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ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACTS FROM HORSERADISH (*ARMORACIA RUSTICANA* L.) METAMORPHOSED ROOTS ON SEVERAL CEREALS

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ABSTRACT

This paper study the allelopathic effects of different dilution (5, 15, 25, 50, 75 and 100%) of aqueous extracts from *Armoracia rusticana* L. metamorphosed roots on caryopses germination ability and seedling growth of *Triticosecale* Witt., *Triticum aestivum* L. and *Hordeum vulgare* L. Allyl glucosinolate namely sinigrin exists in significant content in horseradish roots and release by enzymatic hydrolysis, when the plant tissue is disrupted, the aglycon allelopathic compounds (allyl isothiocyanate, allyl cyanide, 2,3-epithiopropane cyanide). The 5% and 15% dilution of aqueous extracts from horseradish metamorphosed roots stimulated kernel germination ability at all cereal species and accumulation of the fresh and dry biomass at triticale and wheat but not in the barley case. The increasing of the aqueous extracts concentration from 25% to 100% accentuated the inhibitory effects. The roots were more affected by the extracts than coleoptiles or leaves. The stimulatory/inhibitory effects of allelopathic aqueous mixture from horseradish roots are in relation with their concentration and with receptor species sensibility. The allyl isothyocianate is the main bioactive compounds in the aqueous extracts of *Armoracia rusticana* L. metamorphosed roots. Is indicated the utilization of this compounds from black mustard essential oil.

Key words: allelopathy, *Armoracia rusticana* L., aqueous extract, allyl isothyocianate, *Triticosecale* Witt., *Triticum aestivum* L. and *Hordeum vulgare* L., seed germination, seedling growth.

INTRODUCTION

In the last decades allelopathic research was focused for finding biofertilizers and bioherbicides (Morimoto, 2004; Weston, 2005). Many researcher studied the effects of aqueous extracts from different parts of plants on the growth and development of other species (Kazinczi et al., 2004; Paul and Sultana, 2004; Uremis et al., 2005; Javaid et al., 2006; Xingsiang et al., 2009; Qian et al., 2010). This paper describes the allelopathic interference of aqueous extracts from *Armoracia rusticana* L. metamorphosed roots on germination ability and seedling growth of *Triticosecale* Witt., *Triticum aestivum* L. and *Hordeum vulgare* L.

MATERIAL AND METHODS

The certified materials used in our experiments were represented by cereals caryopses of *Triticosecale* Witt., *Triticum aestivum* L. and *Hordeum vulgare* L. The germination ability of grains was 90% in triticale, 92% in wheat and 80% in barley. The aqueous extracts from horseradish (*Armoracia rusticana* L.) metamorphosed roots were prepared as follows: fresh roots were grated by a shredder and were covered with distilled water. The ratio fresh shredded root material (g) and distilled water (ml) was 1:2. This mixture was left to macerate at 25°C for 12 h in covered Erlenmeyer glasses. Allyl glucosinolate, namely sinigrin, exists in significant amount in horseradish roots (Stoin et al., 2007) and releases allyl isothiocyanate (volatile major pungent compound) and other aglycon compounds (allyl cyanide and 2,3-epithiopropane cyanide), sugar products and potassium sulfate (Shahidi and Gabon, 1990), by the action of myrosinase (enzymatic hydrolysis) (Stoin et al., 2008), when the plant tissue is disrupted. The macerate was

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squeezed and aqueous etract was filtered through filter paper. This extract was considered 100% and was preserved for short time (3 days) in dark glass at 4°C.

Allyl isotiocianate is unstable and gradually decomposes in approximately ten days, in aqueos solution at room temperature, to paraffin like hydrocarbon and sulfur. Moreover, N, N'-diallylthiourea is produced by action of water (Kawakishi and Namiki, 1969). The aqueous extract considered 100% was diluted with distilled water to 5%, 15%, 25%, 50% and 75% (Corbu et al., 2007).

The germination of caryopses was performed in sterile colourless plastic casseroles. Sterilization of the casseroles was made by submersing them in sodium hypochlorite solution 1% for 12 h and washed with tap water in abundance. Pre-disinfection treatment of grains was made with ethylic alcohol 96° for 30 seconds and after this procedure grains were disinfected by submersing them in sodium hypochlorite solution 2% for 1 minute and then washing with sterile water (Sauer and Burroughs, 1986). The bottom of casseroles was covered with filter paper which was moistened with 25 ml of different dilution of the aqueous extracts (5, 15, 25, 50, 75 and 100%) and with the same volume of distilled water for controls. For all experimental variants and control, lots 50 caryopses were placed in each casserole. The germination ability was detemined in six replicates. The covered coourless casseroles were then placed in a germination cabinet at 25-27°C in the dark. Germination ability was determined after 3 days and in the casseroles with plantlets for biometry - two replicates- 20 ml of the different dilutions from aqueous extract or distilled water (for control) were added. For seedling growth, after 5 day of germination, the embryonic and adventitious root length, coleoptiles and first leaf length (mm), fresh and dry biomass of the plantlets (mg) was determined. For dry biomass plantlets were dehydrated at 80°C for 24 hours. Statistical analysis included: arithmetic mean (M), standard deviation (s_d) and Student's test of significance (SigmaPlot 2001 software). The significance level was set at $P<0.05$ or $P<0.001$. The response index (RI) was calculated according Williamson and Richardson (1988). RI=1-C/T (when T≥C) and RI=T/C-1 (when T<C), where T is the treatment response and C is the control response. RI value ranges from +1 to -1, with RI>0 meaning stimulation, RI<0 meaning inhibition.

RESULTS

The experimental results that reveal allelopathic effects of different dilutions (5, 15, 25, 50 and 75%) of aqueous extracts (100%) from *Armoracia rusticana* L. metamorphosed roots on caryopses germination ability and seedling growth of *Triticosecale* Witt., *Triticum aestivum* L. and *Hordeum vulgare* L. are listed in table 1.

After 5 days germination on filter paper moistened with aqueous dilution 5% and 15%, triticale germination ability, coleoptile length, fresh and dry biomass were stimulated (Table 1). The length of embryonic and adventitious roots was inhibited, but insignificantly ($P>0.05$) (RIs of the roots was -0,09, respetively -0,06) in the case of 5% dilution and significant ($P<0.001$) for 15% (RIs of the roots was -0,21 and -0,22 respectively). In the case of 25% dilution – for all parameters except germination ability and coleoptiles length – insignificant and significant inhibition were registered (Table 1). For all parameters the significant inhibitions ($P<0.001$) were progressively enhanced with increasing the aqueous extracts concentration from 50% to 100%. In the case of wheat, the dilutions of 5% and 15% produced insignificant or significant stimulation for germination ability ($P>0.05$), coleoptiles length ($P>0.05$), fresh ($P<0.05$) and dry biomass ($P>0.05$). The roots were more susceptible because significant inhibition was registered (Table 1). The germination ability of wheat was insignificantly stimulated ($P>0.05$) by 25% dilution of aqueous extract from horseradish metamorphosed roots and seedling growth was significantly inhibited in comparison with control (Table 1). The increasing of the aqueous extracts concentration from 50% to 100% progressively enhanced the significant inhibition of all parameters studied by us.

Table 1. Values of the response index (RI) and significance level
(a = insignificant P>0.05; b = significant P<0.05; c = strongly significant P<0.001)

Cereals	Parameters	Dilution (%) of the aqueous extract from *Armoracia rusticana* L. metamorphosed roots					
		5%	15%	25%	50%	75%	100%
		RI-P					
Triticosecale Witt.	Germination	+0.03a	+0.03a	+0.06a	-0.18b	-0.35c	-0.47c
	Embryonic root length	-0.09a	-0.21c	-0.58c	-0.72c	-0.86c	-0.99c
	Adventitious root length	-0.06a	-0.22c	-0.54c	-0.65c	-0.83c	-0.92c
	Coleoptile length	+0.05b	+0.06b	+0.05a	-0.14c	-0.71c	-0.81c
	First leaf length	+0.07a	-0.06a	-0.19c	-	-	-
	Fresh biomass	+0.18b	+0.06a	-0.15b	-0.65c	-0.81c	-0.83c
	Dry biomass	+0.13a	+0.03a	-0.03a	-0.40c	-0.57c	-0.60c
Triticum aestivum L.	Germination	+0.01a	+0.04a	-0.02a	-0.15b	-0.46c	-0.68c
	Embryonic root length	-0.16b	-0.36c	-0.54c	-0.67c	-0.80c	-0.90c
	Adventitious root length	-0.19c	-0.35c	-0.50c	-0.64c	-0.74c	-0.87c
	Coleoptile length	+0.03a	+0.02a	-0.21c	-0.48c	-0.67c	-0.88c
	First leaf length	-0.03a	-0.06a	-	-	-	-
	Fresh biomass	+0.17b	+0.12b	-0.17b	-0.41c	-0.58c	-0.77c
	Dry biomass	+0.14a	+0.03a	-0.17b	-0.32c	-0.44c	-0.58c
Hordeum vulgare L.	Germination	+0.08b	+0.11b	-0.05b	-0.24c	-0.44c	-0.54c
	Root length	-0.28c	-0.40c	-0.48c	-0.72c	-0.86c	-0.87c
	Coleoptile length	+0.06a	+0.05a	+0.06a	-0.16b	-0.68c	-0.78c
	First leaf length	-0.07a	-0.09a	-0.12a	-	-	-
	Fresh biomass	-0.03a	-0.02a	-0.07a	-0.41c	-0.77c	-0.82c
	Dry biomass	-0.11a	-0.11a	-0.14b	-0.32c	-0.61c	-0,62c

The germination ability in barley was significantly stimulated (P<0.05) by 5% and 15% dilution of aqueous extract from horseradish metamorphosed roots, but seedling growth was inhibited in comparison with control. Significant inhibition (P<0.001) was registered in the case of root length (RIs of the roots were -0.21 for 5% dilution and -0.40 for 15% respectively) (Table 1). If the barley grains were germinated for 5 days on filter paper moistened with 25% dilution, RIs of the coleoptiles length was +0.06 (P>0.05). Insignificant or significant inhibitions were registered for germination ability, roots length, first leaf length, fresh and dry biomass (Table 1). The RIs experimental values showed inhibition of all parameters (P<0.001) in the case of barley germination and plantlets growth on more concentrated aqueous extracts (50, 75 and 100%).

DISCUSSION

The stimulatory or inhibitory effects of aqueous extracts of horseradish metamorphosed roots on seed germination and seedling growth in triticale, wheat and barley is concentration dependent. The 5% and 15% dilution of aqueous extracts prepared by us stimulated germination ability in all species studied and biomass accumulation in triticale and wheat, but not in barley. From 25% to 100% concentration of aqueous extracts the inhibitory effects increased as the concentration increased. The 5% and 15% aqueous extracts of *Armoracia rusticana* L. metamorphosed root decreased the root length, but increased the coleoptiles length. It is known that roots are more susceptible than shoots to allelochemicals (Obaid and Qasem, 2005; Qian et al., 2010).

The allyl isothyocianate is the main bioactive compounds in the aqueous extracts of *Armoracia rusticana* L. metamorphosed roots. The main chemical component of black mustard oil has no less than 92% allyl isothiocyanate (http://www.essentialoils.co.za/essential-oils/mustard.htm#Chemical).

The actual use of aqueous extracts from metamorphosed horseradish roots would be less recommended because several variables may occur during their preparation: the amount of sinigrin in the roots varies depending on the ontogenetic stage of the plant (Corbu et al., 2007); the shredding degree of the material influences the release of myrosinase and this enzyme decomposes the sinigrin depending on the temperature conditions it is subjected to (Sahidi and Gabon, 1990; Stoin et al., 2008); the aqueous solutions must be freshly prepared as the allyl isothiocyanate decomposes (Kawakishi and Namiki, 1969). The limit between the stimulating and inhibitory concentrations must be established with high accuracy for each species. Any mistake in concentration may lead to bioinhibition instead of biostimulation and vice versa. If stimulating concentrations for grain crops are inhibitory for certain weeds, aqueous solutions with allyl isothiocyanate might be used as bioherbicide. Recent researches aim at using essential oils extracted from plants for their bioactive compounds (Cavalieri and Caporali, 2010; Mutlu et al., 2010, Jinbao et al., 2010).

CONCLUSIONS

The germinating ability of triticale, wheat and barley seed was stimulated by the 5% and 15% dilutions of the aqueous extract of the metamorphosed horseradish roots. The increase in the concentration of the aqueous extracts (50%, 75%, and 100%) caused significant and progressive inhibition of germinating ability in all three species.

The seedling roots of the three species studied by us proved to be sensitive to the action of the bioactive compounds, even at 5% and 15% dilutions of aqueous extracts of horseradish; significant inhibitions of their growth in length was noticed.

The coleoptiles and first leaves of triticale, wheat and barley seedlings were less sensitive to the action of allyl isothiocyanate than the roots, and stimulation of their growth occurs under the action of 5%, 15% and even 25% dilutions of aqueous extracts of metamorphosed horseradish. The aqueous extracts of higher concentrations (50%, 75%, 100%) caused statistically significant inhibitions of the growth in length of the coleoptiles in triticale, wheat and barley.

The experimental results regarding the fresh and dry biomass of the seedlings revealed stimulations of these parameters only in triticale and wheat and at 5% and 15% dilutions.

Higher concentrations produced only inhibitions of these two parameters, inhibitions that advanced from insignificant to significant as the concentration of bioactive substances in the aqueous extracts increased.

One must accurately establish the limit between the stimulatory and inhibitory concentrations for each species. In certain concentrations, the allyl isothiocyanate can be used as natural biostimulator or herbicide, if some weeds will prove to be more susceptible to its action than crop plants.

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