THE PHYTOTOXICITY OF THE AQUEOUS EXTRACTS FROM FRUITS AND SEEDS OF SOME *APIACEAE* SPECIES

Şipoş Monica*, Bandici Gheorghe, Chimpan Camelia*****

* University of Oradea, Biology Department, Science Faculty, 1Universitatii St., Oradea, Romania, e-mail: siposmonica70@yahoo.com
** University of Oradea, Agronomy Department, Environmental Protection Faculty, 26 Gen. Magheru St., 410048, Oradea, Romania
*** University of Oradea, M.Sc. student in Biology, Science Faculty, 1Universitatii St., Oradea, Romania

Abstract

The present study was conducted to investigate the phytotoxicity of biologically active mixture that was extracted from fruits and seeds of some Apiaceae species (Petroselinum crispum L. var. radicosum, Pastinaca sativa L., Anethum graveolens L. and Apium graveolens L.). The germination ability and seedling growth of biotest species Sinapis alba L. and Raphanus sativus L. var. Saxa were investigated. The aqueous extracts of Apiaceae achenes displayed their allelopathic effect on seeds germination and seedling growth in the following descending order of phytotoxicity: celery, parsley, dill and parsnip. Mustard, in comparison to radish, is more sensitive to the action of biologically active substances from the aqueous extracts of Apiaceae species and, therefore, it is more indicated to be used as biotest specie.

Key words: phytotoxicity, fruits, *Petroselinum crispum* L. var. *radicosum*, *Pastinaca sativa* L., *Anethum graveolens* L. , *Apium graveolens* L.

INTRODUCTION

There is a mixture of bioactive compounds in the fruit of some *Apiaceae* such as dill, parsnip, parsley and celery. Thus, one can point out:

- Fatty acids: palmitic, oleic, linoleic, petroselinic in *Apiaceae* (dill, parsley, parsnip, celery).

- Flavonoids and polyphenolic acids: the parsley leaves contain apiin, luteolin and apigenin glycoside as well as apiol and myristicin as antioxidants (Fejes et al., 1998). In dill there are hydroxycinnamic acid derivatives (caffeic acid, chlorogenic acid), rutin, quercetin (flavonoids from leaves and fruit), kemferol (flavonoids in fruit) (Orţan et al., 2009). A strong bioactive compound found in the fruit of the celery is the p-Coumaric acid, soluble in water (http://www.herbalextractsplus.com/celery-seed.cfm).

- Terpene hydrocarbons: they are numerous and varied and have been studied in detail in the aerial parts of *Apiaceae* (Ostav et al., 2003). Triterpenes, such as oleanolic acid and ursolic acid, and sterol - beta-sitosterol - were identified in the fruits of dill (Orţan et al., 2009). Monoterpenes and sesquiterpenes are also present (Zorca et al., 2007). 40-

60% carvone have also been identified in the dill. It has been shown that monoterpenes are soluble in water, even if in a smaller proportion (Weidenhamer et al., 1993).

- Coumarin and furanocoumarin: Imperatorin (31.8%), bergapten (18.1%), isopimpinellin (5.6%), xanthotoxin (35.5%), sphondin (8.5%) (Berembaum et al., 1984) and angelicin 0.5% (Berenbaum, 1991) were identified in the fruit of *Pastinaca sativa*. There are imperatorin and bergapten in the fruit of parsley (Fejes, 1998). Scopoletin is present in all parts of the dill. There is coumarin in the aerial part of the plan, while in the fruit one can find scopoletin, umbelliferone, bergapten, and xanthotoxin (Orțan et al., 2009). Bergapten and scopoletin were identified in the fruits of celery (http://www.herbalextractsplus.com/celery-seed.cfm).

The solubility of the bioactive compounds is variable. For example, the coumarin and furanocoumarin solubility is good in alcohol (Usmanov et al., 1969) and very good in acetone (Eidler et al., 1975; Razavi et al., 2010); the ethylic alcohol is indicated for terpenes, flavones and phenolic acids (Sayadi et al., 2010). The terpenes can be easily extracted using alcohol, yet they are slightly soluble in water (Cal, 2006, Weidenhamer et al., 1993).

Our research has been focused on observing the effect of the biologically active aqueous mixtures, which were extracted from the seeds and fruit of some species belonging to the family *Apiaceae* (*Anethum graveolens* L., *Pastinaca sativa* L., *Petroselinum crispum* L. var. *radicosum* şi *Apium graveolens* L.) on the bioassay species *Sinapis alba* L. and *Raphanus sativus* L., Saxa variety. Seed germination and the growth of vegetative organs of seedlings (embryonic roots and hypocotyls) were observed in the bioassay species, under the action of various dilutions (2, 5, 10, 20 and 40%, which represented the experimental variants V_1-V_5) of the aqueous extracts mentioned above.

MATERIAL AND METHOD

The study was conducted using white mustard seed (*Sinapis alba* L.), with germination ability higher than 90% and radish seeds of Saxa variety (*Raphanus sativus* L., Saxa variety), with a germination ability of about 75%.

The germination ability of seeds used in our experiments was determined in compliance with the methodological norms recommended by the International Seed Testing Association (ISTA).

Stock solutions, representing the extracts with bioactive compounds from the fruit and seeds of parsnip, dill, parsley and celery, were obtained by introducing 25 g of fruit in 150 ml of distilled water (1: 6 ratio), where

they were kept for 54 hours, at room temperature. The extraction of organic compounds in different solvent increased with temperature. Dilutions with distilled water were made from this solution (2, 5, 10, 20 and 40%, the experimental variants V_1-V_5) and they were used for the study of their phytotoxicity. Mustard and radish seeds were germinated in Petri glass dishes with a diameter of 11cm, on filter paper moistened with 10 ml of the extract dilutions. 25 seeds were placed in each Petri glass dish, both for treated groups and control groups, represented by the seed germinated on filter paper, moistened with 10 ml of distilled water. Four replicates were prepared for each experimental variant and control samples. The Petri dishes were covered and were kept at room temperature to encourage germination (T = 21-23 ° C) in the dark. Four clear plastic containers, parallel lots to the Petri dishes, were placed to germinate in order to establish the influence of dilutions from *Apiaceae* fruit extracts on the germination ability of mustard and radish seeds. 50 seeds were placed in each casserole dish, on filter paper moistened with 20 ml of different dilutions and with 20 ml of distilled water for control samples. The germination ability was established after three days of germination. The length of embryonic roots and hypocotyls, both in treated groups and control samples, was measured after 5 days of germination. The biometrics was carried out on seedlings that grew in two replicates in Petri dishes (50 seedlings) for each experimental variant.

Statistical analysis (arithmetic media, standard deviation and Student's test) was performed using SigmaPlot 2001 software. The significance level was set at $P<0.05$ or $P<0.001$. The response index (RI) was calculated according to Williamson and Richardson (1988). RI=1-C/T (when $T \geq C$) and RI=T/C-1 (when $T<C$). T is the treatment root or shoot elongation response and C is the control response. RI value range from -1 to +1, $RI>0$ means stimulation, $RI<0$ means inhibition.

RESULTS AND DISCUSSION

As for the mustard seeds, the germination ability was significantly inhibited using V_4 (20%) and V_5 dilutions (40%) of the aqueous extract of celery achenes and at V_5 dilution (40%) of the aqueous extract of parsley and dill. Also, the germination of radish seeds was significantly inhibited using the V_5 dilution (40%) of the aqueous extract of celery (Table 1.).

Table 1.

Germination ability of the mustard and radish seeds germinated on different dilutions of aqueous extracts of *Apiaceae* achenes (b- significant inhibition).

Variants	Control sample and aqueous extracts of different dilutions					
	M	V_1(2%)	V_2(5%)	V_3(10%)	V_4(20%)	V_5(40%)
Mustard with dill	94%	91%	91%	87%	93%	77%b
Radish with dill	75%	79%	79%	71%	71%	72%
Mustard with parsnip	92%	89%	87%	86%	85%	87%
Radish with parsnip	74%	68%	71%	68%	68%	62%
Mustard with parsley	91%	95%	91%	94%	90%	73%b
Radish with parsley	79%	78%	74%	69%	68%	73%
Mustard with celery	94%	96%	93%	92%	77%b	69%b
Radish with celery	78%	77%	78%	69%	67%	49%b

The average increase in length of the embryonic root of mustard seedlings, in experimental variants V_1-V_3 of dilutions from **dill** extract, showed significant stimulation ($p < 0.001$) compared to the control sample, IR=+0.54(V_1), +0.51(V_2) and of +0.84 (V_3). Instead, the dilution of 40% of the aqueous extract from dill achenes - representing the experimental version V_5 - manifested its phytotoxic effect on the growth of seedlings of white mustard (IR = -0.57) (Table 2). As for the radish, in the case of the experimental variants $V_1 - V_4$, statistically insignificant stimulations and inhibitions were recorded ($p > 0.05$), compared to control samples, regarding the increase in length of the embryonic roots. The dilution of 40% of aqueous extract of dill achenes - representing the experimental version V_5 - manifested its phytotoxic effect on the growth in length of the roots of radish seedlings IR = -0.31 ($p < 0.001$). The embryonic roots of mustard seedlings were more sensitive to the action of bioactive substances extracted using water from dill achenes, compared with embryonic roots of radish seedlings. Thus, in the case of the experimental variants $V_1 - V_3$, no significant stimulations of the growth in length of the embryonic roots were recorded and, in the experimental variant V_5, the inhibition was more pronounced.

As it can be seen in Table 2, in the case of germination of mustard and radish seeds using the dilutions of 2% (V_1), 5% (V_2), 10% (V_3) and 20% (V_4) of the aqueous extract from the **parsnips** fruit and seeds, stimulations and inhibitions of the growth in length of embryonic roots were recorded, but they were not statistically significant. Alternatively, for the experimental variant V_5, one can see statistically significant inhibitions, compared to the control sample (IR = -0.21, $p < 0.05$).

In the experimental variants V_1 – V_3 of radish and mustard seed germination on substrates moistened with aqueous extracts from **parsley** fruit and seeds, statistically insignificant stimulations and inhibitions were recorded ($p > 0.05$) in the average increase in the length of embryonic roots of the seedlings. Conversely, the inhibitions were statistically significant in the experimental variants V_4 – V_5 ; IR = -0.33 in mustard (V_4) ($p < 0.001$) and -0.38 (V_5) ($p < 0.001$); IR = -0.19 in radish (V_4) ($p < 0.05$) and -0.36 (V_5) ($p < 0.001$) (Table 2).

For mustard seed germination using the dilutions of 2% (V_1) and 5% (V_2) of the aqueous extract of **celery** fruit and seeds, there were statistically significant (V_1) and insignificant stimulations (V_2) of the increase in the length of embryonic roots. Conversely, in the experimental variants V_3 -V_5, one can notice the statistically significant inhibitions in relation to the control sample, in the average length of embryonic roots of the mustard seedlings (Table 2). In the seed germination of radish using 2% dilutions (V_1) and 5% (V_2) of the aqueous extract of celery fruit, significant stimulations of the increase in length of the embryonic roots were recorded ($p < 0.05$). In V_4 -V_5 experimental versions one can see, in relation to the control sample, significant inhibitions ($p < 0.05$) in the growth of the average length of embryonic roots of radish seedlings, IR = -0.15 (V_4), and respectively -0.30 (V_5). It is obvious that the embryonic roots of the mustard seedlings are more sensitive to the action of bioactive substances extracted with water from celery achenes, as compared to the embryonic roots of the radish seedlings (Table 2).

The average increase in height of hypocotyls of the mustard seedlings resulted from germinated seeds on substrate moistened with the aqueous extracts from **dill** achenes was significantly stimulated ($p < 0.001$) in the case of the experimental variants V_1 - V_3 , IR=+0,20(V_1), +0,21(V_2), respectively of 0.20 (V_3). In the experimental versions V_4 -V_5 one can notice, in relation to the control sample, statistically insignificant stimulations in terms of the growth of the average height of mustard seedlings hypocotyls (Table 2). As for the radish plants, one can see in Table 2 the fact that the growth of hypocotyls was insignificantly stimulated in the case of the experimental variants V_1 - V_3, and significantly stimulated ($p < 0.05$) in the case of germination of radish seeds on aqueous extracts from dill achenes, 20% (V_4)) and 40% (V_5) dilutions. These results demonstrate that, just like the embryonic roots, the hypocotyls of mustard seedlings are susceptible to the action of the bioactive compounds found in the aqueous extracts of dill, in comparison to those of radish.

The average increase in height of hypocotyls of mustard and radish seedlings, resulting from the germination of seeds on aqueous extracts of **parsnip** achenes using the 2% (V_1), 5% (V_2), 10% (V_3), 20% (V_4) and 40%

(V_5) was stimulated in all experimental variants. The hypocotyls of mustard seedlings proved to be more susceptible to the action of bioactive substances, recording statistically significant progress since using the dilution of 10% (V_3), IR = + 0.27 ($p < 0.001$), while those of radish marked significantly higher values at 20% dilution (V_4), IR = + 0.26 ($p < 0.05$).

Regarding the growth of mustard and radish seedlings on filter paper moistened with dilutions of aqueous extract of **parsley** achenes, statistically insignificant stimulations (V_1 -V_2) and significant ones (V_3 –V_5) were recorded, regarding the increase in height of seedlings hypocotyls (Table 2). With regard to the growth of mustard seedlings on filter paper moistened with dilutions of 2% (V_1) , 5% (V_2), 10% (V_3), 20% (V_4) and 40% (V_5) from the aqueous extract of the **celery** achenes, the average increase in height of celery seedlings hypocotyls in the treated groups was significantly boosted, in relation to the control sample, in all experimental variants.

Radish appears to be less sensitive to dilutions of 2% (V_1), 5% (V_2), 10% (V_3) and 20% (V_4) of the aqueous extract of celery achenes; statistically insignificant stimulations were recorded in the increase in height of seedlings hypocotyls. Only in the experimental variant with a dilution of 40% (V_5), the average increase in height of the treated radish hypocotyls seedlings was significantly stimulated in relation to the control sample, IR = + 0.26 ($p < 0.05$) (Table 2).

The average increase in the height of hypocotyls of mustard and radish seedlings, in the bioassay species, was stimulated by bioactive substances from aqueous extracts of the studied *Apiaceae* achenes.

Embryonic roots of the bioassay species of seedlings were more sensitive to the action of bioactive substances in aqueous extracts of *Apiaceae* fruit compared to hypocotyls. In the experimental variants V_4 and V_5 mostly statistically significant inhibitions were recorded, regarding the average growth in length of embryonic roots.

Table 2.

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)

Aqueous extracts of *Apiaceae* achenes	Dilutions	Biotest Species			
		Mustard		Radish	
		Root length RI/P	Shoot height RI/P	Root length RI/P	Shoot height RI/P
Anethum graveolens L.	V_1 2%	+0,54c	+0,20c	-0,07a	+0,05a
	V_2 5%	+0,51c	+0,21c	+0,08a	+0,11a
	V_3 10%	+0,84c	+0,20c	+0,18b	+0,02a
	V_4 20%	- 0,01a	+0,03a	-0,05a	+0,18b
	V_5 40%	-0,57c	+0,01a	-0,31c	+0,14b
Pastinaca sativa L.	V_1 2%	-0,03a	+0,06a	+0,04a	+0,12a
	V_2 5%	-0,17a	+0,08a	-0,07a	+0,17a
	V_3 10%	-0,07a	+0,27c	-0,04a	+0,16a
	V_4 20%	-0,08a	+0,35c	-0,10a	+0,26b
	V_5 40%	-0,21b	+0,15b	-0,21b	+0,40c
Petroselinum crispum L. var. *radicosum*	V_1 2%	+0,01a	+0,02a	+0,06a	+0,03a
	V_2 5%	-0,06a	+0,02a	+0,08a	+0,04a
	V_3 10%	-0,01a	+0,13b	-0,13a	+0,14b
	V_4 20%	-0,33c	+0,15b	-0,19b	+0,43c
	V_5 40%	-0,38c	+0,15b	-0,36c	+0,14b
Apium graveolens L.	V_1 2%	+0,26b	+0,16b	+0,20b	+0,09a
	V_2 5%	+0,05a	+0,21b	+0,19b	+0,10a
	V_3 10%	-0,19b	+0,14b	-0,01a	+0,17a
	V_4 20%	-0,64c	+0,16b	-0,16b	+0,02a
	V_5 40%	-0,70c	+0,17b	-0,30b	+0,26b

CONCLUSIONS

The aqueous extracts of *Apiaceae* achenes displayed their allelopathic effect on seeds germination and seedling growth in the following descending order of phytotoxicity: celery, parsley, dill and parsnip.

Mustard, in comparison to radish, is more sensitive to the action of biologically active substances from the aqueous extracts of *Apiaceae* species and, therefore, it is more indicated to be used as biotest specie.

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