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Exudation of Allelopathic Substances in Buckwheat (*Fagopyrum esculentum* Moench)

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Root exudates of the common buckwheat, especially phenolic compounds, were studied. Their contents, both in the soil during the growing season and in agar medium during germination, were determined by HPLC and GC-MS. The allelopathic activity of the soil from a buckwheat stand was evaluated, as well. Palmitic acid, squalene, epicatechin, vitexin, a gallic acid derivative, and a quercetin derivative were the main compounds of the agar medium. In the soil, palmitic acid methyl ester, vanillic acid, rutin, a gallic acid derivative, and a 4-hydroxyacetophenone derivative were identified. The effects of vitexin, squalene, epicatechin, 4-hydroxyacetophenone, and vanillic and gallic acids were tested on eight plant species. Inhibitive effects were observed in the cases of 4-hydroxyacetophenone and vanillic and gallic acids. Comparisons of the identified compounds and inhibitive effects of soil extracts indicated that palmitic acid and the gallic acid derivative probably have an important function in the allelopathic root response of buckwheat.

KEYWORDS: Allelopathy; epicatechin; *Fagopyrum esculentum*; palmitic acid; root exudates; squalene; rutin; vanillic acid; vitexin; weeds

INTRODUCTION

Plant roots influence the physical, chemical, and biological conditions of the soil in the rhizosphere through exudation of a wide range of organic substances: sugars, amino acids, lipids, enzymes, etc. Root exudates play a fundamental role in the mineral nutrition of plants. They also contain chemical signals that control the rhizosphere processes enhancing nutrient uptake and assimilation. Therefore, rhizosphere processes are the key to improved plant nutrition and increased crop yields (*1*). In the case of biotechnology applications, root exudates can be utilized in the human diet (e.g., as food additives), because some of them are important antioxidants (*2*, *3*).

Root exudates are defined as substances released into the substrate by healthy and intact plant roots. Excreted compounds exert an inhibitory growth function within adjacent rhizospheres (regulation of the soil microbial community) and may affect development of other plants, if they grow in the vicinity (allelopathy) (*4*). Germinating seeds may secrete allelochemicals, as well. The inhibition of germination has been observed at various levels of intensity; this phenomenon demonstrates the selectivity of these natural excreta, similar to the effects of synthetic herbicides (*5*).

Phenolic compounds have an especially important position in this phenomenon (*4*). The phenolic substances retained by cells had chemical properties different from those located in the root exudates (*5*). They are synthesized by the plants, and, like the chemical defenses against stress and other species, they also have some physiological functions (*6*).

Allelopathy may be an alternative to the chemical control of weeds in agro-ecosystems. Progress in cultural methods of weed control has included the use of weed-suppressing cover crops and the identification of specific crop traits for weed suppression (*7*). Common buckwheat (*Fagopyrum esculentum* Moench), belonging to the family Polygonaceae, is a species especially spread within the temperate zone of Eurasia. It is the only cultivated species of buckwheat in Europe, which is often grown as a cover crop. Most of the studies oriented to buckwheat allelopathy were conducted by using organic solvents to extract the allelochemicals from the buckwheat tissues (fresh or dried), such as the leaves, stems, or roots. Only limited information is available on the root exudates released from living buckwheat plants into the environment. Root exudates of buckwheat stimulated the growth of oat (*Avena sativa* L.), but had inhibitive effects on lupine (*Lupinus albus* L.) (*4*). In this paper, the root exudates, especially the phenolic compounds of common buckwheat growing both in soil under natural environmental conditions and in agar medium in laboratory conditions, were studied.

MATERIALS AND METHODS

Field Cultivation. Common buckwheat plants (variety Pyra, Czech Republic) were grown in four replications on plots (size of one plot =

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Table 1. Basic Meteorological Data during the Period 2004 and 2005

	year	May	June	July	Aug	Sept	annu av
mean daily air temp (°C)	2004	12.5	16.3	18.3	19.2	13.7	8.9
	2005	14.4	17.7	19.0	16.8	14.8	8.8
	LTN[a]	13.0	16.2	17.7	17.1	13.5	8.2
total precipitation (mm)	2004	65.7	101.4	52.3	47.5	48.9	655.5
	2005	64.7	68.3	162.3	157.3	98.3	798.3
	LTN	70.1	93.0	77.8	78.8	47.5	582.8

[a] Long-term normal.

10 × 1.25 m) in Ceske Budejovice (48° 57′ 42″, 14° 28′ 05″, 380 masl, sandy-loam brown soil) in years 2004 and 2005. The soil was medium acid (pH 5.6), medium (16 mg kg^{-1}) in mineral nitrogen, low (141 mg kg^{-1}) in potassium, and high (114−142 mg kg^{-1}) in phosphorus. Buckwheat achenes were sown after oat (*Avena sativa* L.) in 12.5 cm wide rows by seed drill for precise drilling with a density of 200 seeds per square meter in May 2004 and 2005. No mechanical or chemical treatments were applied during the crop seasons. Sums of precipitation and average temperature are given in **Table 1**.

Soil depths (0−100 mm) were sampled next to the buckwheat plants at the stages of branching, flowering, and before harvest. Samples of soil from the field before sowing and from another field, without a buckwheat stand, were used as the control. Simultaneously with the soil samples, plant samples were also taken for the authentification of the compound found in the plants. For further processing of the samples, see Kalinova et al. (*8*).

Laboratory Cultivation. The method of laboratory cultivation was adapted from that of Wu et al. (*9*). A glass beaker (300 mL) containing 33 mL of 0.3% w/v agar solutions (no nutrients) was autoclaved. Six surface-sterilized (sodium hypochloride and ethanol) and pregerminated seeds were aseptically sown in two rows. The experiment had four repetitions. The beaker was wrapped with Parafilm and kept at the temperature of 23 ± 1 °C, with a 14 h light photoperiod. After 12 days, the buckwheat seedlings were removed from the agar and the medium was lyophilized.

Sampling of Root Exudates from the Soil. The soil was screened using a 2 mm sieve, and then exudates were extracted by two different agents (hot distilled water and methanol).

(1) The sample was mixed with boiling distilled water, and then the mixture was sonicated for 10 min and extracted for 30 min at 60 °C. After centrifugation (3500 rpm/10 min/22 ± 1 °C), the sediment was washed twice with distilled water, and then the supernatants were mixed and lyophilized. The residue, after lyophilization, was dissolved in methanol.

(2) The soil sample was shaken with 100% methanol for 3 × 30 min at laboratory temperature, and then the sample was centrifuged. The supernatants were mixed, filtered through cotton wool and anhydrous sodium sulfate, and then concentrated on a rotary evaporator.

Collection of Root Exudates from Agar. The agar medium was extracted by 10 mL of 90% methanol for 15 min at the laboratory conditions (22 ± 2 °C). The samples were stirred intensively during extraction. The extracts were filtered through cotton wool and sodium sulfate. Then, the extraction was repeated twice with 5 mL of methanol. All methanol extracts were blended. After the methanol extraction, the agar samples were extracted by 3 mL of ethyl acetate for 15 min at laboratory conditions. Further treatment was identical with the methanol extraction; the amount of ethyl acetate for repeated filtration was 2 mL. Both methanol and ethyl acetate extracts were concentrated by nitrogen flow, and then they were analyzed by HPLC and GC-MS.

HPLC and GC-MS Analysis. Samples were analyzed by a HPLC HP 1050 (Hewlett-Packard), DAD detector (HP 1040, Hewlett-Packard), and Phenomenex Luna C18(2), 3 μm, 2 × 150 mm column. Mobile phase A contained 5% acetonitrile + 0.1% orthophosphoric acid; mobile phase B contained 80% acetonitrile + 0.1% orthophosphoric acid. The gradient was from 5% B to 35% B in 55 min and from 35% B to 60% B for 5 min; flow rate was 0.25 mL/min. Phenolic compounds were detected at 220 nm (data were measured in the range of 190−600 nm).

GC-MS analyses were performed on a Finnigan GCQ instrument using a Zebron ZB-5 column, 30 m i.d. × 0.25 mm, and stationary phase thickness was 0.25 μm with the following temperature program: initial temperature of 60 °C for 1 min, then gradient at 20 °C/min to 180 °C, followed with the gradient at 1.5 °C/min to 275 °C. The linear inlet helium velocity was set to 30 cm/s. Each peak in the chromatogram was evaluated using XCalibur mass spectrometry software, and the obtained mass spectra were compared with the spectra from the NIST library. Only measured spectra having the highest probability of a match with the spectra in the library were recorded.

Materials. Standards of rutin, palmitic acid, squalene, quercetin, (−)-epicatechin, (+)-catechin, (−)-epicatechin gallate, vanillic acid, gallic acid, quercetin-3-D-galactoside, quercetin-3-β-D-glucoside, 4-hydroxyacetophenone, ethyl acetate, orthophosphoric acid, and agar were purchased from Sigma-Aldrich. Other standards, vitexin and orientin, were purchased from Extrasynthese. Vitexin was diluted in a mixture of methanol and dimethyl sulfoxide (18:2). The dimethyl sulfoxide concentration in the sample, which was used for the biotests, was not higher than 1%, and this concentration did not influence the growth of tested seeds. The solvents ethanol, methanol, dimethyl sulfoxide, and acetonitrile were purchased from Merck.

Biotests with Soil from Buckwheat Stand. The allelopathic effect of the soil samples from a buckwheat stand was tested on 20 pregerminated lettuce seeds (seeds with 1−2 mm visible radicle, after 24 h), sown on double layers of filter paper, covered with 50 g of dry soil, moistened with 10 mL of distilled water, and then covered with plastic film for the laboratory test with soil. Soil without any stand was used as a control. All four repetitions were incubated in the dark at 22 ± 2 °C for 3 days. The growth intensity of seeds (length of root and hypocotyl) was evaluated 3 days after sowing. This method was adapted from that of Conklin et al. (*10*).

Biotests with Compounds. Vanillic acid, gallic acid, 4-hydroxyacetophenone, vitexin, and squalene were tested. The standard of each tested compound was diluted in 90% methanol. Different concentrations of tested compounds were applied on filter paper (Filpap KA1). Treatment with methanol, only, was used as the control (in the case of vitexin, also a mixture of methanol and dimethyl sulfoxide). The methanol was then evaporated, and 3 mL of distilled water was added onto the filter paper. The tested concentrations decreased from 10 to 0.001 mM in water. Seeds of lettuce (*Lactuca sativa* L. cv. 'Capitata'), white mustard (*Sinapis alba* L.), yarrow (*Achilea milefolium* L.), ribwort plantain (*Plantago lanceolata* L.), Dutch clover (*Trifolium repens* L.), perennial ryegrass (*Lolium perenne* L.), barnyard grass [*Echinochloa crus-galli* (L.) P.B.], and redroot pigweed (*Amaranthus retroflexus* L.) were sown on filter paper in Petri dishes (9 cm diameter). The Petri plates were kept in the dark at the temperature of 22 ± 2 °C. Every dish was covered with Parafilm to reduce evaporation. The lengths of roots and hypocotyls were measured after 3 days. There were at least five replicates for each concentration.

Data Analysis. The percentage influence of the extract or a tested compound was calculated using the following equation: percentage influence (%) = (obtained value − control value) × 100/control value. The data were evaluated using analysis of variance (Statistica 6.0 software). The effective concentration required for 50% inhibition (EC_{50}) was established on the basis of fitted regression equations.

RESULTS AND DISCUSSION

Biotests with Soil. The soil allelopathic activity was statistically significant at the flowering stage in 2004 and 2005 and at the branching stage in 2004 (**Table 2**). The release of the effective substances into the soil during germination probably causes the strong soil allelopathic activity at the beginning of the season. Kalinova et al. (*11*) recorded a 36% inhibition of lettuce growth caused by buckwheat achenes on the fourth day of their germination. The inhibition of lettuce growth was more evident in the case of lettuce root. The intensities of soil allelopathic activity were different between years. Inhibitive effects of the samples in 2004 were enhanced by the dry weather, this factor causing a higher stress impact on the plants. In 2005, weather conditions were more suitable for the growth of buckwheat (more precipitation). The stronger activity, at the

Table 2. Percentage Influence of Buckwheat Soil on Lettuce Growth (Mean ± SD)[a]

	mean radicle length (mm)		mean hypocotyl length (mm)	
stage	2004	2005	2004	2005
branching	−30.50 ± 3.156 a **	−16.97 ± 10.383 a ns	4.96 ± 3.901 b *	5.35 ± 10.229 a ns
full flowering	−15.00 ± 8.122 b *	−34.71 ± 10.655 a *	−11.76 ± 8.021 a *	5.30 ± 7.270 a ns
before harvest	3.54 ± 5.229 c ns	4.98 ± 2.462 b ns	10.14 ± 8.524 b ns	10.45 ± 4.521a ns

[a] Differences among varieties after Tukey HSD are given by letters following data. ns, nonsignificant; **, $P < 0.01$; *, $P < 0.05$.

flowering stage, in 2005 compared to 2004 was probably caused by soil sampling after a shower in 2005. These results indicate the importance of leachates from the aboveground biomass in the allelopathy of buckwheat.

Compounds in the Soil. Rutin, vanillic acid, and a compound with a spectrum similar to that of gallic acid (HPLC) and palmitic acid (GC-MS) were both identified and quantified in soil extracts. By the comparison of extracts from different stages of the growing period and the control in both 2004 and 2005, it is possible to consider that these compounds most probably were buckwheat exudates.

Rutin is the main flavonoid of buckwheat. Kalinova et al. (*8*) recorded that rutin is present in all parts of the buckwheat plant, especially in the leaves and flowers at the flowering stage. Therefore, it is not surprising that it was also established in high amounts in the soil from the buckwheat stand. In both periods, rutin amount was increased during all of the growing period. In agar medium, rutin was not present. Watanabe and Ito (*12*) described how the rutin content gradually increased during the early development of buckwheat seedlings. Therefore, we can assume that rutin production comes from necrotic parts of the buckwheat plant or from leachates, but not from root exudates. The presence of rutin could influence the growth of other plants. According to Liang et al. (*13*), rutin concentrations of 20−100 μg/mL in the dark, or 80−100 μg/mL in the light, exhibited growth inhibition of mung bean seedlings and suppressed indoleacetic acid accumulation in hypocotyls of the crop.

Vanillic acid was present in the water extracts of the soil samples in both periods, and the amount increased through the duration of the growing season. In both periods, the highest contents were established before harvest. In agar medium, vanillic acid was not found. Similarly, Whitehead et al. (*14*) recorded relatively low concentrations of vanillic acid from soil, but it was increased in monocultures. According to Li et al. (*15*), vanillic acid is one of the most common phenolic acids available in the soil, together with ferulic and *p*-coumaric acids. Vanillic acid was found in many crops. Kushima et al. (*16*) identified vanillic acid as one of the potent allelochemicals from germinating watermelon seeds. The amount of vanillic acid determined in the soil from the buckwheat stand was comparable with the results of Wu et al. (*17*), who established concentrations of 0.00061−0.01753 μg of vanillic acid/mL of agar by 17-day-old wheat seedlings. In the plants, we determined the presence of vanillin in buckwheat leaves by GC-MS analysis. As agar medium did not contain vanillic acid (similarly as in the case of rutin), we can assume that this compound may be a product of decomposition.

From other compounds present in the methanol soil extract, a compound with a spectrum similar to that of gallic acid was identified in both periods, during all of the growing season. The quantity and the quality of the compound were not determined. The presence of gallic acid in buckwheat has been described (*18*). The gallic acid content ranging from 0.01 to 0.02% was recorded in the upper parts of common buckwheat (*18*).

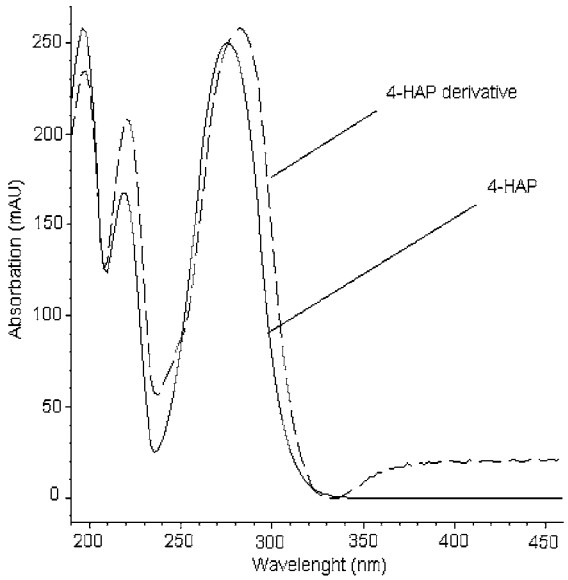

Figure 1. UV–vis spectrum (200–450 nm) of the detected 4-HAP derivative.

By GC-MS analysis of the methanolic extracts of soil, a derivative of palmitic acid as palmitic acid methyl ester was detected in samples, taken at the stage of branching, in both periods. Palmitic acid was detected in the buckwheat roots, stems, and leaves. Tsuzuki et al. (*19*) identified four fatty acids, including palmitic acid, in *Fagopyrum cymosum* seedlings, and they explained that these compounds are allelochemicals of buckwheat. The possibility that other fatty acids, such as palmitic, stearic, and arachidonic acids, have allelopathic effects on other plants follows from several studies. Wu et al. (*17*) observed that palmitic acid at a concentration of 250 ppm caused a slight, but significant, inhibition in the growth of rice seedlings. According to Khanh et al. (*20*), palmitic acid at 100−200 ppm inhibited the germination and growth of *Echinochloa crus-galli*, but significantly less so than the other compounds, lactones and coumarin.

A 4-hydroxyacetophenone derivative was detected in the methanol extract of the soil at the stage of branching, only in 2005 (**Figure 1**). A similar compound (3,4-dimethoxyacetophenone) was detected in buckwheat leaves by GC-MS analysis. 4-Hydroxyacetophenone derivatives are known, especially from needles of coniferous trees, but they have also been found in other plants [e.g., Bohlmann and Ehlers (*21*) identified a *p*-hydroxyacetophenone derivative in *Artemisia monosperma* Del., and Háznagy et al. (*22*) isolated *p*-hydroxyacetophenone from *Cynanchum vincetoxicum* (L.) pers]. The amount of the 4-hydroxyacetophenone derivative identified in the soil from the buckwheat stand was higher (**Table 3**) than in the roots of *Picea abies* (0.0001−0.0010 mg mL^{-1} of agar) (*6*). However, 4-hydroxyacetophenone could also be one of the main metabolites of bisphenol degradation (*23*). Therefore, it is necessary

Table 3. Content of Identified Compounds in the Soil Extracts from a Buckwheat Stand (Milligrams per Kilogram)

compound	2004	2005
rutin	0.008297–0.031424	0.007488–0.026949
4-HAP derivative		0.00378
vanillic acid	0.00091–0.00234	0.00093–0.00748

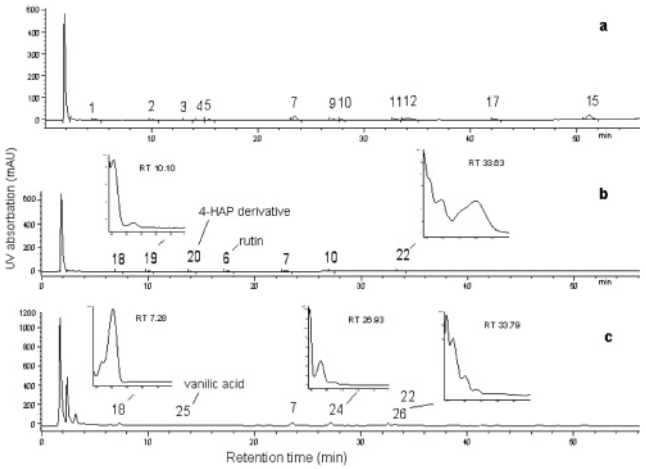

Figure 2. HPLC studies of the soil extracts: (**a**) control, methanol extract of the soil, before sowing in 2004; (**b**) methanol extract of the soil, sampled at flowering stage in 2005; (**c**) water extract of the soil, sampled at flowering stage in 2004. UV–vis spectrum (200–450 nm) of the individual compound, given at retention times of 7.28, 10.10, 26.93, 33.63, 33.79.

to verify the presence of the 4-hydroxyacetophenone derivative in buckwheat plants.

Some of the identified constituents could not be quantified because they could not be purified in our laboratory (**Figure 2**).

Compounds in the Agar Medium. Vitexin, epicatechin, and quercetin derivative, compounds with a spectrum similar to that of gallic acid (HPLC; **Figure 4**), as well as squalene and palmitic acids (GC-MS (**Figure 3**), were identified in the agar medium. The quantities of those compounds identified in the extracts of root exudates of buckwheat are shown in **Table 4**. In the agar medium, rutin and vanillic acid, which were present in the soil samples, were not found.

The content of vitexin was the third highest after squalene and palmitic acid. Because vitexin was not detected in the soil, this compound is probably produced, principally, at the beginning of growing season, during germination. This also confirms the results of other studies, which had described vitexin content in buckwheat. Dietrych-Szostak and Oleszek (*24*) identified vitexin (14.92 mg/100 g) in achenes, as the second main flavonoid, after rutin. We determined similar results on vitexin content (15.8 mg/100 g) in buckwheat achenes. Watanabe and Ito (*12*) found five compounds in extracts of buckwheat during the early stage of development: vitexin, isovitexin, orientin, isoorientin, and rutin; but only rutin was detected after the 19th day. In the agar medium, orientin and rutin were not found.

Epicatechin was excreted in the lowest amount in buckwheat (**Table 4**). The concentration of epicatechin in buckwheat root exudates was similar to the amount of epicatechin in root exudates of Norway spruce (*P. abies*) recorded by Tomova et al. (*6*). Epicatechin was detected only at the beginning of growth. It is possible to assume that epicatechin also occurs later in the soil, but probably at lower concentrations than the limits of detection. Kalinova et al. (*8*) recorded the highest content of epicatechin at the full-flowering stage in the flowers and leaves of *F. esculentum*. According to Trotin et al. (*2*), a hairy roots culture of *F. esculentum* synthesized (+)-catechin, (−)-epicatechin, (−)-epicatechin-3-*O*-gallate, procyanidin B_2, and procyanidin B_2-3′-*O*-gallate. Epicatechin gallate and catechin were not found in the agar medium.

In the agar medium, another quercetin derivative other than quercetin-3-D-galactosid (quercetin-3-β-D-glucosid) was determined. Bais et al. (*25*) reported that quercetin was not phytotoxic. The same gallic acid derivative, as in the soil samples, was also found in the agar medium. Therefore, these compounds could be part of the allelopathic exudates of buckwheat.

In the agar medium, palmitic acid was identified in the highest amount. As palmitic acid methyl ester was determined in relatively high amount in the soil, it is very probable that it plays an important role in the allelopathy of buckwheat.

The amount of squalene in germinating achenes of buckwheat was second highest among the detected compounds. According to Kalinova et al. (*8*), squalene was identified in all parts of *F. esculentum* plants; the highest amount was obsereved in leaves. The content of squalene in buckwheat plants increased through the stage of full flowering. However, this compound was not found in the soil samples. Haudenschild and Hartmann (*26*) described how plants terminated sterol biosynthesis in response to either pathogen or elicitor challenge. The sensitivity to a stress factor could be one of the reasons for squalene's absence in the soil.

Biotests with Compounds. Allelopathic effects of five compounds (squalene, vitexin, vanillic acid, gallic acid, and 4-hydroxyacetophenone) were tested on eight plants. Inhibitive effect on the growth of lettuce was evident with vanillic acid, gallic acid, and 4-hydroxyacetophenone.

Vanillic acid had a significant inhibitive effect on the growth of all eight plants (**Table 5**). We observed a higher inhibition of vanillic acid on root elongation than on hypocotyl elongation in all plants tested. The greatest difference between the inhibition of the roots and hypocotyls was determined in *T. repens*. Rizvi and Rizvi (*27*) reported that vanillic acid inhibited N_2 fixation of bluegreen alga, (*Anabaena cylindrica*) important in rice fields. Therefore, we can assume that vanillic acid could have a similar effect as in the case of nitrifying bacteria in *T. repens*, as well. According to Sampietro et al. (*28*), vanillic acid was more phytotoxic to root elongation of lettuce and four weeds (*Amaranthus quitensis* L., *Sida rhombifolia* L., *Brassica campestris* L., *Bidens subalternans* L.) than ferulic and synapic acid. They suggested that vanillic acid inhibited dehydrogenase activity and the mitotic index, also reducing the chlorophyll content in lettuce.

The effective concentrations ranged from 250 to 2000 μM. The hypocotyls of *T. repens*, *P. lanceolata*, and *A. retroflexus* were the most resistant plants. On the contrary, *S. alba* and *L. sativa* were the most sensitive plants. Rizvi and Rizvi (*27*) reported the effective concentration of vanillic acid as 5×10^{-4} M for lettuce growth. This finding is very close to our results (**Table 5**).

The inhibitive effect of gallic acid on the growth of *T. repens* and *A. retroflexus* compared to the control was not statistically significant ($P > 0.05$). The effective concentration of gallic acid for other plants ranged from 27 to 3865 μM (**Table 6**).

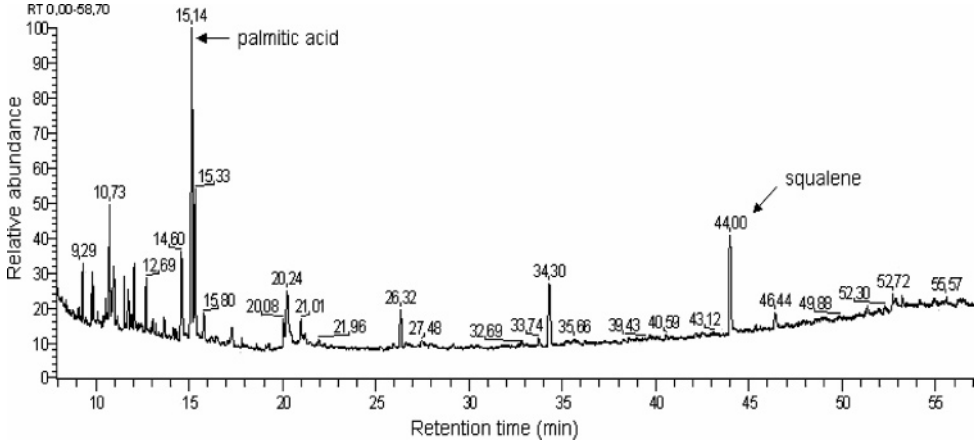

Figure 3. GC-MS studies of the methanol extract of agar with buckwheat root exudates.

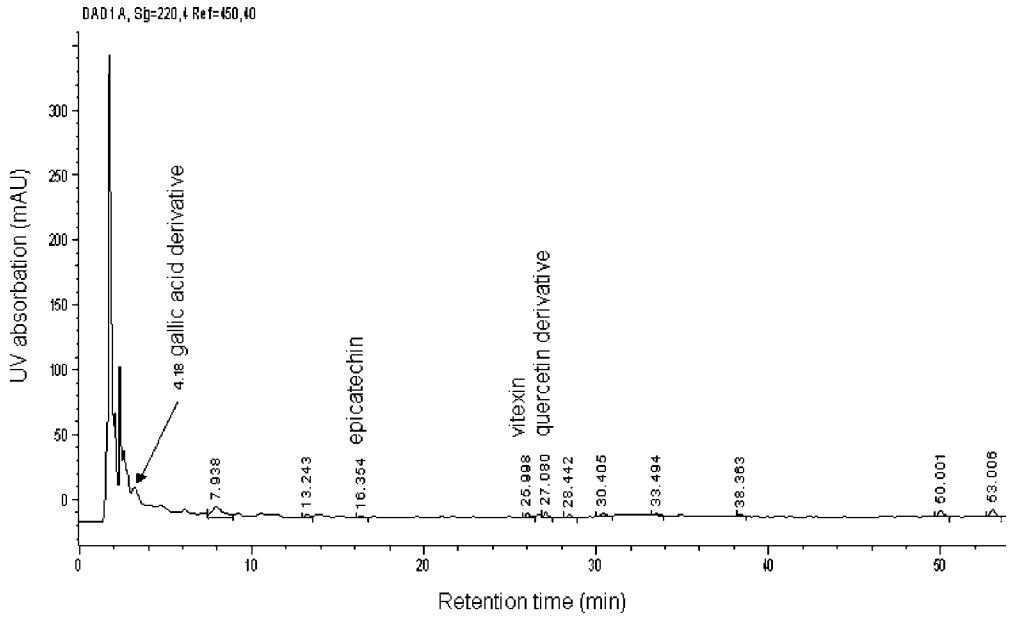

Figure 4. HPLC studies of the methanol extract of agar with buckwheat root exudates.

Table 4. Average Content of Identified Compounds in the Agar Medium, after Buckwheat Germination

compound	μg/plant	μg/mL of agar
epicatechin	0.0105	0.0019
palmitic acid	3.2786	0.5415
quercetin derivative[a]	0.0286	0.0052
squalene	0.1110	0.0202
vitexin	0.0918	0.0165

[a] Calculated on the basis of quercetin.

A stronger unambiguous influence of gallic acid on the root, which was established in vanillic acid, was not evident. Gallic acid was described as an effective inhibitor of germination in the leachate from leaves of numerous plants (*4*).

L. sativa and *S. alba* were found to be the most sensitive plants (123−137 μM for roots) to vanillic acid. *E. crus-galli* and *L. perenne* were moderately sensitive (600−840 μM). Iquabal et al. (*18*) recorded the value of $EC_{50} = 29-59$ μM for roots of *Brassica juncea* Czesn. and *L. sativa* and 589 μM for *L. multiflorum* Lam. and *E. crus-galli*. The use of agar instead of filter paper may be the reason for slightly lower values. *P. lanceolata* was the most resistant plant (10^{-3} M), as in the case of vanillic acid. Regiosa et al. (*29*) determined that a higher concentration (10^{-2} M) of gallic acid reduced the growth of *P. lanceolata* and *A. retroflexus*, and a lower concentration had stimulative effects; however, they used two different temperatures and light and dark conditions in a day.

Table 5. Influence of Different Concentrations (1–10000 μM) of Vanillic Acid on Weed Growth

plant	plant part	sig[a]	equation	$R^{2\,b}$	EC_{50} (μM)
A. milefolium	root	**	$y = -11.711 \ln x + 20.505$	0.92	411
	hypocotyl	**	$y = -15.694 \ln x + 52.951$	0.84	710
A. retroflexus	root	**	$y = -27.312 \ln x + 143.71$	0.94	1202
	hypocotyl	**	$y = -21.189 \ln x + 98.457$	0.85	1103
E. crus-galli	root	**	$y = -12.738 \ln x + 23.05$	0.76	308
	hypocotyl	**	$y = -8.5351 \ln x + 17.864$	0.71	875
L. perenne	root	**	$y = -10.215 \ln x + 9.7082$	0.89	325
	hypocotyl	**	$y = -9.9861 \ln x + 13.737$	0.81	590
L. sativa	root	**	$y = -16.196 \ln x + 40.59$	0.93	268
	hypocotyl	**	$y = -9.3381 \ln x + 10.227$	0.97	628
P. lanceolata	root	**	$y = -27.443 \ln x + 148.89$	0.89	1405
	hypocotyl	**	$y = -15.143 \ln x + 61.867$	0.86	1615
S. alba	root	**	$y = -9.6204 \ln x + 3.4638$	0.83	263
	hypocotyl	**	$y = -8.4901 \ln x + 1.861$	0.82	450
T. repens	root	**	$y = -14.747 \ln x + 38.241$	0.95	396
	hypocotyl	**	$y = -21.611 \ln x + 114.05$	0.83	1980

[a] Significance of difference of tested compound from the control: **, $P < 0.01$. [b] R^2 is the proportion of common variation in the two variables, i.e., the "strength" of the relationship.

Table 6. Influence of Different Concentrations (1–3000 μmol) of Gallic Acid on Weed Growth

plant	plant part	sig[a]	equation	$R^{2\,b}$	EC_{50} (μM)
A. milefolium	root	**	$y = -24.304 \ln x + 112.07$	0.90	780
	hypocotyl	**	$y = -23.884 \ln x + 104.32$	0.79	640
E. crus-galli	root	**	$y = -34.238 \ln x + 180.71$	0.96	840
	hypocotyl	**	$y = -27.768 \ln x + 133.47$	0.81	740
L. perenne	root	**	$y = -17.941 \ln x + 64.822$	0.78	600
	hypocotyl	**	$y = -22.851 \ln x + 84.369$	0.88	360
L. sativa	root	**	$y = -6.3573 \ln x - 18.706$	0.84	137
	hypocotyl	**	$y = -11.236 \ln x - 12.879$	0.89	27
P. lanceolata	root	**	$y = -14.333 \ln x + 69.808$	0.93	3865
	hypocotyl	**	$y = -8.5481 \ln x + 10.3$	0.73	1158
S. alba	root	**	$y = -7.9593 \ln x + 20.862$	0.80	123
	hypocotyl	**	$y = -24.54 \ln x + 102.78$	0.85	500

[a] Significance of difference of tested compound from the control: **, $P < 0.01$. [b] R^2 is the proportion of common variation in the two variables, i.e., the "strength" of the relationship.

Table 7. Influence of Different Concentrations (1–10000 μmol) of 4-Hydroxyacetophenone on Weed Growth

plant	plant part	sig[a]	equation	$R^{2\,b}$	EC_{50} (μM)
A. milefolium	root	**	$y = -26.511 \ln x + 140.43$	0.85	1317
	hypocotyl	**	$y = -59.903 \ln x + 402.69$	0.82	1915
A. retroflexus	root	**	$y = -14.187 \ln x + 38.376$	0.87	505
	hypocotyl	**	$y = -13.314 \ln x + 29.054$	0.87	378
E. crus-galli	root	**	$y = -14.013 \ln x + 37.672$	0.85	520
	hypocotyl	**	$y = -10.937 \ln x + 27.478$	0.84	1193
L. perenne	root	**	$y = -9.4291 \ln x + 10.114$	0.85	575
	hypocotyl	**	$y = -22.333 \ln x + 84.324$	0.94	410
L. sativa	root	**	$y = -21.892 \ln x + 85.767$	0.96	493
	hypocotyl	**	$y = -21.454 \ln x + 85.39$	0.98	550
P. lanceolata	root	**	$y = -9.2725 \ln x + 9.6213$	0.80	618
	hypocotyl	**	$y = -26.276 \ln x + 136.94$	0.80	1230
S. alba	root	**	$y = -9.2232 \ln x + 13.435$	0.88	963
	hypocotyl	**	$y = -9.5523 \ln x + 9.9803$	0.70	530
T. repens	root	**	$y = -13.124 \ln x + 31.582$	0.81	500
	hypocotyl	**	$y = -16.396 \ln x + 65.857$	0.92	1171

[a] Significance of difference of tested compound from the control: **, $P < 0.01$. [b] R^2 is the proportion of common variation in the two variables, i.e., the "strength" of the relationship.

4-Hydroxyacetophenone inhibited the growth of all tested plants (**Table 7**), and the EC_{50} ranged from 378 to 1915 μM, dependent on the plant part and species tested. *L. perenne*, *L. sativa*, and *A. retroflexus* were established as the most sensitive and *A. milefolium*, *P. lanceolata*, and *E. crus-galli* as the most resistant.

The roots of *P. lanceolata* were inhibited more by 4-hydroxyacetophenone than by either vanillic or gallic acids. On the contrary, the growth of *L. sativa* was most sensitive to gallic acid, of the compounds tested. In the cases of the other plants, the roots were more resistant to gallic acid and the hypocotyls to vanillic acid.

The influence of different concentrations of squalene [$1 \times (10^{-3}-10^{-6})$ M] on the growth *A. retroflexus*, *E. crus-galli*, *L. perenne*, *P. lanceolata*, *A. milefolium*, and the root of *T. repens*, compared to the control, was not statistically significant ($P > 0.05$). Only stimulation was observed in the case of hypocotyl elongation of *S. alba*, *L. sativa*, and *T. repens*. For the influence of squalene upon root elongation of *S. alba* and *L. sativa*, the effective concentration could be established by the following equations: $y = -3.4695 \ln x + 24.387$ ($R^2 = 0.92$, $EC_{50} = 400000$ μM); $y = -3.4695 \ln x + 24.387$ ($R^2 = 0.92$; $EC_{50} = 1400000000$ μM). The results indicate either no or a stimulative effect of squalene on plant growth.

Vitexin [$1 \times (10^{-3}-10^{-6})$ M] had either no or a stimulative effect on the growth of plants tested in the dark. The growth of *A. retroflexus*, *E. crus-galli*, and *P. lanceolata* was not significantly influenced by vitexin ($P > 0.05$). Only stimulation was established in the case of *T. repens* growth and hypocotyl elongation of *L. sativa* and *S. alba*. The fitted regression equation found for the root growth of *S. alba* was $y = -10.376 \ln x + 61.06$ ($R^2 = 0.96$; EC_{50} = about 44000 μM), and that for *L. sativa* was $y = -5.6606 \ln x + 15.157$ ($R^2 = 0.93$; EC_{50} = about 100000 μM). Basile et al. (*30*) observed a 50% inhibition of seed germination in *R. sativus* by vitexin with a concentration of 0.2×10^{-6} M. However, these results were obtained in the light after 24 and 48 h, whereas in the dark no significant effect was established. The presence of vitexin in achenes, the absence in other plant parts, and the different behavior in diverse light conditions indicate the possible function of vitexin in germination.

In our laboratory, we were not able to determine which epicatechin enantiomer is present in common buckwheat. According to Trotin et al. (*2*) a hairy root culture of *F. esculentum* synthesized (−)-epicatechin. Therefore, for the biotests we used (−)-epicatechin, which is widespread in plants, in contrast to (+)-epicatechin, which is a minor component of a few species, such as *Camellia sinensis* (tea plant) (*24*). We established a nonsignificant effect of (−)-epicatechin [$1 \times (10^{-3}-1 \times 10^{-6})$ M] upon the growth of *A. retroflexus* and elongation of the hypocotyl in *E. crus-galli*, *L. perenne*, *T. repens*, *S. alba*, and *A. milefolium* ($P > 0.05$). A significant stimulative effect was observed in root elongation of *E. crus-galli*, *L. perenne*, *T. repens*, *P. lanceolata*, *L. sativa*, and *S. alba*, plus hypocotyl elongation of *P. lanceolata* ($P < 0.05$). Fitted regression equations were found for the root elongation of *A. milefolium* ($y = -2.1085 \ln x + 0.4384$; $R^2 = 0.74$; $EC_{50} = >100000000$ μM) and hypocotyl elongation of *L. sativa* ($y = -1.9471 \ln x + 8.0257$; $R^2 = 0.76$; $EC_{50} = >100000000$ μM). The results indicated no inhibitive effects of (−)-epicatechin. Also, according to Bais et al. (*24*), (−)-epicatechin (250 μg/mL) does not inhibit the germination of seven different plants.

Comparison of the identified compounds, their effects as well as the inhibitive effects of soil extracts from the buckwheat stand, indicates that a compound similar to gallic and palmitic acids (possibly a derivative of 4-hydroxyacetophenone), probably has an important function in the allelopathic root response of common buckwheat. Vanillic acid and rutin could be important as cumulative substances in the soil during buckwheat development. Further studies are needed to establish the impact of these compounds under natural conditions.

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