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THE INFLUENCE OF SUNFLOWER AND MUSTARD LEAF EXTRACTS ON THE GERMINATION OF MUSTARD SEEDS

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The extracts from sunflower and mustard leaves were separated using SPE-Columns. The mustard seeds were germinated on water (24 h) and subsequently on crude extracts or separate fractions. The heat production rate was measured by isothermal calorimetry at 21°C and changes in seed cotyledons by FT-Raman spectroscopy. Crude extracts strongly inhibited seed germination. The water and 'methanol' fractions of mustard and sunflower extracts have a similar influence on the pattern of heat efflux. FT-Raman spectroscopy showed that extracts caused changes in cotyledons mainly in the content of fatty acids, carotenoids and flavonoids. Isothermal calorimetry and Raman spectroscopy are useful for the study of allelopathic interactions.

Keywords: *allelopathy, FT-Raman spectroscopy, heat production rate, isothermal calorimetry, mustard, non-destructive analysis, sunflower*

Introduction

Sunflower (*Helianthus annuus* L.) can actively influence the growth of surrounding plants. Dried sunflower residues and leaf powder incorporated in the soil reduced the growth of sorghum, soybean and sunflower itself [1, 2]. Leather [3] demonstrated selective phytotoxicity of sunflower residues towards weeds. Although the inhibitory effect of phytotoxins in sunflower leaf extracts or crop residues on the germination of various seeds has been reported previously [4], their mode of action has not yet been demonstrated.

It was shown in our earlier paper [5] that aqueous extracts of sunflower leaves completely inhibited the germination of mustard seeds. The inhibition of germination was associated with alterations in reserve metabolism and generation of energy in the catabolic phase of germination. Degradation of lipids was suppressed by sunflower foliar extracts.

Since undisturbed reserve mobilisation is an important requirement for germination as well as subsequent plant growth and development, we investigated the influence of various extracts from sunflower and mustard leaves (as autoallelopathy) on catabolic activity during mustard seed germination. Total catabolic activity was measured as the heat production rate (in mW) of germinated seeds in an isothermal calorimeter. Additionally, changes in the chemical composition of cotyledons provoked by the presence of sunflower and mustard phytotoxins were observed. For this purpose, FT-Raman spectroscopy was employed.

Experimental

Plant material

Mustard seeds (cv. Nakielska) were pre-germinated in a 9 cm diameter plastic Petri dish on filter paper moistened with distilled water for 24 h at 21°C in darkness.

Preparation of extracts

The leaves of sunflower (cv. Ogrodowy) and mustard (cv. Nakielska) were air-dried, ground to powder and the crude aqueous extracts were prepared by shaking with water for 24 h in darkness at room temperature. The concentration of each extract was 5% (*w*/*v*). The aqueous extracts from mustard and sunflower leaves were separated using SPE (Solid Phase Extraction) into water and 'methanol' fractions. The SPE-column (Octadecyl - C18 Polar Plus, 6 mL, 3000 mg) was conditioned with 2 mL of methanol (POCh) and rinsed with 4 mL of distilled water; subsequently, the crude extract was injected (1 mL) on the column. Water fraction was collected. The column was then washed with 1 mL of methanol and the methanol fraction was collected. This fraction was evaporated to dryness under nitrogen. The residue was dissolved in 1 mL of distilled water and called the 'methanol' fraction.

Calorimetric measurements

The heat production rate (HPR) was measured in mW in an isothermal calorimeter (BioActivity Moni-

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

tor 2277, Thermometric, Järfälla, Sweden). In the experiment, ampoules (volume: 20 mL) with lids were used, which enabled air exchange. Five pre-germinated seeds were put into an ampoule on filter paper with 300 μL of each extract. The ampoules were allowed to equilibrate for 30 min and placed in the measurement chamber. In the reference ampoule only the filter paper moistened with the extract was placed. The seeds germinated on filter paper with 300 μL of distilled water were used as an absolute control. Measurements were recorded continuously for 24 h in 21°C and five repetitions were carried out. The HPR was expressed per gram of dry mass.

Fourier transform Raman spectroscopy measurements

For FT-Raman measurements, five seeds were taken which germinated on each kind of the extracts investigated. FT-Raman spectra were recorded using a Nicolet NXR 9650 FT-Raman Spectrometer equipped with an Nd:YAG laser emitting at 1064 nm and a germanium detector cooled with liquid nitrogen. The spectrometer was provided with an *xy* stage, a mirror objective and a prism slide for the redirection of the laser beam. The measurements were performed with a spectral resolution of 4 cm^{-1} in the range of 100 to 4000 cm^{-1}. All spectra were accumulated from 64 scans, measured with a laser power of 150 mW using an unfocused laser beam of a diameter of approx. 100 μm. Raman spectra were recorded by the Omnic/Thermo Scientific software programme. For each treatment five spectra were collected.

Chemometrics

Hierarchical cluster analysis was performed for the FT-Raman spectra using the Opus/Bruker package (5.1). The spectra were not baseline corrected. The cluster analysis was performed separately for mustard and sunflower extracts on the averaged spectra for the whole wavenumber ranges using Ward's algorithm. The spectral distances for the mustard extracts were calculated with the standard algorithm after applying vector normalization. For the sunflower extracts the cluster analysis was carried out with the factorization algorithm using the first three factors.

Results and discussion

Seed heat emission

Isothermal calorimetry is an excellent tool for measuring the heat production rate in plant tissue. In this method, the total heat flow from an organism is measured. In the environment, the chemical compounds are released from one living plant and they influence the growth and development of others [6]. Thus, these allelopathic compounds certainly cause changes in the metabolism of plants and their heat production rate, which can be easily detected by isothermal calorimetry.

The heat production rate–time curves of mustard seeds exposed to mustard extracts at 21°C are shown in Fig. 1. The HPR of control seeds (germinated on water) increases continuously during the 24 h of germination. The differences in the HPR are observed between the seeds germinating on various mustard extracts and are already evident in the first hour of the measurement. The highest value of HPR is observed for the seeds germinating on the crude extract and amounts to 20.22 mW g_{DW}^{-1}. The HPR for these seeds goes through a maximum between 10 and 16 h. The curve for the seeds germinated on the water fraction of mustard extract shows that germination of seeds is strongly inhibited. The difference in the HPR value is about 3.5 mW g_{DW}^{-1}. It is supposed that allelopathic compounds are present in the water fraction, which can influence seed growth and change their metabolism. A different situation is observed for the 'methanol' fraction of the mustard extract. At the beginning, the metabolic pathways go on. The HPR in a maximum of 6 h is over 8 mW g_{DW}^{-1}. Then, HPR goes down rapidly and reaches the minimum between 12 and 16 h. The metabolic activity is strongly inhibited and the HPR value is almost zero. Subsequently, the HPR rises again after 16 h. At the end of the measurement, the HPR value is almost the same for the water fraction (6.92 mW g_{DW}^{-1}) as for the 'methanol' fraction (5.27 mW g_{DW}^{-1}).

Figure 2. shows the heat production rate–time curves of the mustard seeds which germinated on sunflower extracts at 21°C. In this case, a very similar phenomenon is observed as for the mustard extracts, which reflects the form of the HPR curve. However, the maximum HPR for the seeds germinated on the crude sunflower extract goes through a maximum between 8 and 16 h, and the highest value of HPR for these seeds is 4.51 mW g_{DW}^{-1}. This could be explained by the content of allelopathic compounds in this crude extract. Chemical studies of sunflowers have shown that several bioactive compounds are present in this plant: flavonoids [7], terpenoids [8], norsesquiterpenoids [9], sesquiterpene lactone [10, 11], annuionones [12, 13] and two new families of sesquiterpenoids: heliespiranes [14] and heliannanes [15–17]. The minimum for the 'methanol' fraction is between 10 and 12 h.

The similarity in the forms of curves for mustard and sunflower extracts can be associated with the chemical composition of the individual fractions. In

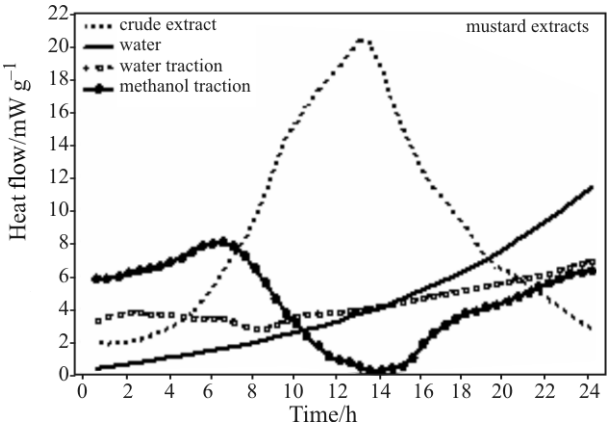

Fig. 1 The heat production rate–time curves for mustard extracts

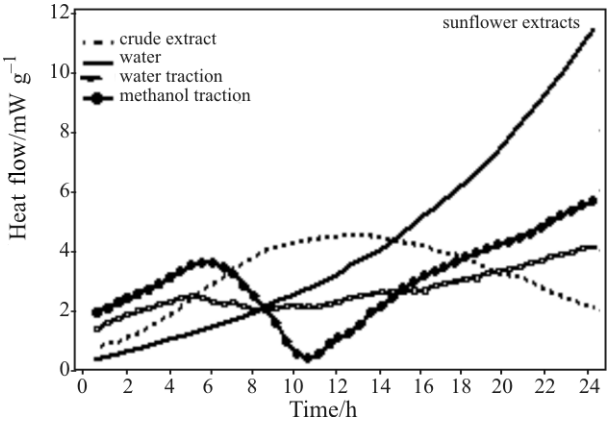

Fig. 2 The heat production rate–time curves for sunflower extracts

other words, the same groups of allelopathic compounds from different extracts influence the heat production rate–time in a similar way.

FT-Raman spectra and cluster analysis

The FT-Raman spectra obtained from the mustard seeds germinated on the extracts from mustard, as well as from sunflower leaves, are shown in Figs 3 and 4, respectively. The most characteristic wavenumber range with several distinct bands is seen between 1700 and 1200 cm^{-1}. The prominent band at about 1440 cm^{-1} together with signals near 1655, 1300 and 1266 cm^{-1} can be assigned to fatty acids, but also lignin can be correlated with the signals near 1600–1650 cm^{-1}. Additionally, a characteristic flavonoid band near 1590 cm^{-1} can be detected. The Raman measurements allowed us to successfully investigate the degree of unsaturation of the fatty acids. The ratio of the scattering intensity arising from the C=C stretching vibration (1600 cm^{-1}) to that obtained from the CH_2 scissoring mode (1444 cm^{-1}) could be used to reliably predict the iodine values of unconjugated vegetable oils. Determination of the total degree of unsaturation can also be obtained by calculating the ratio of the intensity of the band at ~1270 cm^{-1} to that of the band at ~1300 cm^{-1}.

The most significant difference between the spectra shown in Figs 3 and 4 can be observed between the seeds germinated on water and various extracts. It is related mainly to the ratio of 1270 and 1300 cm^{-1} bands, which can be correlated with the degree of unsaturation of the fatty acids in these samples. The application of Cluster Analysis on the averaged spectra obtained from the various mustard seeds (Figs 5 and 6) confirms the above statement. Distinct discrimination between the seeds germinated on water and the individual extracts was achieved. These results indicate that changes in plant constituents caused by germination conditions can be related to the characteristic Raman signals.

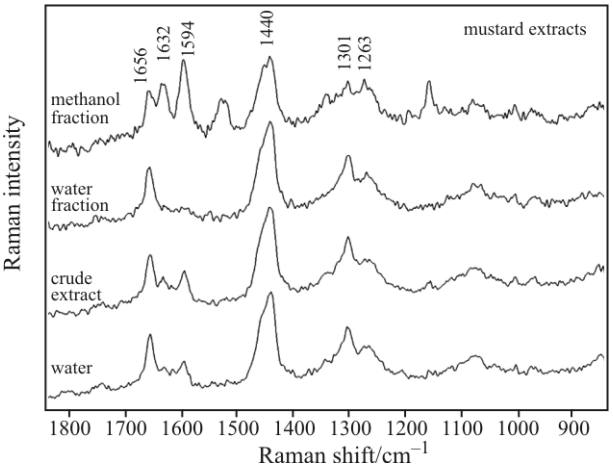

Fig. 3 FT-Raman spectra from the mustard seeds germinated on the mustard extracts

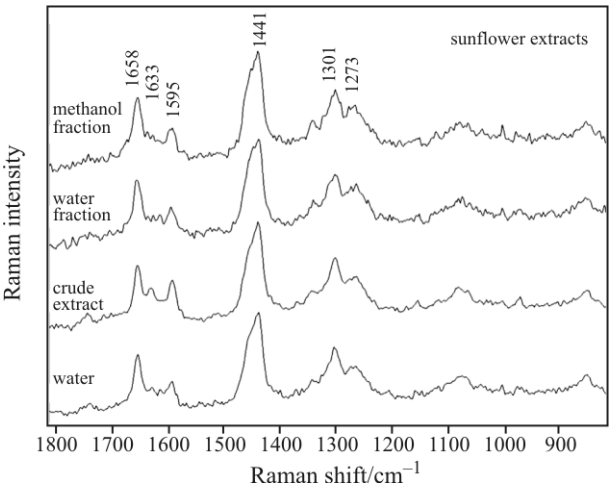

Fig. 4 FT-Raman spectra from the mustard seeds germinated on the sunflower extracts

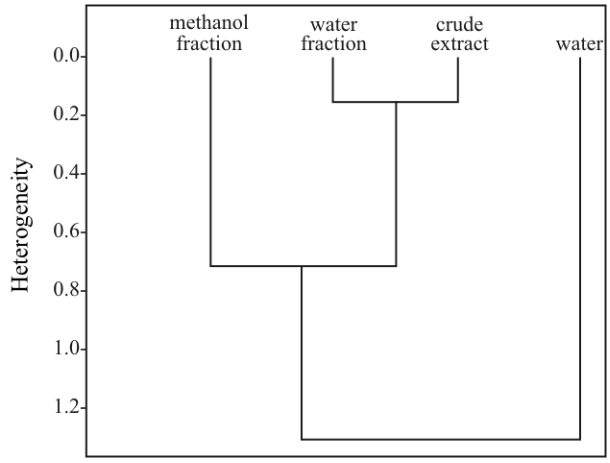

Fig. 5 The cluster analysis performed for spectra of mustard extracts (100–4000 cm^{-1})

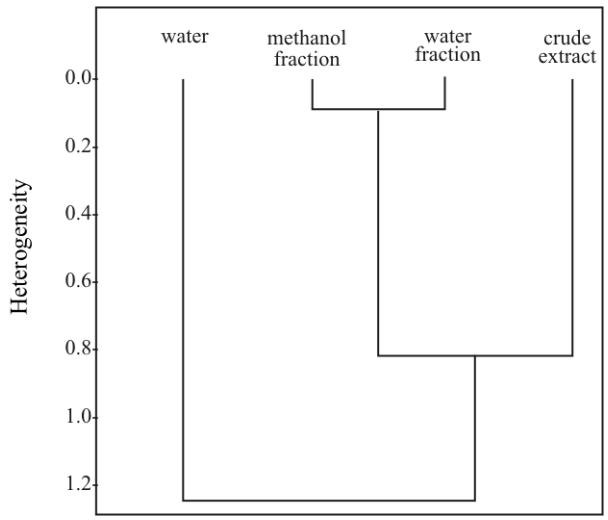

Fig. 6 The cluster analysis performed for spectra of sunflower extracts (100–4000 cm^{-1})

Conclusions

Isothermal calorimetry is a useful method for studying interactions between plants and allows the monitoring of the allelopathic effects. The heat production rate–time curves of mustard seeds can be correlated with different extracts used for seed germination and, consequently, with different allelopathic compounds which influence the metabolism of seeds. The seeds germinated on the mustard extract have higher values of metabolic activity than those germinated on the sunflower extract.

FT-Raman spectroscopy with the hierarchical cluster analysis is a good tool for comparing the chemical composition of seeds treated with mustard and/or sunflower extracts. The spectra of mustard seed cotyledons showed that extracts mainly influenced the degree of saturation of fatty acids, but also in the same way the metabolism of flavonoids.

References

1 S. Irons and O. Burnside, Weed Sci., 30 (1982) 327.
2 M. K. Schon and F. Einhellig, Bot. Gaz., 143 (1982) 505.
3 G. R. Leather, Plant Soil, 98 (1987) 17.
4 A. A. P. M. Azania, C. A. M. Azania, P. L. C. A. Alives, R. Palaniraj, H. S. Kadian, S. C. Sati, L. S. Rawat, D. S. Dahiya and S. Narwal, Allelopathy J., 11 (2003) 1.
5 E. Kupidłowska, A. Gniazdowska, J. Stępień, F. Corbineau, D. Ginel, A. Skoczowski, A. Janeczko and R. Bogatek, J. Chem. Ecol., 32 (2006) 2569.
6 E. L. Rice, Allelopathy, Academic Press, New York 1984, p. 1.
7 F. A. Macias, J. M. G. Molinillo, A. Torres, R. M. Varela and D. Castellano, Phytochemistry, 45 (1997) 683.
8 F. A. Macias, A. Torres, J. L. G. Galindo, R. M. Varela, J. A. Alvarez and J. M. G. Molinillo, Phytochemistry, 61 (2002) 687.
9 F. A. Macias, R. M. Varela, A. Torres, R. M. Oliva and J. M. G. Molinillo, Phytochemistry, 48 (1997) 631.
10 F. A. Macias, A. Torres, J. M. G. Molinillo, R. M. Varela and D. Castellano, Phytochemistry, 43 (1996) 1205.
11 A. P. de Luque, J. C. G. Galindo, F. A. Macias and J. Jorrin, Phytochemistry, 53 (2000) 45.
12 F. A. Macias, A. Lopez, R. M. Varela, A. Torres and J. M. G. Molinillo, Phytochemistry, 65 (2004) 3057.
13 T. Anjum and R. Bajwa, Phytochemistry, 66 (2005) 1919.
14 F. A. Macias, R. M. Varela, A. Torres and J. M. G. Molinillo, Tetrahedron Lett., 39 (1998) 427.
15 F. A. Macias, R. M. Varela, A. Torres and J. M. G. Molinillo, Tetrahedron Lett., 40 (1999) 4725.
16 S. Ghosh, K. Tuhina, D. R. Bhowmik and R. V. Venkateswaran, Tetrahedron, 63 (2007) 644.
17 A. Roy, B. Biswas, P. K. Sen and R. V. Venkateswaran, Tetrahedron Lett., 48 (2007) 6933.

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