Journal of Agricultural, Food and Environmental Sciences Vol 76 No 5 (2022) 35-44
DOI: https://doi.org/10.55302/JAFES22765035m

ALLELOPATHIC POTENTIAL OF SOME WEEDS IN THE RICE FIELDS OF THE KOCHANI REGION

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ABSTRACT

During the period 2021-2022, the allelopathic effect of three weed species *Scirpus mucronatus* L., *Scirpus maritimus* L. and *Heteranthera reniformis* Ruiz & Pav. was studied under laboratory conditions at the Institute of Forage Crops, Pleven from the rice fields in the Kochani region and determining the allelopathic tolerance of four rice varieties Ronaldo, Opale, San Andrea and Halilbay grown in the Republic of Macedonia. It was established that the developed in vitro test ensures the obtaining of reliable results for the allelopathic effect of *S. mucronatus*, *S. maritimus* and *H. reniformis* and the allelopathic tolerance of the Ronaldo, Opale, San Andrea and Halilbay rice cultivars included in the study. The use of agar-gel as a carrier of allelochemicals and development environment has sufficient water supply and compacted structure to support the optimal development of the accessions included in the experiment development index (*GI%)* varied from 21.5 to 129.2%) and proof of allelopathic interference in rice. In terms of allelopathic tolerance to weed species, rice varieties can tentatively be ranked in the following order: Halilbay ($GI_{\%\ average}$ 47.5%) → Ronaldo and Opale ($GI_{\%\ average}$ 62.3%) → San Andrea ($GI_{\%\ average}$ 94.1%).

Key words: allelopathy, interaction, inhibition, stimulation.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's oldest crops cultivated for food by humans (Dunna & Bidhan, 2013; Alhach, 2018). According to summarized studies by Muthayya et al., 2014 and Ziarati et al., 2022, rice production and consumption provides up to fifty percent of the dietary caloric supply of more than one third of the human population. Kamoshita et al. (2014) identified more than 1800 weed species growing in paddy fields and weeding the crop worldwide. Regardless of the continental location, the local climatic and edaphic conditions during rice cultivation, the species composition of the weeds, the degree of weeding of the crop, the integrated systems used for their management, the species of the genus *Echinochloa, Cyperus, Scirpus* are defined as cosmopolitan weeds in rice. (Berendji et al., 2008; Schaedler et al., 2015; Jabran, 2017). Similar are the results obtained in the experimental work of Pacanoski & Glatkova (2009); Dimitrovski et al. (2015); Glatkova et al. (2019). According to the authors, the weed associations of rice fields in the Republic of Macedonia in Kochani region is represented by the weed species *Cyperus rotundus* L., *Echinochloa crus-galli* (L.) Beauv., *Heteranthera limosa* (Sw.) Willd., *Echinochloa macrocarpa* (L.) P. Beauv., *Scirpus mucronatus* (L.) J. Jung & H. K. Choi, *Ammania coccinea* Rottb., *Rotala ramosior* (L.) Koehne and *Leersia oryzoides* (L.) Sw., etc.

A number of studies have been carried out on the dynamics of weed species and the degree of weed control in rice fields, establishing their harmfulness, the critical period of weed control of the crop (Mola & Belachew, 2015; Mondal et al., 2017; Yawale et al., 2019). It has been established that weeds in rice fields worldwide is a major limiting factor for realizing stable yields from the crop and for deteriorating the quality of the obtained grain. Rice grain losses have been shown to decrease in the range (from 10 to 15%) due to the competitive effect of weeds while the conditions of uncontrolled weeds, yield reduction can reach up to 60%, and in some cases the yields can be complete compromise (100%) (Zoschke 1990; Baltazar & DeDatta, 1992; Brim-DeForest et al., 2017; Karn et al., 2020). The selectivity and efficacy of a number of herbicides have been established for weed control in paddy fields (Kuk et al., 2001; Singh et al., 2016; Ahmed at al., 2021). According to the summary studies of Berendji et al. (2008), Weih et al. (2008), Kato-Noguchi & Peters (2013) a number of compounds, such as phenolic acids, fatty acids, phenylalkanoic acids, hydroxamic acids, terpenes, indoles and the labdane-related diterpenoid momylactones, have been identified as potential allelochemicals identified in rice plants. The allelopathic effect of root exudates from different varieties of rice on the germination and initial development of test plants (lettuce, wheat, rice, clover, etc.) has been proven and the allelopathic potential of the crop has been established (Yu An et al., 2015; Ho et al., 2020; Chang et al., 2022). Most of the studies related to the allelopathic potential of rice and mainly focused on the relationship between rice and common barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) (Yongqing et al., 2014), but there is a lack of data on the allelopathic effect of other weeds on germination and initial development of rice cultivars.

In this aspect, the aim of the study is to investigate and compare the allelopathic potential of some weed species on the germination and initial development of rice varieties cultivated and grown in the Republic of Macedonia for the Kochani region.

MATERIALS AND METHODS

To establish the allelopathic potential of the weed species *Scirpus mucronatus* L.; *Scirpus maritimus* L. and *Heteranthera reniformis* Ruiz & Pav. In the Institute of Forage Crops - Pleven, a laboratory experiment was carried out under controlled conditions. The experiment included four varieties of rice – Ronaldo, Opale, San Andrea and Halilbay, the seeds of which were harvested in the experimental field of the Institute of Agriculture – Skopje, Department for rice in Kochani (locality "Mishjak"). Roots or aboveground dry biomass of each weed species were cut to 0.5-3.0 cm length and dried to constant dry weight at 60 ± 3^0 C and then ground. Biomass of each weed species was placed in Petri dishes (90 mm) at concentrations - 0.0 (control), 0.04, 0.08, 0.16 and 0.32 w/v % and pipetted 20 ml (0.75%) agar-agar, with added 1 ml/l thymol $C_{10}H_{14}O$ (Marinov-Serafimov and Golubinova, 2015). The Petri dishes were then placed in a thermal chamber for 72 hours at 23.0^0 C ($\pm 2.0^0$ C). The seeds of the rice varieties included in the study were pre-soaked for 24 hours in cold water extracts with equivalent concentrations of the weed species. After that, twenty-five seeds of the varieties included in the study were placed on the media and thus prepared, in each petri dish. The accessions were incubated in a thermostat in the dark at a temperature of 23.0^0 C $\pm 2.0^0$ C for five days. Each variant is a bet in ten replicates. The following parameters were determined:

Seed germination ($SG_{\%}$) was determined by the Equation (1).

$$SG_{\%} = \frac{Number\ of\ seedgerminated}{Totalnumber\ of\ seed\ plated}.100 \quad (1)$$

Seedling length (cm) were measured through a measuring line;

Inhibition index ($IR_{\%}$) was determined an adapted formula by Hsu et al. (2007)**,** Equation (2).

$$IR_{\%} = 100 - \frac{(E2.100)}{E1} \quad (2)$$

where: *E1* – measurement of the control treatment (seed germination, % or seedling length, cm); *E2* – measurement of in each treatment (seed germination, % or seedling length, cm); 100 – coefficient. $IR_{\%}$ "-" indicates stimulation or "+" inhibition effects according to control treatment.

Lethal concentration 50 (LC_{50}) and stimulating concentration 50 (SC_{50}) values was calculated using Probit analysis based on Finney's method according to Srinivasan (2004);

Development index ($GI_{\%}$) of rice cultivars was determined by the Equation (3) (Gariglio et al., 2002).

$$GI_{\%} = \left[\left(\frac{G}{G_0}\right).\left(\frac{L}{L_0}\right)\right].100 \quad (3)$$

where *G* - survival plants in each treatment, %; G_0 - survival in the control variants, respectively %; *L* – average length (cm) of seedlings in treatment transformed into percentage as against the control treatment; L_0 – average length (cm) of the seedlings in the control treatment taken as 100%.

The $SG_{\%}$ for all trial variants was determined after prior *arcsin* transformation according to Anant (1996).

$$Y = arcsin\sqrt{(\frac{X_{\%}}{100})} \quad (4)$$

where $x_{\%}$ – germinated seeds for each treatment (%).

The experimental results were processed mathematically - statistically by the method of dispersion analysis (ANOVA) with a probability less than 5% (i.e. $P<0.05$) for statistical significance.

RESULTS AND DISCUSSION

The dry root and aboveground biomass of the weed species *S. mucronatus*, *S. maritimus* and *H. reniformis* included in the study; had an indifferent, stimulating and inhibiting effect on seed germination of the tested rice varieties. The inhibition index ($IR_{\%}$) on seed germination ($SG_{\%}$) in the tested rice varieties depending on the applied weed dry root biomass ranged from -67.5 to 33.6%, and from aboveground biomass ranged from -67.5 to 55.4 % (Table 1).

The integral impact of the applied dry weed (root and aboveground) biomass on the $IR_{\%}$ of the seed germination of the tested rice varieties can be conditionally ranked in the following ascending order: *S. mucronatus* ($IR_{\%}$ from -67.5.5 to 29.1%) → *S. maritimus* ($IR_{\%}$ from -67.5 to 33.0%) → *H. reniformis* ($IR_{\%}$ from -67.5 to 55.4 %). Depending on the established inhibitory effect (without the variants with a statistically proven stimulating effect) of the dry weed biomass (root or aboveground) included in the study, they can be conditionally grouped into three groups: *First group* (inhibition of seed germination up to 21.0%) including aboveground weed biomass of *S. mucronatus; Second group* (inhibition of seed germination from 22.0 to 35.0%) including root weed biomass of *S. mucronatus* and *H. reniformis* and aboveground biomass of *S. maritimus* and *Third group* (inhibition of seed germination from 35.0 up to 45.0%) – for weed root biomass of *S. maritimus* and aboveground biomass of *H. reniformis*. Regarding the percentage content of the weed biomass and development medium (agar-agar) of the test plants, it is evident that as its content increases to 0.16 - 0.32 w/v %, the percentage of germinated seeds in the test decreases disproportionately -plants compared to the control variant, the differences being statistically proven reduced at $P<0.05$. An exception was found

at the lower applied concentrations of 0.04÷0.08 w/v % dry weed biomass where the differences statistically unproven (Table 1). This dependence can be explained by the presence of allelochemicals at the weed biomass concentrations tested. Allelochemicals are known to possess toxicity with protoplasmic action (Weih et al. 2008), which at higher concentrations cause an inhibitory effect on seed germination, while lower concentrations have a stimulatory to weak inhibitory effect on the studied parameter. The differences in the inhibitory and/or stimulatory effect of the tested concentrations of dry (root or aboveground) weed biomass on the seeds germination of the test plants, on the one hand, can be explained by the diffusion of soluble allelochemicals in the development medium (agar-agar), and on the other with the different crude protein content of the seeds. Similar results were reported by Putnam et al. (1983), according to which extraction of dry weed biomass extracts allelochemicals that are not released in rice agrophytocoenoses, and according to Amb & Ahluwalia (2016), crude protein content determines different sensitivity to the inhibitory effect of weed extracts on seed germination of the test plants. The results obtained when determining the LC_{50} on seed germination of the rice varieties included in the study are similar, depending on the type of applied weed biomass (Table 2).

Table 1. Influence of weed biomass on seed germination in rice varieties by agar-agar method

Weeds		C_m w/v%	Rice varieties			
			Ronaldo	Opale	San Andrea	Halilbay
			SG% *(IR%)*	*SG%* *(IR%)*	*SG%* *(IR%)*	*SG%* *(IR%)*
S. mucronatus	dry root biomass	0.00	67.2^{c} (0.0)	63.4^{c} (0.0)	53.7^{a} (0.0)	71.6^{f} (0.0)
		0.04	67.2^{c} (0.0)	67.2^{c} (-6.0)	67.2^{c} (-25.1)	67.2^{e} (6.1)
		0.08	67.2^{c} (0.0)	90.0^{f} (-41.9)	77.1^{d} (-43.5)	67.2^{e} (6.1)
		0.16	60.0^{b} (10.7)	77.1^{d} (-5.9)	71.6^{cd} (-33.2)	50.8^{a} (29.1)
		0.32	56.8^{b} (15.5)	77.1^{d} (-5.9)	71.6^{cd} (-33.2)	50.8^{a} (29.1)
S. maritimus		0.04	63.4^{bc} (5.6)	53.7^{ab} (15.3)	90.0^{e} (-67.5)	71.6^{f} (0.0)
		0.08	63.4^{bc} (5.6)	56.8^{b} (10.5)	90.0^{e} (-67.5)	56.8^{b} (20.6)
		0.16	53.7^{b} (20.3)	56.8^{b} (10.5)	71.6^{d} (-33.2)	67.2^{e} (6.1)
		0.32	45.0^{a} (33.0)	56.8^{b}(10.5)	90.0^{e} (-67.5)	67.2^{e} (6.1)
H. reniformis		0.04	67.2^{c} (0.0)	60.0^{c} (5.4)	63.4^{b} (-18.1)	63.4^{d} (11.4)
		0.08	56.8^{b} (15.5)	60.0^{bc} (5.4)	63.4^{b} (-18.1)	63.4^{d} (11.4)
		0.16	67.2^{c} (0.0)	56.8^{b} (10.5)	63.4^{b} (-18.1)	53.7^{ab} (24.9)
		0.32	56.8^{b} (15.5)	42.1^{a} (33.6)	63.4^{b} (-18.1)	60.0^{c} (16.2)
S. mucronatus	dry aboveground biomass	0.00	67.2^{d} (0.0)	63.4^{c} (0.0)	53.7^{b} (0.0)	71.6^{f} (0.0)
		0.04	67.2^{d} (0.0)	63.4^{c} (0.0)	90.0^{e} (-67.5)	67.2^{ef} (6.1)
		0.08	71.6^{e} (-6.5)	63.4^{c} (0.0)	90.0^{e} (-67.5)	63.4^{e} (11.4)
		0.16	67.2^{d} (0.0)	60.0^{bc} (5.4)	63.4^{c} (-18.5)	56.8^{d} (20.6)
		0.32	77.1^{e} (-14.7)	63.4^{c} (0.0)	56.8^{b} (-5.7)	56.8^{d} (20.6)
S. maritimus		0.04	60.0^{c} (10.7)	63.4^{c} (0.0)	77.1^{d} (-43.5)	53.7^{c} (24.9)
		0.08	56.8^{c} (15.5)	47.9^{a} (24.5)	56.8^{b} (-5.7)	67.2^{ef} (6.1)
		0.16	60.0^{c} (10.7)	53.7^{b} (15.3)	71.6^{d} (-33.2)	53.7^{c} (24.9)
		0.32	47.9^{bc} (28.8)	53.7^{b} (15.3)	77.1^{d} (-43.5)	50.8^{c} (29.1)
H. reniformis		0.04	56.8^{bc} (15.5)	63.4^{c} (0.0)	90.0^{e} (-67.5)	56.8^{d} (20.6)
		0.08	56.8^{bc} (15.5)	67.2^{d} (-0.6)	77.1^{d} (-43.5)	56.8^{d} (20.6)
		0.16	45.0^{b} (33.0)	50.8^{b} (20.0)	45.0^{ab} (16.2)	45.0^{b} (37.1)
		0.32	30.0^{a} (55.4)	42.1^{a} (33.6)	39.2^{a} (27.0)	39.2^{a} (45.2)

Legend: C_m - Weight/Volume percentage concentration (w/v %); a, b, c, d, e statistically proven differences at $P<0.05$; $SG_{\%}$ - seeds germination, %; ($IR_{\%}$) - inhibition index in which $IR_{\%}$ "-" indicates stimulation or "+" inhibition effects according to control treatment

LC_{50} values ranged from 0.66 to 4.81 w/v % for applied root dry weed biomass and from 0.30 to 6.92 w/v % for aboveground dry weed biomass. A reciprocal stimulatory SC_{50} concentration ranging from 0.07 to 1.11 w/v % was also found. The differences in the LC_{50} and SC_{50} values of the tested weed biomasses (root and aboveground) can be explained, on the one hand, by the variable nature of the allelopathic effect, which is probably due to the different solubility of the allelochemicals present, and on the other hand by the different sensitivities of the test plants. The most sensitive to the allelopathic effect of the weed species included in the study in terms of laboratory seed germination is the variety Ronaldo - LC_{50} - from 0.30 to 2.04 w/v %, followed by Halilbay - LC_{50} from 0.46 to 4.81 w/v %, and relatively least sensitive is Opale variety LC_{50} is in the range of 0.47 to 6.92 w/v %, while in San Andrea a stimulatory effect is found (SC_{50} from 0.07 to 1.11 w/v %).

Table 2. The effective concentration of weed biomass on inhibition LC_{50} and stimulation SC_{50} of seed germination in rice varieties

Weeds		Rice varieties			
		Ronaldo	Opale	San Andrea	Halilbay
S. mucronatus	dry root biomass	LC_{50} 1.51 (0.39÷5.88) SC_{50} *NS**	LC_{50} *NS** SC_{50} 0.49 (0.26÷0.92)	LC_{50} *NS** SC_{50} 0.81 (0.09÷7.14)	LC_{50} 0.67 (0.32÷1.39) SC_{50} *NS**
S. maritimus	dry root biomass	LC_{50} 0.66 (0.33÷1.33) SC_{50} *NS**	LC_{50} <80.3 SC_{50} 0.0005 (0.0007÷3.36)	LC_{50} *NS** SC_{50} 0.07 (0.04÷0.11)	LC_{50} 1.37 (0.40÷4.67) SC_{50} *NS**
H. reniformis	dry root biomass	LC_{50} 1.37 (0.37÷5.02) SC_{50} *NS**	LC_{50} 0.73 (0.34÷1.58) SC_{50} *NS**	LC_{50} *NS** SC_{50} 1.11 (0.33÷3.83)	LC_{50}4.81 (0.20÷18.01) SC_{50} *NS**
S. mucronatus	dry aboveground biomass	LC_{50} *NS** SC_{50} 0.85 (0.39÷1.87)	LC_{50} 6.92 (0.14÷41.4) SC_{50} *NS**	LC_{50} *NS** SC_{50} 0.16 (0.13÷0.19)	LC_{50} 2.51 (0.30÷21.04) SC_{50} *NS**
S. maritimus	dry aboveground biomass	LC_{50} 2.04 (0.28÷14.84) SC_{50} *NS**	LC_{50} 1.22 (0.32÷4.73) SC_{50} *NS**	LC_{50} *NS** SC_{50} 0.26 (0.18÷0.49)	LC_{50} 1.16 (0.26÷5.15) SC_{50} *NS**
H. reniformis	dry aboveground biomass	LC_{50} 0.30 (0.20÷0.45) SC_{50} *NS**	LC_{50} 0.47 (0.31÷0.72) SC_{50} *NS**	LC_{50} 0.64 (0.32÷1.25) SC_{50} 0.21 (0.18÷0.25)	LC_{50} 0.46 (0.20÷1.04) SC_{50} *NS**

Legend: LC_{50} - the concentration required to inhibit germination seeds by 50% at P<0.05; SC_{50} - the concentration required to stimulate germination seeds by 50% at P<0.05; NS - no stimulatory or inhibitory effect was found in. LC_{50} or SC_{50}.*

The growth of sprouts in the test plants under the influence of allelochemicals present in the weed biomass, follows the trends established in relation to the laboratory seed germination depending on the allelopathic effect of the weed biomass (root or aboveground) included in the study with the difference that they are more clearly expressed Table 3. The inhibition index (*IR%*) on seedling growth in the tested rice cultivars depending on the applied dry weed root biomass ranged from 1.2 to 67.9% and for aboveground dry biomass ranged from 9.7 to 81.9%. It is evident from Table 3 that it establishes a general trend of a disproportionate decrease in the length of sprouts in the tested varieties of rice with an increase in the concentration (from 0.08 to 0.32 w/v %) of the weed biomass in the medium for their development - from 0.2 to 19.3 times the root biomass and from 0.5 to 3.5 times aboveground weed biomass compared to the lowest applied concentration of 0.04 w/v %. An exception to the described dependence was found at the lowest applied concentration of 0.04 w/v % dry root biomass of *H. reniformis* in the rice cultivar Opale, which caused a weak statistically unproven stimulatory effect.

Table 3. Influence of weed biomass concentration on seedling *length* (SL_{cm}) in rice varieties

Weeds		C_m w/v%	Rice varieties							
			Ronaldo		Opale		San Andrea		Halilbay	
			SL_{cm}	$(IR_{\%})$	SL_{cm}	$(IR_{\%})$	SL_{cm}	$(IR_{\%})$	SL_{cm}	$(IR_{\%})$
S. mucronatus	dry root biomass	0.00	8.2^{e}	(0.0)	16.1^{e}	(0.0)	3.5^{c}	(0.0)	7.1^{f}	(0.0)
		0.04	8.1^{e}	(1.2)	14.9^{d}	(6.9)	1.9^{ab}	(45.8)	3.4^{c}	(52.6)
		0.08	8.2^{e}	(0.0)	12.2^{c}	(23.7)	3.0^{c}	(15.2)	2.9^{b}	(59.3)
		0.16	7.9^{d}	(2.6)	12.1^{c}	(24.4)	2.8^{bc}	(19.2)	2.8^{b}	(61.0)
		0.32	5.4^{b}	(33.6)	12.4^{c}	(22.7)	1.3^{a}	(62.5)	2.3^{a}	(67.9)
S. maritimus		0.04	5.7^{b}	(29.6)	13.6^{cd}	(15.4)	3.4^{c}	(1.7)	2.8^{b}	(61.2)
		0.08	6.9^{c}	(16.0)	12.4^{c}	(22.9)	2.5^{b}	(29.2)	4.9^{d}	(30.7)
		0.16	6.0^{bc}	(26.3)	10.2^{bc}	(36.4)	3.2^{c}	(8.6)	6.1^{e}	(14.9)
		0.32	4.0^{a}	(50.8)	12.2^{c}	(24.2)	2.3^{b}	(33.2)	3.0^{b}	(58.5)
H. reniformis		0.04	7.2^{d}	(11.2)	16.9^{e}	(-5.0)	2.2^{b}	(36.7)	3.3^{b}	(53.3)
		0.08	6.4^{c}	(16.0)	12.4^{c}	(23.0)	3.0^{c}	(14.0)	6.2^{e}	(12.6)
		0.16	6.3^{c}	(26.3)	8.3^{ab}	(48.0)	2.1^{b}	(40.7)	6.4^{e}	(10.8)
		0.32	5.1^{b}	(50.8)	7.0^{a}	(56.6)	2.6^{bc}	(26.6)	5.0^{d}	(29.9)
S. mucronatus	dry aboveground biomass	0.00	8.2^{g}	(0.0)	16.1^{i}	(0.0)	3.5^{d}	(0.0)	7.1^{f}	(0.0)
		0.04	6.3^{f}	(23.1)	12.4^{g}	(22.9)	2.1^{a}	(39.0)	4.6^{bc}	(36.2)
		0.08	7.3^{fg}	(10.9)	11.6^{f}	(28.0)	2.9^{c}	(17.8)	2.8^{a}	(60.3)
		0.16	5.6^{e}	(31.2)	8.6^{e}	(46.4)	2.7^{c}	(23.5)	5.1^{c}	(28.2)
		0.32	5.4^{e}	(33.7)	9.3^{e}	(42.1)	2.3^{b}	(34.7)	5.1^{c}	(28.2)
S. maritimus		0.04	6.4^{f}	(21.6)	13.4^{h}	(16.4)	2.2^{b}	(36.4)	6.4^{d}	(9.7)
		0.08	6.6^{f}	(18.5)	13.1^{h}	(18.6)	2.3^{b}	(35.0)	2.8^{a}	(61.0)
		0.16	5.8^{e}	(28.8)	7.8^{cd}	(51.7)	2.5^{b}	(29.2)	4.1^{b}	(42.2)
		0.32	4.9^{d}	(40.4)	6.8^{c}	(57.7)	2.1^{b}	(39.0)	4.0^{b}	(44.6)
H. reniformis		0.04	2.5^{b}	(69.9)	8.2^{d}	(48.9)	2.7^{c}	(22.9)	4.3^{b}	(39.4)
		0.08	3.4^{c}	(58.3)	4.8^{b}	(70.3)	2.6^{bc}	(24.9)	5.1^{c}	(28.1)
		0.16	1.8^{a}	(77.4)	5.1^{b}	(68.5)	2.5^{b}	(28.4)	3.1^{ab}	(56.4)
		0.32	1.8^{a}	(77.4)	2.9^{a}	(81.9)	1.2^{a}	(67.0)	2.6^{a}	(63.5)

Legend: C_m - Weight/Volume percentage concentration (w/v %); a, b, c, d, e statistically proven differences at P<0.05; SL – seedling length, cm; $(IR_{\%})$ - inhibition index in which $IR_{\%}$ "-" indicates stimulation or "+" inhibition effects according to control treatment

The differences in LC_{50} values on seedling t growth of rice cultivars depending on the applied concentrations of dry weed biomass can be explained by the presence of allelochemicals and the different sensitivity of the test plants. The most sensitive to the allelopathic effect of the weed species included in the study in terms of LC_{50} values can be conditionally arranged in the following ascending order: Halilbay - LC_{50} from 0.02 to 0.32 w/v % → Ronaldo LC_{50} - from 0.01 to 1.37 w/v % → San Andrea - LC_{50} - from 0.21 to 1.65 w/v % → Opale LC_{50} - from 0.21 to 2.32 w/v %. The mechanism of seedling growth inhibition due to the direct effect of allelmochemicals is probably due to a reduction in cell division (Iman et al., 2006). Therefore, seed germination in rice can be considered as a relatively less sensitive period of individual plant development, while sprout growth allows it to be used as a potential test to determine the allelopathic effect of weed biomass under laboratory conditions, due to the direct contact in the growth medium (agar-agar) during the bioassays.

Table 4. The effective concentration of weed biomass on lethal concentration 50 (LC_{50}) of seedling length in rice varieties

Weeds		Rice varieties			
		Ronaldo	Opale	San Andrea	Halilbay
		LC_{50}			
S. mucronatus	dry root biomass	0.49 (0.17÷1.44)	2.32 (0.02÷30.9)	0.21 (0.06÷0.66)	0.02 (3.7E-05÷13.39)
S. maritimus	dry root biomass	1.37 (0.37÷5.02)	1.26 (0.03÷59.6)	0.48 (0.05÷4.39)	0.16 (0.07÷0.35)
H. reniformis	dry root biomass	0.79 (0.03÷19.33)	0.21 (0.13÷0.36)	0.50 (0.01÷22.37)	0.32 (0.10÷1.02)
S. mucronatus	dry aboveground biomass	0.80 (0.02÷26.16)	0.41 (0.06÷3.18)	0.61 (0.01÷66.75)	0.24 (0.05÷1.21)
S. maritimus	dry aboveground biomass	0.76 (0.02÷31.20)	0.21 (0.11÷0.39)	1.65 (8.2E-08÷35.95)	0.18 (0.08÷0.42)
H. reniformis	dry aboveground biomass	0.01 (9.3E-05÷2.00)	0.35 (0.01÷0.14)	0.24 (0.05÷1.21)	0.13 (0.05÷0.39)

Legend: LC_{50} - the concentration required to inhibit seedling length by 50% at $P<0.05$

The development index (*GI%*) depends on the same factors and follows the observed relationships in terms of laboratory seed germination and seedling growth of the rice cultivars included in the study (Table 5).

According to the development index(*GI%*) of plant depending on the applied dry root or aboveground weed biomass of the weed species included in the study, the relatively lowest development index was reported for the variety Halilbay (*GI%*average 49.2% for roots and 45.7% aboveground biomass) followed by Ronaldo (*GI%average* 72.1% roots) and Opale (*GI%average* 49.9% aboveground) ≈ Opale (*GI%average* 75.5% roots) and Ronaldo (*GI%average* 52.5% aboveground), while in cultivar San Andrea, regardless of the type and concentration of the dry weed biomass applied, a high *GI%* average plant development index of 99.7% on the roots biomass and 88.5 for the applied aboveground biomass was found, respectively, which also determines a high allelopathic tolerance of the variety to the weed species included in the study. Therefore, the observed differences in the tested rice varieties regarding the allelopathic effect of the weed biomass included in the study can also be explained by genetic differences, since the comparisons between them were made under the same controlled conditions.

Table 5. Effect of weed biomass on development index ($GI_{\%}$) on the rice varieties

Weeds		C_m w/v %	Rice varieties			
			Ronaldo	Opale	San Andrea	Halilbay
			$GI_{\%}$			
S. mucronatus	dry root biomass	0.04	98.8	98.6	67.7	44.5
		0.08	100.0	108.2	121.7	38.2
		0.16	87.0	91.9	107.6	27.7
		0.32	56.1	94.0	50.0	22.8
S. maritimus		0.04	66.5	71.7	164.6	38.8
		0.08	79.3	69.1	118.6	55.0
		0.16	58.9	56.9	121.7	80.0
		0.32	32.9	67.9	111.8	39.0
H. reniformis		0.04	88.8	99.4	74.8	41.4
		0.08	66.5	72.8	101.5	77.5
		0.16	77.5	46.5	70.0	67.0
		0.32	52.4	28.8	86.6	58.8
		Average	72.1	75.5	99.7	49.2
S. mucronatus	dry aboveground biomass	0.04	76.9	77.1	102.2	59.9
		0.08	94.8	72.0	137.8	35.2
		0.16	68.8	50.7	90.3	56.6
		0.32	58.7	57.9	69.1	57.0
S. maritimus		0.04	70.0	83.6	91.3	67.8
		0.08	68.8	61.4	68.7	36.6
		0.16	63.5	41.0	94.3	43.4
		0.32	42.5	35.8	87.6	39.3
H. reniformis		0.04	25.4	51.1	129.1	48.1
		0.08	35.2	31.4	107.7	57.1
		0.16	15.1	25.2	60.0	27.4
		0.32	10.1	12.0	24.1	20.0
		Average	52.5	49.9	88.5	45.7

Legend: C_m - Weight/Volume percentage concentration (w/v %)

CONCLUSIONS

The developed *in vitro* test ensures the obtaining of reliable results for the allelopathic effect *of S. mucronatus, S. maritimus and H. reniformis* and the allelopathic tolerance of the Ronaldo, Opale, San Andrea and Halilbay rice cultivars included in the study. The use of agar-gel as a carrier of allelochemicals and development environment has sufficient water supply and compacted structure to support the optimal development of the accessions included in the experiment ($GI_{\%}$ from 21.5 to 129.2%) and proof of allelopathic interference in rice. In terms of allelopathic tolerance to weed species, rice varieties can tentatively be ranked in the following order: Halilbay ($GI_{\%average}$ 47.5%) → Ronaldo and Opale ($GI_{\%average}$ 62.3%) → San Andrea ($GI_{\%average}$ 94.1%).

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