamino salt (commercially available Deherban A®; Chromos, Zagreb, Croatia). No herbicide was added for control. The herbicides were diluted in 300 ml of sterile Knop's Na-Fe-EDTA medium and glasses

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were covered with aluminum foil. The plants were exposed for four weeks to daily sun light (14.3 ± 1.5 hours) and temperature 23 ± 4.1°C. Observations of the change in growth (length of plants and number of green leaves), of drying the leaves, drying of stems and injuries in development were made weekly. After four weeks, the biomass was determined as wet weight and the amount of chlorophyll$_a$ (Chl_a) and chlorophyll$_b$ (Chl_b) was estimated after the method of Hodgins and Huystee (1986) by measuring the absorbance at wavelenghts 645 and 663 nm on UV-VIS Perkin Elmer 552 spectrophotometer. The results of the fourth week were plotted against the log of herbicide concentration to obtain EC_{50} values (effective concentrations of 50% inhibition). Student's "t" test (Fisher, 1950) was employed to calculate the statistical significance between the control and experimental values.

RESULTS AND DISCUSSION

Sterilization of spores at described conditions insignificantly reduced their germination (data not shown). The sterilization of whole plants was repoted for *Salvinia rotundifolia* (Gaudet and Koh, 1968) an *Salvinia auriculata* (Rao and Narayana, 1968).

By exposing the young plants to the herbicide metolachlor, the most affected were the growth of leaves (Fig. 1) and amount of Chl_a and $_b$ in comparision to wet weight and length of stems (Table 1). The apparent EC_{50} values were (mg/L): 0.075, 0.08, 0.05, 0.15 and 0.55, respectively. Heuer et al. (1991) reported that 0.05 mg/L of metolachlor after 3 weeks reduced the growth of cucumber plants shoots and roots by 30-50%. Morphological changes of *Salvinia natans* at concentration of metolachlor 0.1 mg/L were observed after two weeks as thin side sprouts with undeveloped leaves, where after four weeks small leaves appeared. At a concentration of 1 mg/L of metolachlor, the plants after the first week were dwarfed with deep green downwards folded leaves. In the second week, 32 ± 8% leaves (the aldest) were dry and after four weeks, all the leaves were dry, but the stems remained green.

In comparison to metolachlor, the 2,4-D was about 10 times less active (Fig. 1, Table 1) and also different morphological changes were observed. The EC_{50} for growth of leaves, wet weight, length of stems and amount Chl_a and $_b$ was (mg/L): 6, 6.5, 6.5, 0.3 and 0.3, respectively. From these results it is evident, that 2,4-D mostly affected the amount of chlorophylls. At concentration of 0.01 mg/L of 2,4-D also the ratio Chl_a/Chl_b was changed (Table 1). Morphological changes at the concentration of 10 mg/L of 2,4-D were observed after the first week as curled roots, hairless leaves of half width and sunken new sprouts. In the second week the developed stems were winded and some new

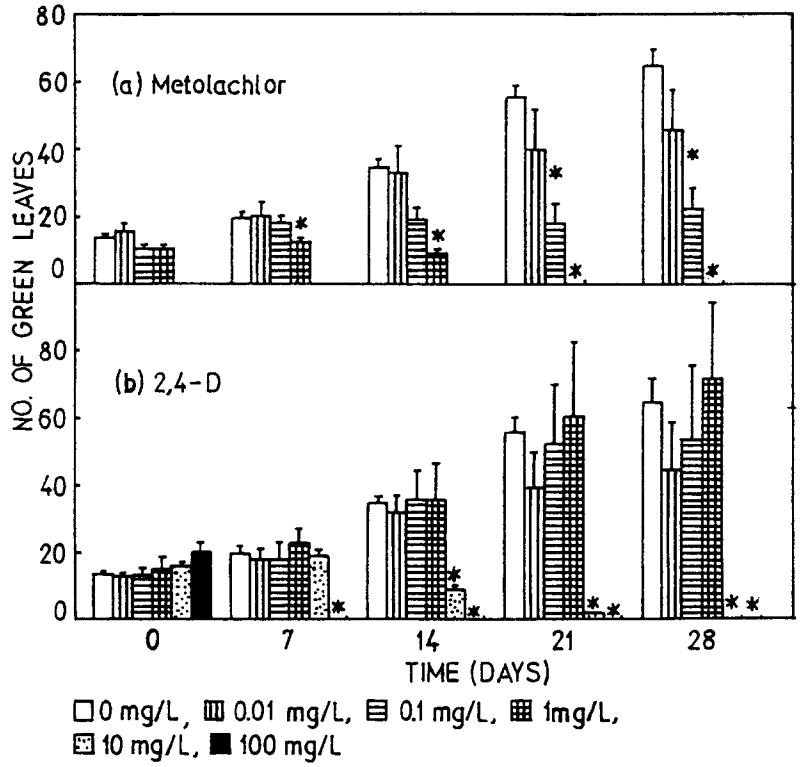

Figure 1. Leaf number of *Salvinia natans* at various concentrations of metolaclor (a) and 2,4-D (b). Values are mean ± S.E. of triplicate. * = $p < 0.05$.

Table 1. Effect of metolachlor and 2,4-D on wet weight, length and amount of chlorophyll$_a$ and $_b$ in *Salvinia natans* after four weeks of exposure. Each value is arithmetic mean ± S.D. of triplicate. * = $p<0.05$.

Herbicide	Herbic. conc. (mg/L)	Wet weight (g)	Length (cm)	Chlorophyll$_a$ (µmol)	Chlorophyll$_b$ (µmol)
Metolachlor	0.0	0.21±0.03	11.0±1.8	152±21	71±10
	0.01	0.30±0.14	10.6±6.6	119±55	46±21
	0.1	0.22±0.01	11.0±4.5	69±38*	29±16*
	1.0	0.04±0.00*	2.3±0.8*	6± 0*	3± 0*
2,4-D	0.01	0.17±0.01	9.0±4.6	80±47	36±21*
	0.1	0.23±0.14	11.3±8.0	107±75	48±34
	1.0	0.30±0.17	11.3±7.5	34±20*	14± 8*
	10.0	0.05±0.01*	4.6±1.5*	1± 0*	0± 0*

leaves were rounded. At the lower concentration (1 mg/L) no morphological changes and an increase in

wet weight (at $p<0.5$) by decreased amount of Chl a and b (at $p<0.5$) was detected. Similar results were obtained for *Salvinia rotundifolia* in sterile culture, where 2,4-D at the concentration above 1 mg/L inhibited the growth, but at lower concentrations it was increased (Gaudet and Koh, 1968). The amount of chlorophylls there was not measured. 2,4-D at concentrations above 0.5 mg/L also inhibited the growth of *Salvinia auriculata* in sterile medium (Rao and Narayana, 1968).

Salvinia natans in the Petišovci lake showed no signs of morphological changes, as could be expected from the report of Buttle and Harris (1991). In their experiment, the stream flow surrounded by a metolachlor treated field reached peak with the concentration of 125 µg/L of this herbicide, while the water from the tile drainage under the same field reached the peak of 70 µg/L of the same herbicide. These peaks were indeed of short duration. Further questions that need to be answered are related to the effects of a metolachlor-2,4-D mixtures and susceptibility of *Salvinia natans* developmental stages.

REFERENCES

Buttle JM, Harris BJ (1991) Hydrological pathways of metolachlor export from an agricultural watershed. Air Soil Poll 60:315-335

Fisher RA (1950) Statistical method for research work (11th ed) Oliver and Boyd, Edinburgh

Gaudet JJ, Koh DV (1968) Effect of various growth regulators on *Salvinia rotundifolia* in sterile culture. Bull Torrey Bot Club 95:92-102.

Heuer B, Brates N, Saltzman S (1991) Growth and herbicide accumulation in cucumber plants exposed to residual concentrations of metolachlor in their nutrient media. J Environ Sci Health B26:209-217

Hodgins RR, Van Huystee RB (1986) Rapid simultaneous estimation of protoporphyrin and Mg-porphyrins in higher plants. J Plant Physiol 125:311-323

Pierik RLM (1987) In vitro culture of higher plants. Martinus Nijhoff publishers, Dordrecht, Boston, Lancaster

Rao YRM, Narayana R (1968) Effect of mineral nutrition and auxins on *Salvinia auriculata* Aublet, grown in cultures. J Mysore Univ Sect B:136-143

Sen AK, Mondal NG (1987) *Salvinia natans* - as scavanger of Hg(II). Wat Air Soil Poll 34:439-446

Sen AK, Mondal NG (1990) Removal and uptake of copper(II) by *Salvinia natans* from waste water. Wat Air Soil Poll 49:1-6

Von-Denffer D, Ehrendorfer F, Maggdefrau K, Ziegler H (1978) Lehrbuch der Botanik fur Hochschulen. Gustav Fischer Verlag, Stutgart, New York, p 331