

Suitability of several dicotyledonous macrophytes as additional test species for the risk assessment refinement

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Introduction

The species sensitivity distribution (SSD) method is an often used tool in the tier 2 risk assessment (RA) of plant protection products (PPP) in which additional toxicity data of 8 potentially sensitive species can be provided by the applicant (EFSA 2013). For the substance group of auxins, which are only effective on dicotyledonous plant species, it would be appropriate to test only dicotyledonous macrophytes for a SSD as refinement method in the aquatic RA of auxins. Therefore, we tested dicotyledonous macrophytes in a multispecies test system (Fig. 1) in order to gain information on their suitability as additional test species in a SSD approach.

Method

Macrophyte set up

- One individual per glass beaker (250 mL, Fig. 2)
- Use of shoots or shoots with roots (species dependent), 5/10 replicates per species
- 3 layers of sediment (sand, commercial pond soil (peat basis, Co. Floraself), sand)
- Experimental phase of 20 - 26 days



Fig. 2: Plants in beaker



Fig. 1: Multi species test system

Test system set up

- Artificial light (HQI lamps, 80-112 $\mu\text{mol}/\text{m}^2/\text{s}$)
- Light cycle adopted to outdoor conditions
- Electrical conductivity 530 $\mu\text{S}/\text{cm}$
- pH 8.2-8.7
- 0.45 mg/L TN and 0.23 mg/L TP in eluate of pond soil
- Mean water temperature 20 °C

Results

Tab. 1: Relative growth rates (RGR) and coefficients of variation (CV) of tested species

Macrophytes	Source/Handling	Suitable Endpoints	RGR (d^{-1})	CV (%)	Usability
<i>Ceratophyllum demersum</i>	Natural lake, 10 x 10 cm shoots without side shoots	Length main shoot	0.09 (14 d)	12	😊
		Total length	0.07 (26 d)	19	
		Fresh weight	0.03 (26 d)	12	
<i>Ranunculus aquatilis</i>	Co. Naturagart, 10 x 10 cm shoots without side shoots	Length main shoot	0.08 (21 d)	15	😐
		Fresh weight	0.01 (21 d)	39	
<i>Nymphoides peltata</i>	Own culture, 5 x 5 cm of rhizome	Total length all shoots	0.22 (20 d)	20	😊
		Sum of leave size	0.05 (20 d)	11	
		Fresh weight	0.02 (20 d)	7	
<i>Hottonia palustris</i>	Co. Naturagart, 10 x 15 cm plants with 5 cm of rhizome	Length main shoot	0.01 (12 d)	9	😐
		Number of side shoots	0.07 (12 d)	16	
<i>Callitriche palustris</i>	Co. Naturagart, 5 x 6.5-9.5 cm plants with rhizome	Length of most shoots	0.05 (20 d)	15	😊
		Fresh weight	0.02 (20 d)	17	
		Root length	0.03 (20 d)	15	
<i>Hygrophila polysperma</i>	Co. Aquarienpflanzenshop, 5 x 10 cm shoots without side shoots	Total shoot length	0.05 (20 d)	8	😊
		Fresh weight	0.03 (20 d)	14	
<i>Ludwigia repens</i>	Co. Aquarienpflanzenshop, 5 x 10 cm shoots without side shoots	Length main shoot	0.02 (21 d)	11	😊
		Number of leaves	0.03 (21 d)	15	
		Fresh weight	0.02 (21 d)	4	
<i>Hygrophila difformis</i>	Co. Aquarienpflanzenshop, 5 x 10 cm shoots without side shoots	Length main shoot	0.03 (20 d)	20	😐
		Number of leaves	0.02 (20 d)	8	
		Fresh weight	0.02 (20 d)	15	
<i>Veronica beccabunga</i>	Co. Naturagart, 5 x 10 cm shoots without side shoots	Length main shoot	0.05 (20 d)	4	😊
		Number of leaves	0.04 (20 d)	7	
		Fresh weight	0.06 (20 d)	9	

Conclusions

- In addition to the standard macrophytic test species *Myriophyllum spicatum*, 9 additional dicotyledonous macrophyte species were identified to be suitable for the use in a SSD approach (Tab. 1).
- The macrophytes *Persicaria amphibiana*, *Hippuris vulgaris*, *Mentha aquatica*, *Stachys palustris*, *Menyanthes trifoliata*, *Caltha palustris*, *Myosotis scorpioides*, and *Hydrocotyle leucocephala* were also tested but not regarded as suitable for this approach due to too low growth rates or too high CVs for most of the endpoints.

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Photo Sources: <http://www.flowgrow.de/db/wasserpflanzen/ceratophyllum-demersum>, https://www.baumschule-horstmann.de/wasser-hahnenfuss-1386_60129.html, <http://www.gartenteich-ratgeber.com/pflanzen/seerosen/seekanne.html>, <https://de.wikipedia.org/wiki/Wasserfeder>, <https://de.wikipedia.org/wiki/Sumpf-Wasserstern>, https://en.wikipedia.org/wiki/Hygrophila_polysperma, https://en.wikipedia.org/wiki/Ludwigia_repens, https://en.wikipedia.org/wiki/Hygrophila_difformis, https://en.wikipedia.org/wiki/Veronica_beccabunga