

THE ALLELOPATHIC EFFECT PRODUCED BY SYNTHETICALLY AND NATURALLY ALLYL ISOTHIOCYANATE ON SEEDLING GROWTH TO TRITICOSECALE AND TRITICUM AESTIVUM FALADO VARIETY

Monica ȘIPOȘ^{1#*}, Ioana BORZA², Sorina PETRUȚ³

¹University of Oradea, Biology Department, Informatics and Science Faculty, 1Universitatii St., Oradea, Romania,

²University of Oradea, Agriculture-Horticulture Department, Environmental Protection Faculty, 26 Gen. Magheru St., 410048, Oradea, Romania

³University of Oradea, B.Sc. student in Biology, Informatics and Science Faculty, 1Universitatii St., Oradea, Romania
corresponding author e-mail: siposmonica70@yahoo.com

RESEARCH ARTICLE

Abstract

This paper study the effects of microemulsions (0,02% ; 0,01% ; 0,005%; 0,002% and 0,001%) of synthetically Sigma-Aldrich allyl isothiocyanate and of the solutions naturally obtaining (20%, 10%, 5%, 2% and 1%) from horseradish roots. The allyl isothiocyanate is the main bioactive compound in aqueous extracts from metamorphosed horseradish roots. The effect produced on caryopses germination ability and seedling growth of *Triticosecale* and *Triticum aestivum* L. var. Falado was followed. The germination capacity of cereal caryopses - compared to the control - was not modified by any concentrations of synthetically allyl isothiocyanate microemulsion used (0,02%, 0,01%, 0,005%, 0,002% and 0,001%). Only the 20% aqueous extract from horseradish roots caused a decrease in germination of wheat caryopses to 48%. In *Triticosecale* and *Triticum aestivum* variety Falado the 0,02% concentration of microemulsion determined significant inhibitions of seedling growth; 0,01% and 0,005% concentrations caused influences statistically insignificant. The 0,002% and 0,001% concentrations caused significant stimulation of roots and coleoptiles growth. The growth in length of vegetative organs of cereal seedlings was significantly inhibited by aqueous extracts of horseradish roots at 20% and 10% concentrations. In triticale the 5% concentration caused significant stimulations but in wheat this concentration determined significant inhibitions in the growth of seedlings. In the last two variants of aqueous horseradish root extracts 2% and 1% the growth in length of triticale seedlings was significantly stimulated. In wheat Falado variety the influences were insignificant for roots but coleoptile was significantly stimulated of aqueous horseradish extracts 2%.

Keywords: allyl isothiocyanate, *Triticosecale*, *Triticum aestivum* var. Falado, allelopathy

#Corresponding author: siposmonica70@yahoo.com;

INTRODUCTION

Allelopathy is an important mechanism that plays a semnificative role in natural as well as cultivated ecosystems. Allelochemicals are released by a variety of mechanisms (volatilization, decomposition of residues and root exudation). The plants release secondary metabolites (allelochemicals) that influence positive or negative the growth and development of neighboring plants (Cachiță et Corbu, 2010; Hiero et Callaway, 2021, Zang et al., 2020). More recently, a distinction is made between "true allelopathy" (direct release of active compounds) and "functional allelopathy" (compounds transformed by soil microorganisms) (Scavo et al., 2018).

The allyl glucosinolate called sinigrin exists in significant amounts in metamorphosed

horseradish roots and releases allyl isothiocyanate through myrosinase hydrolysis (Stoin et al., 2007, 2008). This volatile, pungent compound is an allelochemical (Corbu et al., 2007).

This study examined the effects of naturally allyl isothiocyanate from aqueous extracts of horseradish root and synthetic isothiocyanate Sigma – Aldrich on the growth of seedlings of two cereals *Triticosecale* and *Triticum aestivum* var. Falado. In fact, all allelochemicals, depending on their concentration, have inhibitory or stimulating effects on plants growth. The boundary between the stimulatory and inhibitory concentrations for each species and variety should be precisely determined (Bandici et al., 2017; Bortîș et Șipoș, 2018; Șipoș et al., 2012;).

MATERIALS AND METHODS

The certified materials used in our experiments were represented by cereals caryopses without chemicals treatment from Territorial Inspectorate for Quality of Seeds and Implanting Material Bihor. The germination ability was determined at *Triticum aestivum* var. Falado (95%-100%) and *Triticosecale* (98-100%).

The synthetic oil of allyl isothiocyanate (Sigma-Aldrich) was dispersed in water by ultrasonication using an Emmi device-4D with the frequency 40 kHz. In an Erlenmeyer flask with a glass cork 0,2 ml of synthetic allyl isothiocyanate and 100 ml of distilled water were introduced. 0,2% microemulsions of allyl isothiocyanate was obtained after ultrasonication for 30 minutes at 30°C. Using the graduated cylinders dilutions 0,02% (I₁), 0,01% (I₂), 0,005% (I₃), 0,002% (I₄), 0,001% (I₅) were performed.

The naturally allyl isothiocyanate solution was produced by scraping tuberous roots of horseradish (*Amaracia rusticana* L.) harvested in the fall. 200 g of scraped material was placed in 1000 ml distilled water. The mixture was left to macerate for 24 hours at

room temperature (20 - 21°C). The aqueous horseradish extract was strained and filtered obtaining a 20% concentration solution (H₁). The following dilutions were prepared: 10% (H₂), 5% (H₃), 2% (H₄), 1% (H₅).

The germination of caryopses in sterile colorless plastic casseroles was performed. The bottom of casseroles with filter paper moisted with 25 ml of different dilution or distilled water (for control) were covered. 50 caryopses were placed in each casserole for all experimental variants and control lots. The germination ability was determined in two replicates. The casseroles were then placed in a germination cabinet at 21-23°C in the dark. The germination ability and seedling growth after 5 days of germination was determinate. The embryonic and adventitious root length and coleoptile height (cm) of 50 plantlet were measured for all experimental variants and control lots. Individual data were statistically processed with the Sigma Plot 2001 software. Statistical analysis included arithmetic media and Student's test. Values of the procentual differences in raport with control (100%) were determined. The significance level of this difference was P>0,05 insignificant P<0,05 significant or P<0,001 strongly significant.

RESULTS AND DISCUSSIONS

The germination capacity of *Triticosecale* and *Triticum aestivum* var. Falado caryopses - compared to the control - was not modified by any concentrations of synthetically allyl isothiocyanate microemulsions used (0,02%, 0,01%, 0,005%, 0,002% and 0,001%). Only the 20% allyl isothiocyanate aqueous extract from horseradish roots caused a decrease in germination of wheat caryopses to 48% (Table 1).

The microemulsion of allyl isothiocyanate at a concentration of 0,02% (I₁) caused insignificant inhibitions of the growth in length of the vegetative organs of triticale plantlets. For triticale the inhibitions were - 10,3% for embryonic roots, -7,9% for adventitious roots and -8,02% for coleoptiles. The wheat variety Falado proved to be more sensitive because the growth of the seedlings was significantly inhibited (-24.49%; -19.55%, respectively -32.61%) (p<0.001).

Table 1.
The germination ability of the caryopses of the *Triticosecale* and *Triticum* var. Falado in control groups (C) and various dilutions of synthetically (I₁ - I₅) and naturally (H₁ - H₅) obtaining allyl isothiocyanate.

Treatment	Variants	Germination ability	
		<i>Triticosecale</i>	<i>Triticum Falado</i>
Allyl isothiocyanate Sigma Aldrich	Control	100%	100%
	I ₁ (0,02%)	100%	96%
	I ₂ (0,01%)	98%	98%
	I ₃ (0,005%)	100%	100%
	I ₄ (0,002%)	100%	98%
	I ₅ (0,001%)	98%	100%
Allyl isothiocyanate in aqueous horseradish extract	Control	99%	95%
	H ₁ (20%)	92%	48%
	H ₂ (10%)	96%	100%
	H ₃ (5%)	98%	98%
	H ₄ (2%)	98%	100%
	H ₅ (1%)	98%	100%

The following two concentrations of synthetic allyl isothiocyanate microemulsions 0,01% (I₂) and 0,005% (I₃) determined in triticale and wheat (Falado) stimulations of the growth in length of the vegetative organs of the seedlings (embryonic roots, adventitious roots, coleoptiles), but influences were statistically insignificant. The concentrations of 0,002% (I₄) and 0,001% (I₅) caused significant stimulation of rootlets and coleoptiles growth (Table 2).

Previous results show that the growth of wheat Cubus and Dropia variety seedlings was significantly inhibited at concentrations of allyl isothiocyanate (0,02%; 0,01%). In the case of experimental concentrations (0,002%; 0,001%) inhibitions were insignificant or growth was stimulated (Bortiş and Şipoş, 2018). In the Lukulus wheat variety seedling growth at the 0,001% concentration allyl isothiocyanate was stimulated. The seedlings growth of Tristan variety triticale evolved in optimum conditions using the concentration of 0,001% allyl isothiocyanate (Şipoş et al., 2016). The concentrations 0,02% and 0,01% allyl isothiocyanate significantly inhibited seedling growth of Marco Polo wheat variety. Instead, the 0,002% and 0,001% concentrations ensured their optimal growth. The Trublion wheat variety was less sensitive to the action of the allelopathic substance allyl isothiocyanate. Only the 0,02% concentration caused significant inhibitions of seedling growth. The other concentrations taken in the study (0,01; 0,005; 0,002 and 0,001%) significantly stimulated the growth in the length of the vegetative organs (Şipoş et al., 2023).

The growth of vegetative organs (embryonic roots, adventitious roots,

coleoptiles) of triticale and wheat (Falado) seedlings was significantly inhibited ($p < 0,001$) by aqueous horseradish extracts H₁ (20%) and H₂ (10%). Thus, in *Triticosecale*, the inhibitions were -90,35% (H₁) and -78,55% (H₂), in terms of embryonic roots; -93,6% (H₁) and -84,9% (H₂) for adventitious roots; -84,2% (H₁) and -77,1% (H₂) for coleoptiles. In wheat (Falado) inhibitions were recorded -90% (H₁) and -87,7% (H₂) for embryonic roots; -86,5% (H₁) and -86,1% (H₂) for adventitious roots and -75,2% (H₁) and -67,5% (H₂) for coleoptiles (Table 2).

In *Triticum aestivum* Falado variety the concentration of 5% (H₃) of aqueous horseradish extracts caused significant inhibitions ($p < 0,001$) of the growth in length of the vegetative organs: embryonic roots (-37,2%); adventitious roots (-29,4%) and coleoptiles (-26,1%). In triticale, the concentration of 5% (H₃) caused significant stimulations ($p < 0,05$) of the growth of the studied vegetative organs.

In the last two variants of aqueous horseradish root extracts 2% (H₄) and 1% (H₅) the growth in length of embryonic, adventitious and coleoptile roots of triticale seedlings was significantly stimulated. In wheat var. Falado the influences were insignificant for roots but coleoptile was significantly stimulated of aqueous horseradish extracts 2% (Table 2).

Our previous research provided similar results regarding the action of aqueous extracts from horseradish roots on the growth of Marco Polo and Trublion wheat seedlings. In the Marco Polo and Trublion wheat variety the concentration 20% from the aqueous extract of horseradish determined significant inhibition in the seedling growth. At Marco Polo the other dilutions 10%, 5% and 2% significantly inhibited the growth of rootlets and coleoptiles showed statistically insignificant stimulations. Only the 1% concentration caused insignificant inhibitions in root length growth and significant stimulations in coleoptile growth. The Trublion wheat variety was also shown to be more resistant to the action of aqueous horseradish extracts. The dilutions 10% and 5% determined significant inhibitions in the roots growth but coleoptiles were significantly stimulated. The 2% concentration caused insignificant inhibitions in roots length growth and significant stimulations in coleoptiles growth. The last dilution of horseradish root aqueous extract 1% were found to be beneficial because significant stimulated the growth of the plantlets (Şipoş et al., 2023).

Table 2.

Values of the procentual differences (%) in raport with control (100%) of arithmetic media of embryonic and adventitious root length and coleoptile height (cm) and significance level (a-strongly significant $P < 0,001$; b-significant $P < 0,05$; c-insignificant $P > 0,05$)

Treatment	Dilutions	Biotest species					
		<i>Triticosecale</i>			<i>Triticum Falado</i>		
		Embryonic root length	Adventitious root length	Coleoptile height	Embryonic root length	Adventitious root length	Coleoptile height
Allyl isothiocyanate	I ₁ - 0,02%	-10,31%c	-7,91%c	-8,02%c	-24,49%a	-19,55%a	-32,61%a
	I ₂ - 0,01%	+5,68%c	+6,66%c	+14,8%b	-2,4%c	+1,01%c	+6,6%c
	I ₃ - 0,005%	+10,3%c	+8,14%c	+9,54%c	+7,19%c	+9,13%c	+1,46%c
	I ₄ - 0,002%	+19,5%a	+15,5%a	+27,1%a	+26,2%a	+23,6%a	+15,01%b
	I ₅ - 0,001%	+33,7%a	+23,7%a	+27,3%a	+20,2%a	+13,45%b	+17,58%b
Aqueous horse-radish extract	H ₁ - 20%	-90,35%a	-93,6%a	-84,24%a	-90 %a	-86,53%a	-75,29%a
	H ₂ - 10%	-78,55%a	-84,98%a	-77,18%a	-87,73%a	-86,1 %a	-67,53%a
	H ₃ - 5%	+19,1%b	+19,7%b	+22,4%b	-37,28%a	-29,46%a	-26,15%a
	H ₄ - 2%	+29,1%a	+15,7%b	+31,9a	-2,58%c	+8,9%c	+16,66%b
	H ₅ - 1%	+18 %b	+15,7%b	+16,2%b	-5 %c	+3,42%c	-0,29%c

CONCLUSIONS

1. The germination capacity of triticale and wheat Falado caryopses was not modified - compared to the control - by any of allyl isothiocyanate concentrations of the microemulsions used: 0,02%, 0,01%, 0,005%, 0,002% and 0,001%. Only the 20% aqueous extract from horseradish roots caused a decrease in germination to 48% at *Triticum aestivum* Falado variety.

2. In *Triticosecale* and *Triticum aestivum* variety Falado the 0,02% concentration of the synthetic allyl isothiocyanate microemulsions caused significant inhibitions of the growth in length of embryonic, adventitious roots and coleoptiles. The 0,01% and 0,005% concentrations caused stimulations or inhibitions of the growth in length of vegetative organs but all influences were statistically insignificant. In contrast, the 0,002% and 0,001% concentration caused significant stimulation of roots and coleoptiles growth.

3. The growth in length of vegetative organs of cereal seedlings was significantly inhibited by aqueous extracts of horseradish roots at 20% and 10% concentrations. In triticale the 5% concentration caused significant stimulations but in wheat this concentration determined significant inhibitions in the growth of seedlings. In the last two variants of aqueous horseradish root extracts 2% and 1% the growth in length of triticale seedlings was significantly stimulated. In wheat Falado variety the influences were insignificant for roots but coleoptile was significantly stimulated of aqueous horseradish extracts 2%.

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