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Sensitivity of the Rooted Macrophyte *Myriophyllum aquaticum* (Vell.) Verdcourt to Seventeen Pesticides Determined on the Basis of EC_{50}

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The runoff of pesticides is a problem in agricultural areas since the chemicals may contaminate surface water, be dissolved in runoff water or be absorbed in soil particles. The residues of pesticides can often be detected in sediment and in water (Khim et al. 2001; Lerch et al. 1999; Pandit et al. 2001).

For the risk assessment of pesticides in most cases, algae have been used as representative of aquatic plants in pesticide registration procedures in many countries. Duckweed are also recommended because they are higher plants and have been shown to be more sensitive for most pesticides than commonly tested algal species (Fairchild et al. 1997; Fairchild et al. 1998; Grossmann et al. 1992). Nevertheless, duckweed are not representative of all plants because they cannot be used to assess the effect of pesticides in sediments. Pesticides can accumulate in the sediment and present a potential risk especially for rooted macrophytes. Thus, a test with a rooted macrophyte is beneficial to the existing pesticide testing requirements and helps protect the non-target aquatic environment. Such a test has been required for a long time (Fairchild et al. 1997; Fletscher, 1991; Freemark et al. 1990; Keddy et al. 1995). Recent investigations demonstrated that some species of *Myriophyllum* are most suitable plants for testing pesticide toxicity. Standardized protocols have been developed for *M. sibiricum* (Roshon et al. 1999) and for *M. aquaticum* (Turgut and Fomin 2001). Before a new test is included in law and regulations, an extensive database is needed to register the range of sensitivity of pesticides on this organism.

The aim of this study was to determine the sensitivity of *M. aquaticum* to 17 pesticides using several endpoints. The contents of chlorophyll a, chlorophyll b, carotenoid and increases in shoot length, root number, total root length, fresh weight, side shoot number and side shoot length were measured. EC_{50} values were determined on the basis of dose response relationships of the active substances in pesticides. The results have been compared with another macrophyte discussed in the literature.

MATERIALS AND METHODS

Myriophyllum aquaticum (Vell.) Verdcourt, a dicotyledonous species, was selected

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as a representative submersed rooted aquatic macrophyte. An axenic culture of *M. aquaticum* was established aseptically, stock cultures were maintained by cutting 1 cm long stems into segments and transferring them into culture vessels (Turgut and Fomin 2001). After 4-6 weeks, 3 cm long axillary buds from stock plants were transferred into the culture tubes (200x25 mm) containing 50 mL of sterile liquid growth medium supplemented with 3% sucrose and filled with 5 g Turface®. All experiments were conducted using 50 mL of sterile Hoagland nutrient medium (Selim et al. 1989), with 30 g/L sucrose added.

Plants were incubated for 14 days at about 25 °C for 16 hr with a light intensity 120-180 μmol m^{-2}. s^{-1} and 18-20 °C during an 8 h dark period following the procedure used by Roshon et al. (1996). After that, plants were exposed to 7 or 8 pesticide concentrations. With the aid of the percentage of pesticide formulation (see Table 1) it was possible to determine the active components. Each concentration was replicated five times. All tested pesticides were purchased from Germany and their chemical class and formulation of the applied pesticides are listed in Table 1. The percent of active ingredients of pesticides in formulations is given in Table 1 but the inert components are unknown. Appropriate control measures were taken in each experiment. The test tubes were covered with sterile plain closures. All pesticides were dissolved in water.

After the beginning of experiment, plant length was established on alternate days. The plant length data (mm) were used to establish growth curves. The area under growth curve was calculated by

$$\text{Area under Curve} = \sum_{i=2}^{n} \frac{IH_{i-1} + IH_i}{2} \cdot (T_i - T_{i-1})$$

Where
IH_i= the increase in height from the start of experiment, and
T= the time at each subsequent measurement point, in hours from time zero (Roshon et al. 1996)

After the fourteen days of exposure, the plants were removed from the test tubes and the shoots, total root length, root number, side shoot number and length were measured by sight and recorded. 50 mg apical segment were weighed and placed into 10 mL of 96% ethanol and stored in the dark at 4 °C for 24 hours and measured with a spectrophotometer at 470, 649 and 665 nm (Lichtenthaler and Wellburn 1989). The EC_{50} values (pesticide concentration required to cause 50% reduction in different endpoints) were calculated using non-linear regression analysis using transformed concentrations of the active components of each pesticide (Sigma Plot version 4.0), following an approach similar to that of Streibig et al. (1993).

RESULTS AND DISCUSSION

In Table 2, the EC_{50}s of tested pesticides show a wide range in pesticide toxicity

Table 1. Chemical classes and formulations of selected pesticides

Chemical	Chemical Class	Active ingredients of formulations
Herbicides		
2,4 D (acid)	Phenoxy	50% EC[a]
Dichlorprop	Aryloxyalkanoic acid	
Dicamba	Benzoic acid	48% EC
Pyridate	Pyridazine	45% WP[b]
Propiquizafop	Propionic acid	10% EC
Terbutryn	Triazine	50% EC
Triflusulfuron methyl	Sulfonylurea	25% SC[c]
Rimsulfuron		25% SC
Metsulfuron methyl		20 % SC
Thifensulfuron methyl		75% SC
Amidosulfuron		75% SC
Glyphosate	Glycine derivate	35.6% EC
Trifluralin	Dinitroaniline	48% EC
Pendimethalin		40% EC
Fungicides		
Chlorothalonil	Chloronitrile	50% EC
Propiconazole	Azole	25% EC
Insecticide		
Parathion	Organophosphorus	50% EC

[a]EC (emulsible concentrate); [b]WP (Wettable powder); [c]SC (suspension concentrate)

to *M. aquaticum*. Depending upon which endpoint parameters were used, toxicity levels can differ very significantly. The results showed that the pigment content (chlorophyll a, chlorophyll b and carotenoid) were mostly more sensitive than the remaining endpoints. The EC_{50} values could be calculated for all pesticides by a non-linear regression model. The observed area under the curve was only more sensitive for some pesticides but it was much more sensitive than the increase in shoot length. The EC_{50} based on shoot length could not be calculated, especially for acid herbicides because of its mode of action as a growth regulator. Increase in fresh weight was an unstable endpoint. Root number showed large variation between replicates.

In most cases, pigment content is a very suitable endpoint for this bioassay, as it is in other aquatic toxicity bioassays. The toxicity of the pesticides, as graded using the chlorophyll a endpoint is: Metsulfuron methyl > Thifensulfuron methyl > 2,4D

> Triflusulfuron methyl >Dichlorprop > Dicamba > Rimsulfuron > Glyphosate > Trifluralin > Amidosulfuron > Pyridate > Propiquizafop > Chlorthalonil > Propiconazole > Pendimethalin > Parathion > Fenpropimorph > Terbutryn.

Acid herbicides have different modes of action, being growth inhibitors (2,4 D and Dicamba), photosynthesis inhibitors (pyridate) and inducing formation of abscission zones (dichlorprop).These pesticides showed high to medium toxicity. The EC_{50} values were calculated between 0.006 to 2.452 mg/L within herbicides and different endpoints in herbicides. 2,4 D showed especially high toxicity to *M. aquaticum* with a EC_{50} by 0.02 – 0.158 mg/L. Roshon and Stephenson (1999) observed that the EC_{50} of *M. sibiricum* varied between 0.13 and 0.28 mg/L in pigment content compared to *M. aquaticum* which was 10 times more sensitive than *M. sibiricum.* Dichlorprop was also very toxic. The calculated EC_{50} for chlorophyll a was 0.07 mg/L. The EC_{50} of dicamba was around 0.1 mg/L for all endpoints, which was about 5 times less effective than 2,4 D. *M. aquaticum* seems to be 10 times more sensitive than *L. minor* (Fairchild et al. 1997). The EC_{50} of pyridate was around 0.6 mg/L based on pigment content whereas root length was slightly more sensitive. Fresh weight and area under the curve were 3 times less sensitive than remaining endpoints. The EC_{50} values for chlorophyll a, b and carotenoid were 1.098, 1.172 and 1.430 mg/L for propiquizafop, respectively, root length was a slightly more sensitive endpoint.

Terbutryn was the least toxic pesticide. It is a triazine herbicide and influences photosynthesis especially photosystem II. The EC_{50} values varied between 67.16 and 68.66 mg/L. The terbutryn did not influence the other endpoints except pigment content. Lockart et al. (1983) noticed that *L. minor* has demonstrated higher sensitivity to terbutryn. The highest concentration by 0.24 mg/L inhibited 40% of growth by *L. minor* but the increased concentrations did not cause any additional effects.

The sulfonylurea herbicides have been widely used in recent years because of their very low application rate. They stop plant cell division by inhibiting biosynthesis of the essential amino acids valin and isoleucine and are often used for broadleaf (dicots) plants. They are absorbed by the roots and foliage with rapid translocation both acropetally and basipetally. The toxicity of sulfonylureas varied from high to medium toxicity. Metsulfuron-methyl (0.000624 mg/L for Chlorophyll a; EC_{50}) was found to be very toxic, whereas thifensulfuron-methyl (0.0088 mg/L for chlorophyll a; EC_{50}) and triflusulfuron-methyl (0.037 mg/L for Chlorophyll a; EC_{50}) were moderately toxic to *M. aquaticum.* Plants were less sensitive to rimsulfuron (EC_{50}; 0.149 mg/L for Chlorophyll a) and amidosulfuron (EC_{50}; 0.325 mg/L for Chlorophyll a). Glyphosate is an EPSP synthase inhibitor and acts on various enzyme systems. It can be classified as a pesticide of medium toxicity and has EC_{50} values between 0.222 to 2.04 mg/L across the endpoints. Shoot growth seems to be more sensitive than in the case of *M. sibiricum* with a EC_{50} by 28.79 mg/L, whereas root number was found to be about 2 times less sensitive (Roshon et al. 1999). Our result is comparable with the frond number of *L. minor*

Table 2. Effective concentrations (EC_{50}) of active components of pesticides tested with *M. aquaticum*. All units are mg/L. Endpoints were determined at 14 d.

Pesticides	Influenced mechanisms	Chlorophyll a	Chlorophyll b	Carotenoid	Area under growth curve	Increase in fresh weight	Increase in shoot length	Total root length	Root number
2.4 D (acid)	**Growth**[a]	0.020	0.022	0.019	nd	nd	nd	0.050	0.158
Dichlorprop	**Formation of abscission zone**[a]	0.070	0.063	0.087	nd	nd	nd	0.106	0.176
Dicamba	**Growth**[a]	0.098	0.099	0.099	nd	nd	nd	0.100	nd
Pyridate	**Photosynthesis**[b]	0.636	0.555	0.653	1.888	1.700	nd	0.485	nd
Propiquizafop	**Fatty acid synthesis**	1.098	1.172	1.430	1.831	2.452	nd	1.063	nd
Terbutryn	**Photosynthesis**[b]	67.160	68.660	67.970	nd	nd	nd	nd	nd
Triflusulfuron methyl	**acetolactate synthase (ALS), amino acid biosynthesis**[a,b]	0.037	0.036	0.045	0.036	0.030	0.018	0.065	0.110
Thifensulfuron methyl		0.0088	0.011	0.012	0.0034	0.003	0.006	0.011	0.006
Metsulfuron methyl		0.000624	0.000882	0.000882	0.001	nd	0.007	0.004	0.003
Amidosulfuron		0.325	0.325	0.325	0.974	0.970	0.974	nd	nd
Rimsulfuron		0.149	0.149	0.149	0.054	0.124	0.097	1.498	nd
Glyphosate	**EPSP synthase**[a,b]	0.222	0.222	0.222	0.221	1.999	2.040	1.998	nd
Trifluralin	**Cell division**[a]	0.323	0.353	0.333	0.274	0.279	0.338	0.418	nd
Pendimethalin		13.970	16.450	16.360	8.550	nd	10.744	nd	24.13
Chlorthalonil	**Chloronitrile**[a]	2.680	2.540	2.550	4.490	nd	2.940	0.480	2.210
Propiconazole	**Azole**[a]	3.886	3.887	3.771	0.218	1.310	0.548	nd	2.069
Parathion	**Organophosphorus**[a]	15.660	15.730	15.060	6.710	8.840	6.930	16.300	nd

[a] from Tomlin (1994) [b] from Ahrens (1994) nd: not determinable

which has an EC_{50} value of 2 mg/L (Hartman and Martin 1985).

Dinitroaniline herbicides trifluralin and pendimethalin -which are absorbed by roots and leaves- disrupt cell division, cell elongation and root development. Their EC_{50} values showed significant variation between 2 herbicides although they belong to the same chemical class and have the same mode of action. Trifluralin had a medium toxicity on *M. aquaticum.* The EC_{50} values varied from 0.323 to 0.418 mg/L, whereas the pendimethalin demonstrated a very low toxicity with an EC_{50} value at 10.74 to 24.13 mg/L. This severe difference in toxicity can be attributed to pendimethalin being degraded by sunlight in aquatic systems. It is also rapidly degraded under anaerobic conditions in sediment (Ahrens 1994).

Propiconazole is a systemic foliar fungicide with broad range activity against fungus and has medium to low toxicity. The EC_{50} values were found to range between 0.218 to 3.887 mg/L. Peterson et al. (1994) observed that the 0.083 mg/L application of propiconazole inhibited 10 % ^{14}C uptake by *L. minor.* Chlorthalonil is also a foliar fungicide and showed a levels of toxicity similar to that of propiconazole. The EC_{50} values varied between 0.480 to 4.49 mg/L. These fungicides have higher toxicity than some herbicides although plants are not target organisms.

Parathion, an organophosphate insecticide which has been widely used against pests and is very often detected in surface water and sediment caused low toxicity in to *M. aquaticum.* The EC_{50} values varied between 6.71 and 16.3 mg/L. It was also found that some other organophosphate insecticides e.g. carbaryl and carbofuran affected some aquatic plants. Application of 3,667 mg/L carbaryl caused 33% inhibition in ^{14}C uptake and 0.667 mg/L carbofuran also inhibited 21% ^{14}C uptake by *L. minor* (Peterson et al. 1994). We conclude that the residues of fungicides and insecticides may have a potential effect on aquatic macrophytes. Therefore, fungicides and insecticides need to be tested for risk during pesticide registration.

Our results demonstrated that *M. aquaticum* shows a similar sensitivity in comparison to duckweed (e.g., Grossmann et al. 1992) and *M. sibiricum* (Roshon et al. 1999) for most tested pesticides. *M. aquaticum* is more sensitive to certain pesticides (acid herbicides and some sulfonylureas) when compared with duckweed. The bioassay with *M. aquaticum* allows one to assess the toxicity of pesticides in water and perhaps, in sediment in the future. *Myriophyllum* can be an important component of microcosm studies, for risk assessment of non-target organisms.

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