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ALLELOPATHIC PROPERTIES OF EXTRACTS AND SOME METABOLITES PRESENT IN THE TISSUES OF COMMON BUCKWHEAT (*Fagopyrum esculentum* MOENCH) SEEDLINGS

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Abstract. The aim of the work was to evaluate the allelopathic effect of water extracts from the above-ground parts of the seedlings of control buckwheat and buckwheat treated with methyl jasmonate vapours (JA-Me), as well as extracts enriched with 2-phenylethylamine (PEA) and its metabolites: phenylacetic acid (PAA) and 2-phenylethanol (PE), on the growth of the seedlings of dicotyledonous (tomato, radish and watercress) and monocotyledonous vegetables (maize). Also their effect on the level of phenolic compounds and flavonoids was determined, as well as the degree of lipid peroxidation in acceptor plant tissues. In all the cases, plant growth inhibition was noted, and the effect differed in relation to the applied extract, the studied species, and the duration of allelochemical stress. Application of JA-Me caused an increase in the content of phenolic compounds and, as an effect, the allelopathic potential of buckwheat tissues. Generally, the roots of the tested acceptor plants were more susceptible to the effect of the studied extracts than the above-ground parts. In the case of maize, the addition of PEA, PAA, and PE strongly inhibited the growth of both the roots and the above-ground plant parts. Different phenomenon occurred, on the other hand, in tomato roots, the growth of which was stimulated by PE. Buckwheat extracts also caused enhancement in lipid peroxidation in maize, although adding PEA, PAA, and PE limited the process. Buckwheat extracts also increased the biosynthesis of phenolic compounds and flavonoids in tomato and maize tissues, which may be related to the activation of the adaptation mechanism to stressful conditions.

Key words: lipid peroxidation, methyl jasmonate, phenylacetic acid, 2-phenylethanol, 2-phenylethylamine

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INTRODUCTION

Plants that grow in natural habitat are constantly subject to stressful conditions. General and induced response to such conditions, both biotic and abiotic, is chemical defence [Bi *et al.* 2007]. This response is of essential significance in the case of plant species that possess allelopathic properties, since it may be related to an increased production and release of allelochemicals [Kato-Noguchi 2011, Maqbool *et al*. 2013]. Part of plant response to stressful conditions is the activation of signal transduction pathway, in which jasmonates may be involved [Wasternack 2007]. Application of jasmonic acid or its methyl ester (JA-Me) stimulates the biosynthesis of various and biologically active secondary metabolites, which may cause an increase in plant allelopathic potential [Wang *et al*. 2005, Uddin *et al.* 2013].

In the tissues of common buckwheat (*Fagopyrum esculentum* Moench), numerous phenolic compounds, flavonoids, fatty acids, and alkaloids were identified, and they showed allelopathic properties [Iqbal *et al.* 2002, Golisz *et al.* 2007a, Kalinova *et al.* 2007]. It was proved that buckwheat extracts affect growth and development processes by inhibiting germination and limiting the growth of mono- and dicotyledonous plants [Golisz *et al.* 2007b, Tatnell *et al.* 2012]. However, the mechanisms of the phytotoxic action of the allelochemicals present in buckwheat on acceptor plants are known only to a limited extent [Golisz *et al.* 2008].

It was recently demonstrated that JA-Me affects the contents of some phenolic acids and flavonoids in buckwheat seedlings [Horbowicz *et al.* 2011a, Kim *et al.* 2011]. The above phytohormone also causes the accumulation of substantial amounts of 2-phenylethylamine (PEA) in buckwheat tissues [Horbowicz *et al.* 2011b]. PEA is an aromatic amine formed as a result of L-phenylalanine decarboxylation [Tieman *et al.* 2006, Horbowicz *et al.* 2011b]. It was demonstrated that PEA metabolism is favourable to the creation of reactive oxygen species (ROS), which may be related to plant defence response to stressful conditions [Kawano *et al*. 2000]. Moreover, PEA may be a substrate in the biosynthesis of 2-phenylethanol (PE) – plant fragrance compound, and phenylacetic acid (PAA), which possesses weak auxin activity [Leuba and LeTourneau 1990, Tieman *et al.* 2007]. However, there is little information on the role of PEA and its metabolites in plants. Therefore, the aim of the work was to evaluate the allelopathic properties of water extracts obtained from control buckwheat seedlings, treated with JA-Me, and also enriched with PEA and PAA and PE in relation to chosen vegetable species: tomato, radish, watercress, and sweet maize. It was also determined whether allelochemical stress induced with the studied extracts affected the level of total phenolic compounds, total flavonoids, and the degree of lipid peroxidation in the acceptor plant tissues. The study may render service to explaining how the components of soil used for common buckwheat cultivation may affect consequently cultivated species.

Selection of the species for testing their response to common buckwheat extracts was motivated by the aim to conduct a relatively wide scope of the study. Therefore, choosing watercress was caused by the fact that the species is often used for the evaluation of the phytotoxic action of different chemicals. Maize is a monocotyledonous species with photosynthesis system different from the remaining studied species. On the other hand, in tomato tissues, metabolism of L-phenylalanine to PEA, and subsequently to PE takes place [Tieman *et al*. 2006; Tieman *et al*. 2007]. Radish is yet an example of the Brassicaceae, a large plant family, very important in Poland.

MATERIAL AND METHODS

Plant material and extracts from the tissues of common buckwheat

Donor species used in the experiment was common buckwheat (*Fagopyrum esculentum* Moench; cultivar Hruszowska). Experiment was carried out in two combinations: with the use of buckwheat seedlings treated with vapours of JA-Me and the control ones. Buckwheat seeds were sown between two layers of moist blotting-paper 10 cm wide, which were then rolled and put in beakers 3 dm^3 in capacity containing 200 cm^3 of water. Seeds were subjected to four-day-long germination in darkness at the temperature of 24±1°C, and then etiolated seedlings were moved for four days to an air-conditioned growth room (temperature 24±2/16±2°C; photoperiod 16/8 h). Light intensity of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was attained by using a high-pressure sodium--vapour lamp. Part of the seedlings was treated with vapours of JA-Me at the concentration of 10^{-4}M, as described earlier [Horbowicz *et al.* 2011a]. After the completion of the experiment, the above-ground buckwheat parts were lyophilized and grinded. In the samples of the control buckwheat seedlings and the ones treated with JA-Me, analyses of total phenolic compounds and total flavonoids were carried out using the methods described below.

In order to obtain water extracts from the lyophilisates of the control buckwheat seedlings and the ones treated with JA-Me, 1% (w/v) mixture of lyophilisate and distilled water was prepared. The mixture was left in darkness for 24 hours at the temperature of 4°C, and then centrifuged, and solutions 0.1% (v/v) in concentration were prepared. Extracts enriched with metabolites, up to obtaining concentrations of 10^{-4}M, were prepared by dissolution of PEA, PAA, and PE (Sigma-Aldrich) in 0.1% extracts from the tissues of control buckwheat.

Effect of buckwheat extracts on the growth processes of the seedlings of chosen species

Effect of 0.1% water extracts from the above ground parts (hypocotyl + cotyledons) of control buckwheat (GK) and buckwheat treated with vapours of JA-Me (GJM) was evaluated, as well as the extracts from the control buckwheat enriched with PEA, PE, and PAA on the growth of roots and the above-ground parts of the seedlings of radish (*Raphanus sativus* L., cultivar Krakowianka), watercress (*Lepidium sativum* L.), sweet maize (*Zea mays* L. ssp. *mays* group *Saccharata*, cultivar Złota Karłowa), and tomato (*Lycopersicum esculentum* L., cultivar Poranek). For the extracts GK and GJM, the control group was plant seedlings grown in distilled water. Effect of extracts with added metabolites was evaluated in relation to plants grown in 0.1% GK extract. Experiments were carried out in a growth room in the above-described conditions. Seeds of the studied species were sown between two layers of moist blotting-paper, which after rolling were placed in beakers 3 dm^3 in capacity containing 200 cm^3 of the tested extracts. After four-day-long germination in darkness at the temperature of 24±1°C, seedlings were moved to a growth room. Experiments were carried out in eight replicates. Measurements of the lengths of the above-ground parts and roots of the studied plants were carried out after four and eight days from the set-up of the experiment, and then plant material was lyophilized and grinded. Material prepared as above was stored tightly closed at the temperature of 4°C in darkness up to the point of biochemical analyses.

Biochemical analyses

In order to determine the total phenol and flavonoid contents, suitable weighted portions of lyophilisates were extracted in 60% methanol (v/v) for 24 h, and then in filtrates spectrophotometric analyses of total phenolic compound content according to the method by Singleton *et al*. [1999] and flavonoid sum according to Ordonez *et al*. [2006] were carried out. Contents were calculated form calibration curve equations prepared with chlorogenic acid and rutin, respectively, for phenol and flavonoid sums.

Degree of lipid peroxidation was analyzed on the basis of malondialdehyde (MDA) determination through the reaction with thiobarbituric acid (TBA) [Hodges *et al.* 1999]. Lyophilized plant material was homogenized in 80% (v/v) ethanol and centrifuged at 3000 *g* for 10 minutes. To 1 ml of the obtained supernatant, 3 ml of 1% polyvinyl-pyrrolidone (PVP) in 0.1% trichloroacetic acid (TCA) was added, and then 0.5% TBA in 20% TCA, at 1:1, and in the case of the control material, 20% TCA, without TBA. Reaction with TBA was carried out for 25 minutes at 95°C, and subsequently the samples were quickly cooled in ice. After centrifuging (3000 *g* for 10 minutes), absorption measurement was carried out at the lengths of 440, 532, and 600 nm (spectrophotometer UV-1800 UV/Vis, Rayleigh). MDA contents were calculated according to the formula given by Hodges *et al.* [1999].

Statistical analysis of the results

The obtained results underwent one-factor analysis of variance (ANOVA) with the computer program Statistica 10.0 (StatSoft Poland). Comparison of the average values between the groups was carried out using the Duncan's test. When homogeneity of variance or distribution normality of the studied groups was not obtained, the Kruskall--Wallis test was carried out with the comparison of average ranks for all the groups. Significance level of $p \leq 0.05$ was applied. Statistical analyses of phenol and flavonoid contents in the tissues of the control buckwheat and buckwheat treated with vapours of JA-Me were carried out with the t-Student test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Presented results made it possible to establish that all the extracts used in the experiments showed an allelopathic effect, although it depended on the acceptor plant species and treatment time. Under the effect of buckwheat extracts, in all the studied species, stronger inhibition of root growth was observed in comparison with the above-ground parts. The obtained results demonstrated that the extract from the control buckwheat (GK) inhibited the growth of tomato seedlings the strongest but did not inhibit the growth of roots and the above-ground parts of radish (Table 1).

Demonstration of the inhibiting effect of buckwheat extracts on plant growth is a confirmation of earlier studies [Tsuzuki and Dong 2003, Golisz *et al*. 2007b, Mioduszewska *et al*. 2013]. Golisz *et al*. [2007b] and Tsuzuki and Dong [2003] suggest that the allelopathic effect of buckwheat extracts on dicotyledonous plants is stronger than on monocotyledonous plants. Present study confirmed higher sensitivity of the seedlings of two species of dicotyledonous vegetables (tomato, watercress) to the effects of buckwheat extracts in comparison with a monocotyledonous plant (maize) but

the effect was not confirmed for radish seedlings. The above results may indicate that growth inhibition is related to a greater extent to a given species of acceptor plant.

Table 1. Effect of water extracts from the tissues of control buckwheat (GK) and buckwheat treated with methyl jasmonate vapours (GJM) on the growth of the primary root and above-ground parts (mm) of chosen plant species after four and eight days of experiment (mean values ±SD)

Tabela 1. Wpływ wodnych ekstraktów z tkanek gryki kontrolnej (GK) oraz gryki traktowanej parami jasmonianu metylu (GJM) na wzrost korzenia głównego i części nadziemnych (mm) wybranych gatunków roślin po 4 i 8 dniach trwania eksperymentu (średnie ±SD)

	Tomato – Pomidor		Radish – Rzodkiewka		Watercress – Rzeżucha		Maize – Kukurydza	
	4 days 4 dni	8 days 8 dni	4 days 4 dni	8 days 8 dni	4 days 4 dni	8 days 8 dni	4 days 4 dni	8 days 8 dni
Primary root – Korzeń główny								
Control water Kontrola woda	32.3±2.1a	81.0±3.9a	14.1±0.8ns	31.4±1.1a	26.7±1.4a	52.2±3.7b	53.8±3.1a	120.3±8.7a
GK	12.0±1.6c	40.5±3.6b	14.2±0.7ns	30.9±1.0a	21.5±1.3b	66.5±5.7a	47.9±2.9ab	104.7±6.2b
GJM	19.6±1.2b	74.8±3.8a	14.4±0.7ns	26.5±1.2b	11.8±0.9c	48.7±4.6b	44.7±2,3b	102.1±6.5b
Above-ground part – Część nadziemna								
Control water Kontrola woda	29.6±1.7a	37.9±1.5a	30.2±1.4ab	43.8±1.8b	38.9±0.9a	44.6±0.8a	28.8±2.5a	67.9±3.5a
GK	19.7±3.3b	31.8±2.6b	28.7±1.6b	47.9±1.9a	35.4±1.0b	41.2±0.9b	29.2±2.4a	68.4±4.0a
GJM	19.1±1.3b	33.3±1.3b	32.5±1.3a	44.8±1.6ab	31.8±1.2c	37.9±1.0c	18.8±1.6b	50.0±2.8b

ns – non-significant differences – różnice nieistotne
results compared in columns (mean values ± standard deviation) marked with various letters are statistically different at $p \leq 0.05$ – wyniki w kolumnach (średnie ± odchylenie standardowe) oznaczone różnymi literami różnią się statystycznie istotnie przy $p \leq 0.05$

It is worth noting that in the majority of the analyzed cases, the growth of roots and above-ground parts of the seedlings of the studied vegetable species was more strongly inhibited after four days of the experiment than after eight days. This phenomenon may result from the activation of adaptive mechanisms in the conditions of allelochemical stress. Plant response to different stress can be, among others, the induction of phenolic compound synthesis. Properties of those compounds make it possible to neutralize reactive oxygen species, inhibit lipid peroxidation, chelate metals, and interrupt free-radical reactions [Sharma *et al*. 2012]. Flavonoids, which are part of this compound group, are also described as substances that make plant adaptation to longer exposition to stress possible [Fini *et al*. 2011].

Results of the present studies indicate that effect of buckwheat extracts on phenolic compound accumulation depended on the species and the applied extract (Tables 2 and 3). Extract GK caused an increase of these compounds content in tomato by 12%, but decreased their accumulation in watercress tissues. On the other hand, in the case of maize, significant decrease in flavonoid content was demonstrated after GK treatment but there was no effect on the total phenolic compound level.

Table 2. Effect of water extracts from the tissues of common buckwheat on the contents of malondialdehyde, total phenolic compounds, and total flavonoids in the tissues of chosen plant species

Tabela 2. Wpływ wodnych ekstraktów z tkanek gryki zwyczajnej na zawartość dialdehydu malonowego, sumy związków fenolowych i flawonoidów w tkankach wybranych gatunków roślin (średnie ± SD)

	Tomato – Pomidor	Radish – Rzodkiewka	Watercress – Rzeżucha	Maize – Kukurydza
Total phenolic compounds, mg·g^{-1} dry matter – Suma związków fenolowych, mg·g^{-1} suchej masy				
Control – Kontrola	10.2±0.3b	21.7±0.3a	36.3±0.6a	32.8±4.7b
GK	10.6±0.2ab	22.2±0.5a	32.5±0.5b	31.8±0.4b
GJM	11.0±0.3a	20.2±0.8b	33.2±0.8b	44.1±2.6a
Total flavonoids, mg·g^{-1} dry matter – Suma flawonoidów, mg·g^{-1} suchej masy				
Control – Kontrola	9.6±0.2c	12.7±0.3a	18.5±0.7ns	22.2±2.4b
GK	10.8±0.5b	12.1±0.3a	17.6±0.4ns	17.9±0.5c
GJM	12.2±0.8a	11.1±0.2b	17.4±0.8ns	27.4±3.1a
Malondialdehyde, nmol·g^{-1} dry matter – Dialdehyd malonowy, nmol·g^{-1} suchej masy				
Control – Kontrola	236.8±43.4ns	127.9±20.5ns	179.7±18.3ns	63.5±11.9b
GK	247.3±31.9ns	127.0±2.7ns	178.4±14.8ns	92.6±1.8a
GJM	188.8±23.7ns	134.8±2.2ns	205.2±24.3ns	82.3±8.7ab

explanations, see Table 1 – objaśnienia pod tabelą 1

Table 3. Effect of water extracts from the tissues of common buckwheat enriched with 2-phenylethylamine (PEA), phenylacetic acid (PAA), and 2-phenylethanol (PE) on the contents of malondialdehyde, total phenolic compounds, and total flavonoids in the tissues of chosen plant species

Tabela 3. Wpływ wodnych ekstraktów z tkanek gryki zwyczajnej wzbogaconych 2-fenyloetyloaminą (PEA), kwasem fenylooctowym (PAA) i 2-fenyloetanolem (PE) na zawartość dialdehydu malonowego oraz sumy związków fenolowych i flawonoidów w tkankach wybranych gatunków roślin

	Tomato – Pomidor	Radish – Rzodkiewka	Watercress – Rzeżucha	Maize – Kukurydza
Total phenolic compounds, mg·g^{-1} dry matter – Suma związków fenolowych, mg·g^{-1} suchej masy				
Control – Kontrola	10.6±0.2b	22.2±0.5a	32.5±0.5b	31.8±0.4b
PEA	10.4±0.5b	21.0±0.4b	35.8±1.6a	39.9±0.6a
PAA	9.7±0.2c	19.1±0.5c	34.6±3.1ab	37.8±6.2a
PE	11.5±0.5a	21.1±0.4b	34.7±0.8ab	40.1±2.9a
Total flavonoids, mg·g^{-1} dry matter – Suma flawonoidów, mg·g^{-1} suchej masy				
Control – Kontrola	10.8±0.5ab	12.1±0,3a	17.6±0,4ab	17.9±0,5b
PEA	11.8±1.6ab	11.4±0,3a	18.2±1.7ab	22.5±0.6a
PAA	10.3±0.5b	10.2±0.7b	16.7±0.6b	20.7±3.2ab
PE	12.3±0.6a	11.6±1.2a	18.7±0.4a	21.7±1.4a
Malondialdehyde, nmol·g^{-1} dry matter – Dialdehyd malonowy, nmol·g^{-1} suchej masy				
Control – Kontrola	247.3±31.9ns	127.0±2.7ns	178.4±14.8ab	92.6±1.8a
PEA	212.4±39.0ns	117.2±5.1ns	223.6±20.3a	58.5±5.5c
PAA	194.2±22.7ns	127.2±4.8ns	188.0±52.5a	70.0±4.2b
PE	186.7±23.0ns	124.7±9.0ns	132.1±12.7b	47.0±6.5d

explanations, see Table 1 – objaśnienia pod tabelą 1

Phenolic compounds, in addition to antioxidant properties, may also display prooxidant effect [Sakihama *et al*. 2002]. Buckwheat tissues are a rich source of those substances, which is acknowledged as a basis of allelopathic properties of that species [Golisz *et al*. 2007a]. Inhibition of the growth of acceptor plants under the effect of buckwheat extract may be therefore related to disturbance in oxidoreduction transformation in their tissues. This condition may lead to the oxidation damage of proteins, lipids, and nucleic acids [Sharma *et al*. 2012]. The first place of the harmful effect of phenols in the cells is cytoplasmic membrane [Politycka 1998]. As a result of phenol activity, phenoxyl radicals may be formed, which may cause lipid peroxidation [Sakihama *et al.* 2002]. The results obtained in the present study demonstrated that after the application of GK extract, over 40% growth in lipid peroxidation process occurred in maize tissues. Similar tendency, although statistically insignificant, was found also under the effect of the extract from buckwheat treated with JA-Me (GJM) in the seedlings of three out of four studied species (Table 2). Baziramakenga *et al.* [1995] observed increase in lipid peroxidation and intensified outflow of electrolytes from soybean (*Glycine max* L.) roots influenced by benzoic acid and cinnamic acid. Similar response to phenolic acid activity was also noted in the case of roots of another plant species [Gmerek and Politycka 2011], although the studies concerned only short-time effect of those compounds on plants. Slightly different results obtained in the present study may result from the application of the extract in low concentrations. On the other hand, they may also indicate plant ability to neutralize the effect of allelochemicals after longer duration of stressful conditions.

Chemical analyses have shown that buckwheat seedlings treated with vapours of JA-Me contained a significantly higher level of total phenolic compound (110.6 $mg \cdot g^{-1}$ dry matter) than non-treated plants (75.8 $mg \cdot g^{-1}$ dry matter), as well as total flavonoids (136.3 $mg \cdot g^{-1}$ and 86.7 $mg \cdot g^{-1}$ dry matter, respectively). Content of phenolic compounds and flavonoids in plants treated with JA-Me increased by 46% and 57%, with respect to the control plants. Similar phenomenon was observed earlier by Bi *et al*. [2007] and Wang *et al.* [2005], who noted an increase in the allelopathic potential of rice and maize treated with JA-Me. The above-mentioned authors explain the stimulation of phenolic compound biosynthesis in the tissues of the studied plants as the effect of increasing transcriptional activity of the phenylalanine ammonia-lyase (PAL) gene. Moreover, it was demonstrated that the extracts from plants treated with JA-Me had strong phytotoxic properties in relation to barnyard grass (*Echinochloa crus-galli* L.). Present research demonstrated that extract from buckwheat treated with JA-Me (GJM) also more strongly inhibited the growth of radish, watercress, and maize seedlings than the extract from the control buckwheat (GK). The exception was only the above-ground parts of radish, in which only slight stimulation of growth was noted. Growth of tomato seedlings was also inhibited by GJM but its effect was weaker in comparison with the control (GK) extracts (Table 1). Increased allelopathic potential of GJM extracts was also responsible for stronger biochemical response in the tissues of acceptor plant seedlings in comparison with GK extract. Such extracts caused an increased accumulation of phenols and flavonoids in tomato and maize tissues, but declined their content in radish tissues (Table 2). Enhanced phytotoxic properties of the GJM extract may therefore be an effect of the increase in phenolic compounds content, in particular flavonoids, among which rutin is considered as the major allelochemical of common buckwheat [Golisz et al. 2007a]. However, JA-Me may also increase the allelopathic potential of buckwheat extract by stimulation of biosynthesis of such compounds as

2-phenylethylamine (PEA) and its possible metabolites: 2-phenylethanol (PE) and phenylacetic acid (PAA) [Horbowicz *et al*. 2011b, 2013].

In available literature, there is a lack of data on the allelopathic properties and physiological functions of PEA in plants. Results of the present study indicate that extract enriched with PEA strongly inhibited seedling growth of all the studied plant species except watercress, in which the growth of the above-ground parts was stimulated to a small degree (Table 4). Extract supplemented by PEA inhibited the growth of the above-ground parts and roots of the studied species, but in the case of maize its stronger effect on the above-ground parts was noted. The greatest growth inhibition was noted in tomato seedlings, particularly after four days of treatment, whereas after eight days of plant exposition to this factor, the effect decreased.

Table 4. Effect of water extracts from the tissues of common buckwheat enriched with 2-phenylethylamine (PEA), phenylacetic acid (PAA), and 2-phenylethanol (PE) on the growth of the primary root and above-ground parts (mm) of chosen plant species

Tabela 4. Wpływ wodnych ekstraktów z tkanek gryki zwyczajnej wzbogaconych 2-fenyloetyloaminą (PEA), kwasem fenylooctowym (PAA) i 2-fenyloetanolem (PE) na wzrost korzenia głównego i części nadziemnych (mm) wybranych gatunków roślin

	Tomato – Pomidor		Radish – Rzodkiewka		Watercress – Rzeżucha		Maize – Kukurydza	
	4 days 4 dni	8 days 8 dni	4 days 4 dni	8 days 8 dni	4 days 4 dni	8 days 8 dni	4 days 4 dni	8 days 8 dni
Primary root – Korzeń główny								
Control Kontrola	12.0±1.6b	40.5±3.6b	14.2±0.7a	30.9±1.0a	21.5±1.3a	66.5±5.7a	47.9±2.9a	104.7±6.2ns
PEA	5.6±0.7c	41.8±2.9b	11.3±0.8b	26.7±1.1b	16.1±1.0b	37.9±1.9b	40.4±2.6bc	101.3±4.4ns
PAA	13.6±1.9b	46.8±4.8b	12.8±0.7ab	25.2±1.2b	7.9±0.7c	44.7±3.4b	45.4±2.7ab	104.0±6.9ns
PE	23.6±1.8a	73.0±4.1a	12.8±0.7ab	26.3±1.1b	14.3±0.6b	28.6±1.4c	37.9±2.5c	96.6±6.2ns
Above-ground part – Część nadziemna								
Control Kontrola	19.7±3.3a	31.8±2.6a	28.7±1.6a	47.9±1.9a	35.4±1.0b	41.2±0.9c	29.2±2.4a	68.4±4.0a
PEA	10.8±1.9b	26.4±1.8b	23.1±1.7b	40.9±1.9b	38.1±0.7a	43.3±0.6b	19.5±1.9b	55.8±2.7b
PAA	11.7±2.2b	26.6±2.4b	23.9±1.5b	42.3±2.2b	30.7±0.9c	37.1±0.9d	14.1±1.9c	50.8±3.1bc
PE	19.5±2.0a	33.6±1.3a	28.2±1.2a	44.6±1.4ab	38.8±0.8a	45.0±0.6a	18.7±2.0b	48.7±3.0c

explanations, see Table 1 – objaśnienia pod tabelą 1

PAA is known as a compound that demonstrates weak auxin activity but there are also reports on its allelopathic properties. Anaya *et al.* [1992] demonstrated inhibition of the roots growth of *Amaranthus leucocarpus* and *Echinochloa crus-galli* by PAA at the concentrations of 50 ppm and 100 ppm. Moreover, this compound, present in the post-harvest residue of maize and rye at the concentration of 25 ppm, inhibited the growth of lettuce roots [Chou and Patrick 1976]. Results of the present work indicate that plant seedlings have different sensitivity to PAA but growth inhibition of the above-ground parts proved to be statistically significant for all the studied species. The effect was the strongest for maize seedlings and the weakest for watercress seedlings. Sensitivity of the roots of the studied plants to PAA was demonstrated, however, only in the case of watercress and radish seedlings.

One of the ways of allelochemicals release to the environment is their evaporation. There is a lot of information of the inhibiting effect of volatile compounds on plants, but

the majority of the studies focus on substances classified as terpenoids [De Martino *et al.* 2010]. There is a lack of information on the allelopathic properties of 2-phenylethanol (PE). Biosynthesis of PE occurs through L-phenylalanine transformation [Tieman *et al*. 2006], and a putative substrate for its biosynthesis was also found in buckwheat seeds [Janeš *et al.* 2009]. According to recent studies, biosynthesis of PE and PAA occurs in the tissues of buckwheat seedlings, and the application of JA-Me significantly enhances those processes [Horbowicz *et al.* 2013]. PE demonstrates antibacterial, antifungal, and antifeedant properties [Berrah and Konetzka 1962; Lester 1965; Eriksson et al. 2008]. Present study showed that PE also has allelopathic properties because it inhibited the growth of radish, watercress, and maize seedlings (Table 3). In the seedlings of watercress and radish, stronger effect of PE on root growth was observed than on the above-ground parts, and in the case of maize, large inhibiting effect occurred for both the roots and the above-ground parts. In contrast with the seedlings of other species, the growth of tomato root was strongly stimulated, both after four and eight days of PE treatment. In the case of the above-ground parts of tomato seedlings, no significant effect of PE was found. This phenomenon may be related to the physiological presence of this compound in tomato tissues, which is a component of its aroma [Tieman *et al.* 2006, 2007]. PE possesses certain antibacterial properties [Uma and Podile 2014] and is an attractant for insects which pollinate flowers [Berrah and Kontezka 1962, Tieman *et al*. 2007].

Use of extracts supplemented with metabolites of PEA significantly affected the contents of phenolic compounds in the maize seedlings, compared to the control plants. Significantly higher accumulation (by circa 15%-25%) of phenolic compounds was noted under the influence of extracts enriched by the PEA, PAA, and PE. Similar tendency but not statistically significant was observed also in the tissues of watercress seedlings. The effect of the studied extracts on radish and tomato seedlings caused, on the other hand, a decrease in phenolic compounds accumulation in the tissues of those plants. Only after the application of the extract supplemented by PE, small but statistically significant increase in their concentration in tomato tissues was noted (Table 4). Metabolic changes that lead to the increased biosynthesis of phenolic compounds are well-known response of the acceptor plants under allelochemical stress [Politycka 1998]. However, similarly to the results of the present study, literature data indicate that the effect of some biotic factors may also cause a decrease in the content of those antioxidants [Bido *et al*. 2010, Mewis *et al*. 2012].

Application of extracts supplemented with the PEA, PAA, and PE caused a diversified effect on lipid peroxidation process in the tissues of the studied plants. Applied extracts resulted in lowering the content of malondialdehyde (MDA) as compared to control plants in the tissues of tomato and maize seedlings, which may indicate inhibition of lipid peroxidation (Table 3). Certain increase in this process was demonstrated only after the application of PEA enriched extract in watercress tissues but it was statistically insignificant. Piotrowska-Niczyporuk and Bajguz [2014] claimed that exogenously used PAA limited the contents of lipid peroxides and H_2O_2 in the tissues of algae (*Chlorella vulgaris*). The phenomenon of cell membrane stabilization and the reduction of lipid peroxidation by polyamines is also known [Groppa *et al*. 2001]. Results of the present experiment suggest a similar effect of both PAA and PE, but this hypothesis requires further studies.

Biosynthesis, as well as the release and effectiveness of allelochemicals may undergo changes under the effect of environmental factors [Inderjit *et al*. 2011, Kato-

Noguchi 2009]. Inderjit and Duke [2003] underscore that it is important to determine phytotoxicity of not only the particular chemicals, but also entire mixtures in regard to their place of action in the acceptor plants. It is particularly important in the case of chemicals belonging to different chemical classes [Inderjit and Duke 2003]. Understanding of those mechanisms may contribute to the use of some plant compounds as natural herbicides, use of plant residue of allelopathic properties or cultivation with underplant, which will make it possible to limit the use of synthetic herbicides [Khanh *et al*. 2005]. Presented studies prove that the 2-phenylethylamine and its metabolites demonstrate an allelopathic effect, although they may play a smaller part in those processes than phenolic compounds. They may, however, enhance the effects of phenols, particularly in the case of monocots. Further research on the effect of the 2-phenylethylamine and its metabolites on physiological processes in plants is continued.

CONCLUSIONS

1. Methyl jasmonate causes an increase in phenolic compound content, which contributes to the increase in the allelopathic potential of common buckwheat.

2. 2-phenylethylamine, phenylacetic acid, and 2-phenylethanol inhibit the growth of tomato, radish, and watercress seedlings, and in particular maize seedlings, which may be a basis for further research on the use of those compounds as natural herbicides.

3. Enriching common buckwheat extracts with 2-phenylethylamine, phenylacetic acid, and 2-phenylethanol declined lipid peroxidation process in tomato and maize seedlings, which may indicate their protective effect on cell membranes, but this hypothesis requires further studies.

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WŁAŚCIWOŚCI ALLELOPATYCZNE EKSTRAKTÓW I NIEKTÓRYCH METABOLITÓW OBECNYCH W TKANKACH SIEWEK GRYKI ZWYCZAJNEJ (*Fagopyrum esculentum* MOENCH)

Streszczenie. W pracy oceniono właściwości allelopatyczne wodnych ekstraktów z nadziemnych części siewek gryki kontrolnej oraz traktowanej parami jasmonianu metylu (JA-Me), a także ekstraktów wzbogaconych 2-fenyloetyloaminą (PEA) oraz jej metabolitami: kwasem fenylooctowym (PAA) i 2-fenyloetanolem (PE) w stosunku do siewek roślin warzywnych dwuliściennych (pomidor, rzodkiewka, rzeżucha) oraz jednoliściennych (kukurydza). Określono także ich wpływ na poziom związków fenolowych i flawonoidów oraz stopień peroksydacji lipidów w tkankach roślin akceptorowych. We wszystkich przypadkach odnotowano hamowanie wzrostu tych roślin, a działanie to różniło się w zależności od zastosowanego ekstraktu, badanego gatunku oraz czasu trwania stresu allelochemicznego. Zastosowanie JA-Me spowodowało podwyższenie zawartości związków fenolowych i w konsekwencji potencjału allelopatycznego tkanek gryki. Generalnie, korzenie testowanych roślin akceptorowych były bardziej wrażliwe na działanie badanych ekstraktów niż części nadziemne. W przypadku kukurydzy dodatek PEA, PAA i PE silnie hamował zarówno wzrost korzeni, jak i części nadziemnych. Odmienne zjawisko wystąpiło natomiast w korzeniach pomidora, których wzrost był stymulowany przez PE. Ekstrakty z gryki powodowały zwiększenie procesu peroksydacji lipidów u kukurydzy, lecz dodanie PEA, PAA i PE ograniczało ten proces. Ekstrakty z gryki wpływały też na zwiększoną biosyntezę związków fenolowych i flawonoidów w tkankach pomidora i kukurydzy, co może być związane z uruchomieniem mechanizmów przystosowania do warunków stresowych.

Słowa kluczowe: jasmonian metylu, 2-fenyloetyloamina, kwas fenylooctowy, 2-fenyloetanol, peroksydacja lipidów

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