

Microwave control of *Reynoutria japonica* Houtt. including ecotoxicological aspect and resveratrol content in rhizomes

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Abstract

Japanese knotweed (*Reynoutria japonica* Houtt.) is Poland's invasive weed, of no efficient control method. The rhizomes of this species are rich in resveratrol. In this work, we evaluated 1) the effectiveness of electromagnetic microwaves (MV) in destroying Japanese knotweed using an original device HOGWEED (MV of 2450 MHz); 2) the ecotoxic effect of the MV on the soil environment; 3) the resveratrol content in knotweed rhizomes after MV treatment. The field studies were carried out in 2022 in southern Poland. Cut plants were MV treated at times: 5.0–25.0 min. The MV efficiency was checked 10 and 56 days after treatment (DAT). After MV treatment, fresh soil samples were taken to analyze their ecotoxicity. As a result, at 56 DAT, knotweed was controlled if MV was used for at least 20.0 min. The MV did not affect the soil ecotoxicity. The MV-treated soils were classified as non-toxic or low-toxic. To analyze the resveratrol content, healthy knotweed rhizomes were dug out and treated with MV in laboratory at times: 2.5–10.0 min, and analyzed for the resveratrol content in HPLC-MS/MS. In result, the resveratrol in the rhizomes significantly decreased following MV exposure in a time-depending manner.

Introduction

Six species of the genus *Reynoutria* Houtt (syn. *Polygonum* Sieb. & Zucc., *Fallopia* Houtt.) are known worldwide, which naturally occur in eastern Asia (in the area from the eastern part of Russia to the Indochina Peninsula) ¹. As introduced plants, they are widespread in vast areas of Europe and North America and listed as particularly invasive alien species (IAS). Due to their spread and environmental impact, they are currently on the Swiss black-list of IAS, considered one of the most dangerous environmental weeds ². Japanese knotweed (*Reynoutria japonica* Houtt., syn. *Fallopia japonica* (Houtt.) Ronse Decr.), Sakhalin knotweed (*Reynoutria sachalinensis* F. Schmidt) and a cross between them - Czech knotweed (*Reynoutria* × *bohemica*) have been found in Poland ^{3,4}. All three taxa of this genus growing in Poland are considered IAS extremely dangerous for native flora ⁵.

Among the invasive knotweeds in Poland, the Japanese knotweed is the most widespread species ⁵. At the same time, according to ⁶, it is one of the hundred most invasive species in the world. A new plant can develop from a rhizome fragment 1 cm long and weighing not more than 0.7 g placed in the soil or water, as well as from a small section of a shoot containing a single node ^{7,8}. The invasion of the species is facilitated by its large size, limits access to light for other species, high regenerative potential, and allelopathic effects ^{9,10}. The dispersion of rhizomes with water, e.g., during river floods, contributes to the spread of plants along river valleys ^{7,4,11}. It also often occurs in, e.g., wasteland, fallows, roadsides, railway embankments, it also enters agricultural areas. As a result, dense canopies of Japanese knotweed, reduce the biodiversity of natural and semi-natural habitats ⁴.

On the other hand, Japanese knotweed is also a source of many valuable biologically active compounds ¹², and is an edible plant in Japan ¹³. Its rhizomes are an excellent reservoir of resveratrol, a polyphenolic phytoalexin (3,5,4'-trihydroxy-trans-stilbene) belonging to the group of stilbenes ¹⁴. The beneficial effect

of resveratrol on cardiovascular, inflammatory, neurodegenerative, metabolic, and age-related diseases has been proven ¹⁵. According to ¹⁶, resveratrol content in Japanese knotweed rhizomes is about 7.4 and 11.1 times higher than in their leaves or stems. It ranges from 2.96 to 3.77 mg g⁻¹ dry weight ^{17,18}.

All invasive knotweeds are difficult to control, as minimum disturbance regimes permit the accumulation of significant below-ground reserves that allow recovery from many physical, biological, chemical, and integrated control methods. According to a few authors, in the case of Japanese knotweed, an approach that works with the seasonal resource translocation between above- and below-ground biomass and adequate herbicide coverage or injections, e.g., glyphosate, is the key to success ^{19,20,21,22}. The other adopted methods are the physical burying of rhizomes and stems ²³. However, all these methods are ineffective, so new methods of Japanese knotweed control are being sought ²⁴.

Microwave radiation has attracted much interest in various environmental studies ^{25,26,27}. This method was used for control of giant hogweed population ²⁸. However, the effects of radiation on soil biological activity are not clear. Some research indicated that microwave radiation applied to soil might harm physicochemical and biological properties ²⁷. Other studies have shown that microwave treatment of soil did not affect soil pH or nutrient availability; however, as the irradiation time increased, the number of colonizing bacteria in the soil decreased significantly ^{29,25}.

The aim of the research was: 1) to assess the effectiveness of microwave radiation in destroying Japanese knotweed plants, taking into account the ecotoxic effect of radiation on the soil environment; 2) analysis of resveratrol content in knotweed rhizomes after microwave treatment.

Material and methods

2.1 Characteristics of the study site and Japanese knotweed population

Field studies were carried out in 2022 on a population of Japanese knotweed (*Reynoutria japonica* Houtt.) of approx. 250 m² area, located near the Vistula embankments in Kraków, south of Poland (N: 50°02'34.31" E: 19°52'43.31", Fig. 1A-B). The plant material was collected respecting the IUCN policy ³⁰ and a required permission for collecting plant samples was obtained from the Municipal Greenery Board in Kraków, PL. Based on ³, the specimens were botanically identified by Dr. B. Grygierzec, then archived in the herbarium resources of the Department of Agroecology and Crop Production of the University of Agriculture in Krakow, that provides access to deposited material.

Figure 1. Location of study site in Poland (A) and Kraków (B).

2.1.1. Chemical characteristics of soil and weather data from the study site

The soil samples taken from the site were air-dried and sieved through a sieve with a mesh size of 2 mm. The following parameters were determined: granulometric composition by the areometric method, pH in 1 mol KCl • dm⁻³ by the potentiometric method, and total organic carbon, nitrogen, and sulfur using the CNS Elementar apparatus Vario MAX cube (Elementar Analysensysteme GmbH). Moreover, the content of assimilable phosphorus and potassium by the Egner-Riehm method was analyzed. The total content of elements (Ca, Mg, K, P, Na, Zn, Cd, Pb, Cu, Ni, Cr, Fe, Mn) in the soil was determined after hot mineralization in a mixture of HNO₃ and HClO₃ acids (3:2 v/v) (supra pure, MERCK) by ICP-OES (Inductively Coupled Plasma Atomic Emission Spectroscopy) on Optima 7300 DV (Perkin-Elmer). Table 1 presents the characteristics of soil in the study site.

Table 1
Characteristics of soil in the study site.

Sand	Loam	Clay	pH	C org.	N	S	P	K	Ca
%	%	%	-	%	g/kg	g/kg	g/kg	g/kg	g/kg
70	26	4	6.06	2.55	1.95	0.38	0.68	2.47	6.21
Mg	Na	Fe	Mn	Zn	Cu	Ni	Cd	Pb	Cr
g/kg	g/kg	g/kg	g/kg	g/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
3.20	0.19	13.23	0.68	153.55	17.35	34.44	0.66	32.20	32.60

Weather data, i.e., daily temperatures and precipitation, recorded every hour from June 28 to August 27, 2022, were collected from the weather station of the Institute of Meteorology and Water Management in Kraków-Balice, located ca. 7.5 km from the study site. The course of the weather is presented in Fig. 2.

2.2 Control of Japanese knotweed using microwaves

On June 29, 2022, the aboveground plant biomass was cut and removed from the ground selected for microwave radiation (MV). The cut shoots remaining in the soil were treated with a self-propelled device HOGWEED, on a tracked chassis with a rotary mower, intended for MV destruction of invasive plants (Fig. 3). The HOGWEED comprises an MV emitter consisting of nine magnetrons generating electromagnetic waves at 2450 MHz, each with a power of 800 W. The average surface power density for the dimensions of the antenna is 30 kW/m².

The MV with an open beam directed at the plants was used at times: 5.0; 7.5; 10.0; 15.0; 20.0, and 25.0 min in 9 repetitions.

The MV treatment of Japanese knotweed plants was carried out in the morning hours when the air temperature was 22.4°C, air humidity 85–89%, and soil moisture, determined by the dryer method in the laboratory, was 88%.

Both before and after MV treatment, the temperature of the plants was measured with a FLIR E60 thermal imaging camera equipped with Wi-Fi (manufacturer: FLIR Systems, Inc., Wilsonville, Oregon, USA), which measures the temperature in the range from -20 to $+120^{\circ}\text{C}$ ($\pm 2^{\circ}\text{C}$). The camera was placed approximately 0.5-1.0 m above the plant stems, and images were taken with a 320 x 240 pixels resolution. Each image was then analyzed with FLIR ResearchIR MAX (manufacturer: Teledyne FLIR LLC, Wilsonville, OR, USA) based on mathematical processing of the pixel color scale—regression analysis in an Excel spreadsheet analyzed average temperatures for each MV time.

The efficiency of radiation control was checked at 10 and 56 days after treatment (DAT). The number of re-growing plants was counted for each MV treatment and replication. Moreover, all MV areas were photographed to document the outcomes.

2.2. Ecotoxicological analyzes of soil treated with microwaves

Soil samples were collected on June 29, 2022, immediately after the plots with plants were MV treated. Nine samples were taken from each MV plot and mixed into one collective sample. In total, six collective soil samples and a control sample were prepared. The soil was stored at $3-4^{\circ}\text{C}$ until further analysis. Soil ecotoxicity was tested using three biotests (Table 2). Two model plant species were used in the Phytotoxkit test: the monocotyledonous *Sorghum saccharatum* (L.) Moench and the dicotyledonous *Sinapis alba* L. After a 3-day incubation of the samples, two parameters were tested: germination inhibition (%) and root growth inhibition (%) ³¹. In the Ostracodtoxkit test, the test organism was the crustacean *Heterocypris incongruens* ³². For this assay, after 6 days of incubation of the samples, mortality and inhibition of *H. incongruens* growth were measured. The Microtox system uses the luminescent bacteria *Alivibrio fischeri*. Exposure of the bacteria to the sample disrupts the metabolic process, reducing the light produced by the bacteria. Measuring the inhibition of bacterial glow in the test sample relative to the control sample is a measure of toxicity evaluation. The toxicity of the soil samples to *Alivibrio fischeri* was tested using the 81.9% Screening Test using the M 500 Analyzer ³⁴. The aqueous extract was prepared by mixing 1 volume of the sample with 10 volumes of redistilled water and shaking mechanically for 24 h. Luminescence was measured before and after a 15-min incubation of the bacterial suspension with the test sample. Screening tests were performed based on the analysis of undiluted samples. The samples' toxicity was assessed based on the percentage value of the toxic effect (PE%) estimated for the performed biotests. The samples were assigned to the appropriate toxicity classes: class I (PE $\leq 20\%$ no significant toxic effect) - non-toxic sample; class II ($20\% < \text{PE} \leq 50\%$ significant toxic effect) - low-toxic sample; class III ($50\% < \text{PE} < 100\%$ significant toxic effect) toxic sample; class IV (PE = 100%) very toxic sample ³⁴.

Table 2
Characteristics of the ecotoxicological biotests.

Test	Model organism	Final parameter	Time
Phytotoxkit	<i>Sinapis alba</i> , <i>Sorghum saccharatum</i>	Inhibition of germination, inhibition of root elongation	72 h
Ostracodtoxkit	<i>Heterocypris incongruens</i>	Death, growth inhibition	6 dni
Microtox	<i>Alivibrio fischeri</i>	Luminescence inhibition	15 min.

2.3. Analysis of resveratrol content in rhizomes after microwave radiation

Healthy Japanese knotweed rhizomes obtained on August 23, 2022, from the field population, were carefully washed under running water and divided into fragments of various lengths (1.7–20.9 cm) and diameters (1.5–3.2 cm) so that their total weight was 500 ± 2 g. The rhizomes were MV treated in the laboratory using one antenna emitting MV of the surface power density of 30 kW/m^2 for 2.5, 5.0, 7.5, or 10.0 min. The device was described in detail by ²⁸. Directly after MV, the temperature of the rhizomes was measured with the FLIR E60 thermal imaging camera (FLIR Systems, Inc., Wilsonville, Oregon, USA) and weighed at laboratory balance (Radwag, PL) with ± 0.01 g accuracy. Next, the bark from the rhizomes was removed, and small (0.5–1.5 cm long) and thin (0.5–1.2 mm) slices were cut with a sterile razor into sterile containers. The material was lyophilized at -40°C in Labconco FreeZone (Kansas City, MO, USA) freeze-dry system for ca. 30 h and sent to analyses for resveratrol content.

The analytical studies were performed using an Agilent Technologies 1260 Infinity II series coupled with Agilent Technologie Infinitylab LC/MSD equipped with an electrospray ionization (ESI) source operating in negative ionization modes. The separation of the target compound was achieved on a Synergi Polar-RP C18 analytical column (250 mm $\times$ 4.6 mm, 4 μm) from Phenomenex (Cheshire, UK). The mobile phase was a mixture of (A) water and (B) acetonitrile, with formic acid 0.1%, at a flow rate of 0.8 mL/min in gradient elution mode. The composition of the mobile phase varied as follows: starting with 20% B then 0–10 min, 70% B; 10–15 min, isocratic condition, 70% B; 15–18 min, 20% B; after which, the column was reconditioned for 10 min. All solvents and solutions were filtered through a 0.2 μm polyamide filter from Sartorius Stedim (Goettingen, Germany). The injection volume was 5 μL . The column temperature was 30°C , and the drying gas temperature in the ionization source was 350°C . The gas flow was 12 L/min, the nebulizer pressure was 55 psi, the capillary voltage was 3000 V, and the fragmentor 70. Detection was performed in single ion monitoring (SIM) mode. The extracted-ion chromatogram of the ion current of the peak m/z 227, corresponding to the (M - H)⁻, was used to quantify resveratrol.

2.3.1. Reagents and standard

The standard of trans-Resveratrol was purchased from Vinci-Biochem (Florence, Italy). At a concentration of 1000 mg/L, individual stock solutions of trans-Resveratrol were prepared by dissolving the pure standard compound in LC-MS grade methanol and storing it in glass-stoppered bottles at 4°C. Standard working solutions at various concentrations, from 0.25 to 2 mg/L, were prepared daily by appropriately diluting the stock solutions with HPLC-grade methanol. LC-MS-grade methanol and acetonitrile were purchased from Merck, while HPLC-grade formic acid 99–100% was bought from JT Baker BV (Deventer, Holland). Deionized water (> 18 MΩ cm resistivity) was further purified using a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). Before HPLC analysis, all samples were filtered with Phenex™ RC 4 mm 0.2 µm syringeless filter, Phenomenex (Castel Maggiore, BO, Italy).

2.3.2. Sample preparation and extraction procedures

Lyophilized *R. japonica* samples were ground with a cutter mill using a 6 mm grid. Afterward, each sample was extracted with ethanol: water mixture (80:20, v/v) in a 1:10 drug-solvent ratio by sonication (FALC ultrasonic bath, Treviglio, Italy) at 25°C and frequency of 40 KHz for 30 min. After centrifugation at 5000 rpm for 10 min with a Thermo Scientific IEC CL10 Centrifuge from Thermo Electron Industries SAS (Chateau-Gontier, France), the sample solutions were filtered through a 0.2 µm syringeless filter before injection into HPLC-MS/MS triple quadrupole. All samples were stored at 15°C until analysis. Each sample was analyzed in duplicate.

2.3.3. Method validation

The HPLC-DAD-ESI-MS analytical method was validated by assessing the linearity, limit of detection (LOD), and limit of quantification (LOQ). Linearity was tested by injecting external standards at different concentrations, then plotting and calculating calibration curves with the respective determination coefficients (R^2). Calibration curves were prepared using the concentration range of 0.25-2 mg/L. Resveratrol showed good linearity as the R^2 was higher than 0.9995. LOD and LOQ were obtained by injecting serial dilutions of the corresponding standard solutions and calculating the signal-to-noise ratio (SNR). LOD, the smallest analyte detectable in the sample, was calculated as the standard concentration with SNR = 3. In contrast, LOQ is the smallest quantity of analyte that can be quantified and corresponds to the analyte amount for which the signal-to-noise ratio is equal to 10. The LOD for resveratrol was 100 ppb while LOQ was 333 ppb. Figure 4 reports the extracted ion chromatogram (EIC) used for the analyte quantification.

Results

Efficacy of Japanese knotweed control using microwaves

One day before the MV treatment, there was a precipitation of 13 mm in the studied area (Fig. 2), which created good conditions for the treatment, as the soil was moist (88%). The average temperature of the control plants, i.e., untreated and the surrounding soil, was 21.9°C. The temperature of MV-treated plants for: 5.0, 7.5, 10.0, 15.0, 20.0, or 25.0 min reached 45.4, 53.7, 67.0, 77.6, 84.8, and 89.6°C, respectively (Table 3).

Table 3
The temperature analysis results of the thermograms from the knotweed and soil treated with microwaves at times 0–25.0 min in a field experiment. The measurements were taken directly after the microwave radiation.

Mean (°C)	21.9	45.4	53.7	67.0	77.6	84.8	89.6
Radiation time (mins.)	0.0	5.0	7.5	10.0	15.0	20.0	25.0
Stdandard Deviation (°C)	0.6	5.2	4.5	8.2	11.6	13.5	18.0
Maximum (°C)	24.3	56.1	64.0	95.9	112.3	137.7	150.2
Minimum (°C)	20.3	29.9	34.0	42.9	42.4	36.3	39.5

The polynomial correlation fit very well, as the determination index was almost equal to 1 (Fig. 5). The temperature of the cut shoots of Japanese knotweed and the surrounding soil surface increased with the increase in the duration of MV time. The temperature increased between the MV for 10 and 25 min by 22.6°C, and between 20 and 25 min only by 4.8°C.

Ten days after MV, the controlling effect of MV depended on the treatment time. MV plants treated for 5.0, 7.5, 10.0, or 15.0 min regenerated, and new shoots of 1–2 cm long appeared. On the contrary, MV plants treated for 20.0 or 25.0 min did not re-grow. During this period, there was precipitation in the study site almost every day, a total of 35 mm, and the average air temperature was *ca.* 19°C (Fig. 2). The photos below present selected radiation plots 10 days after the MV treatment (Fig. 6A-C).

Figure 6. Japanese knotweed 10 days after microwave radiation. Radiation times: A – 5.0 mins., B – 20.0 mins., C – 25.0 mins. In Photo 4 A, red square indicate new, re-growing shoots of Japanese knotweed.

Fifty-six days after the MV, no regrowth of Japanese knotweed was observed on plots where the MV was used for 20.0 or 25.0 minutes (Fig. 7A-B).

Figure 7. Japanese knotweed at 56 days after microwave radiation. Radiation times: A – 20.0 mins., B – 25.0 mins. Empty spots indicate that no new shoots appeared.

Soil ecotoxicity after microwave radiation

Soil ecotoxicity results are presented in Table 4. In the Phytotoxkit test, inhibition of *S. alba* germination ranged from 0 to 21%. The highest germination inhibition was found in the soils treated with MV for 7.5 and 20.0 min. Inhibition of *S. alba* root growth ranged from – 10 to 43%. The greatest inhibition of root growth was shown in the soils MV treated for 20.0 min and the lowest in those treated for 10.0 min. In soils treated for 10.0 min, root growth of the *S. alba* was stimulated. The analyzed soil samples treated for 10.0 min were non-toxic to *S. alba*, and the remaining samples showed low toxicity. The germination inhibition of *Sorghum saccharatum* was 0–8%. In general, 100% germination of the test plants was observed in most soil samples (except for the 7.5 min). The percentage inhibition of *S. saccharatum* root growth ranged from – 25 to 5%. *S. saccharatum* root growth was stimulated in all the MV-treated soil, especially for 10.0 minutes. No significant effect of soil MV treatment on their toxicity for the test plants was found.

In the Ostracodtoxkit test, crustacean (*H. incongruens*) growth inhibition ranged from 13 to 48%, and mortality was zero. The greatest inhibition of crustacean growth was demonstrated in MV soil treated for 25.0 min and in control soil (0 mins). Notably, in all the MV-treated soils, a tendency to reduce ecotoxicity for crustaceans was observed, and the lowest inhibition was shown in the soil treated for 5.0 min. Soil treated for 5.0 mins was non-toxic to crustaceans. The other MV-treated soils were of low toxicity.

Luminescence inhibition of *A. fischeri* in the MV-treated soils ranged from – 9 to 26%. Notably, a statistically significant reduction in soil toxicity was found in soils treated for 5.0, 7.5, and 10.0 min. In soils treated for 15.0, 20.0, and 25.0 min, soil toxicity significantly increased for bacteria compared to the control (not-MV) soil. Luminescence stimulation was demonstrated in soils MV treated for 5.0, 7.5, and 10.0 min. The remaining soils exposed to MV demonstrated a low-toxic effect for bacteria.

Table 4. The effect of microwave radiation on soil ecotoxicity. *IG - inhibition of germination, IR - inhibition of roots growth, M - mortality, GI - inhibition of growth, IL - inhibition of luminescence, ** means with different letters denote differences significant at $p<0.05$.

Radiation	<i>S. alba</i>		<i>S. saccharatum</i>		<i>H. incongruens</i>		<i>A. fischeri</i>	<i>Class toxicity</i>
	IG ¹	IR	IG	IR	M	GI	IL	
	Percentage toxic effect PE%							
0 mins. (control)	0	42	0	5	0	41	9 b ²	II
5 mins.	-	-	-	-	0	13	-9 a	I
7.5 mins.	21	25	8	-14	0	35	-4 a	II
10 mins.	7	-10	0	-25	0	37	-3 a	II
15 mins.	14	21	0	-5	0	35	20 c	II
20 mins.	21	43	0	-7	0	37	22 c	II
25 mins.	14	37	0	-14	0	48	26 c	II

The influence of MV on soil's increased ecotoxicity has not been demonstrated. According to the hazard classification, considering the responses of all soil organisms, soils MV treated for 5.0 mins were classified as the first toxicity class, meaning that they are non-toxic samples that do not threaten the environment. The remaining soils were classified as class II, which means that they are low-toxic and also do not pose a threat to the environment.

Content of resveratrol in the rhizomes of Japanese knotweed treated with microwaves

The duration of MV treatment affected the temperature of the exposed, bare knotweed rhizomes in the laboratory. The temperature of rhizomes increased several times following the shortest MV time. The average temperature of unexposed (control) rhizomes was 20.°C, while the rhizomes MV treated for 2.5 min increased almost 3.5 times (Table 5). The temperature of the rhizomes MV for subsequent periods also increased, compared to the 2.5-min MV time, but not so intensely: by 9%, 15%, and 27% for the 5.0, 7.5, and 10.0-min MV times.

Table 5

In the laboratory experiment, the temperature analysis results from the thermograms of the bare knotweed rhizomes treated with microwaves at times 0–10.0 min. The measurements were taken directly after the radiation.

Time of radiation (mins.)	0.0	2.5	5.0	7.5	10.0
Mean (°C)	20.3	69.4	75.8	79.5	87.9
Std. Dev. (°C)	0.1	13.0	7.4	10.2	10.6
Maximum (°C)	20.8	101.7	90.1	108.8	116.0
Minimum (°C)	19.9	40.2	49.1	42.8	45.8

Following the MV treatments, the changes in rhizomes temperature fit into a polynomial curve that presented a regression equation with a high determination index of 0.92 (Fig. 8).

As a result of the rhizomes MV treatment and the accompanying temperature rises, rhizomes' weight loss was observed, most probably due to water evaporation. The highest weight loss was noted after MV for 10.0 min, by 29%, compared to the non-MV control. In addition, the following losses in rhizome mass following MV were recorded: 21% (7.5 mins), 13% (5.0 mins), and 6% (2.5 mins), compared to non-MV control rhizomes.

The resveratrol content in control (non-MV) rhizomes averaged 164.1 µg/g (Fig. 9). The MV treatment for 2.5 min resulted in a significant 63% decrease in resveratrol content. In the rhizomes MV treated for 5.0 or 10.0 min, there was a further significant decrease in resveratrol content by an average of 94% compared to the control and 83% compared to the rhizomes MV-treated for 2.5 min.

Discussion

The results presented in the paper concerning the control of Japanese knotweed using microwave radiation (MV) at 2450 MHz and a power of 800 watts are pioneering. It was found that the longer the exposure time of cut knotweed plants to MV, the lower their regeneration. It was effective to use longer time ranges of MV, i.e., 20 or 25 min, which generated the plant and soil surface temperature of about 85–90°C. Consequently, the plants did not re-grow 56 days after the MV was applied. Such a long time needed to destroy knotweed plants is most likely due to the ability of plants to regenerate from a deep system of rhizomes and roots that reach up to two meters deep into the soil ³⁵. So far, the MV control technique has been used against another invasive species - Sosnowski hogweed (*Heracleum Sosnowskyi* Manden.), also after the prior cutting of aboveground biomass and exposure of plants to MV with a frequency of 2.45 GHz and a power density of 30.0 kW/m² emitted by the tubular antenna ²⁸. In the case of Sosnowski hogweed, 10 min of MV was enough to destroy the plants and prevent their further regrowth effectively. Previous research on the impact of MV on plants shows that different species show different sensitivity to MV frequency, exposure time, and intensity of MV power. For example, a

stimulating effect was observed with low-power MV irradiation of sequoia plants (*Sequoia Endl.*) *in vitro* culture³⁶. However, in the case of alfalfa (*Medicago sativa* L.), no differences in growth and biomass accumulation were found between plants MV at a frequency of 2.45 GHz and a power density of 0.5–1.2 mW/cm² and plants that were not subjected to such treatment³⁷. Senavirathna and Asaeda³⁸ also showed no effect of MV on the growth parameters of *Myriophyllum aquaticum*, which was exposed to horizontally and vertically polarized continuous MV with a frequency of 2 GHz and a power density of 1.8 W/m. Significant morphological modifications of the common bean (*Phaseolus vulgaris* L.) were caused by long-term MV with a frequency of 915 MHz and a maximum power density of 10 mW/m²³⁹.

Apart from biological factors, environmental factors, particularly soil moisture, are important factors that guarantee the high effectiveness of destroying MV-treated plants. A study by⁴⁰ shows that the higher the soil moisture when treating plants, the higher the effectiveness of the MV because high soil moisture guarantees a high degree of moisture in plant tissues and a significant increase in their temperature. In our research, the shoots' temperature and the substrate's surrounding surface increased to 85–90°C after 20.0–25.0 min of MV exposure. At such a high temperature, irreversible biochemical changes occur in plant tissues, i.e., denaturation of proteins and changes in their composition, changes in the composition of carbohydrates, and significant changes in the metabolism of amino acids and the glyoxylate and galactose pathways, which was observed after the use of MV in tissues *Sosnowski hogweed*²⁸.

The ambiguous impact of MV on the soil environment^{25,29} contributed to the study of its ecotoxicity to soil organisms. Our research used a battery of biotests to assess the ecological risk associated with the microwave method. The organisms used belonged to different taxonomic groups, representing different links in the trophic chain (producers, consumers, decomposers). They were characterized by different sensitivity to the conditions in the tested soil. It is worth noting that each species shows different sensitivity, and none is sensitive to all factors in the sample. In addition, biotests assess the total toxicity of all factors (and substances) present in the tested sample, often acting synergistically or antagonistically^{41,42}. Such an approach in research assessing the risk associated with using MV is crucial⁴³. In this study, in the context of the environmental effects of the presented MV method, their negligible side effect on the ecotoxicity of soils should be considered an important and promising result. According to the hazard classification, considering the responses of all soil organisms, soils MV treated for 5.0 min were classified as samples that did not threaten the environment. The remaining soils were classified as class II, which means that they are low-toxic and also do not pose a threat to the environment.

Our studies showed a significant loss of resveratrol content in knotweed rhizomes directly MV treated in the laboratory, already in the minimum time range of 2.5 min. The content of this compound in the tissues was reduced by more than 60% compared to the control rhizomes. Plants MV treated for 5.0 or 10.0 min had 90% lower resveratrol concentration than the control. At the same time, there were no significant differences in the content of this compound in rhizomes irradiated for 5.0 and 10.0 min. The high temperature during the MV treatment most likely caused the knotweed cells and tissues to be

degraded. That could have impacted, among others, the amount of resveratrol. According to ⁴⁴, at high temperatures, there is hyper fluidity of plant tissue membranes, leading to the outflow of ions, further reducing the activity of many enzymes. A significant reduction in resveratrol in Japanese knotweed rhizomes can also be explained by the degradation of polyphenolic compounds under the influence of high temperatures. However, the lack of significant differentiation of the content of this compound with longer MV exposure was probably the result of lower tissue moisture. The lower the tissue's water content, the lower the degradation of polyphenols. Water molecules quickly absorb MV energy and generate heat, so water molecules move faster than other solvents ⁴⁵. In studies by ⁴⁶, the exposure to MV of 500 W for 8 min and a temperature of 60°C increased the resveratrol and gallic acid degradation with an increase in the percentage of water. Resveratrol and gallic acid were oxidized by free radicals formed by weak hydrogen bond breaks of molecular dipoles with increasing solvent temperature. In turn, the higher the percentage of water, the faster the solvent molecules moved. Physical stresses, such as UV-C radiation, may increase resveratrol content in knotweed tissues, as observed in Japanese knotweed by ⁴⁷. Under the influence of UV-C radiation for 10 minutes, followed by incubation in the dark, the content of resveratrol increased by 2.6-fold and 1.6-fold, respectively, in UV-treated leaves. Differential expression analysis showed that Japanese knotweed strongly induced genes directly involved in resveratrol synthesis under UV-C.

Summing up, the research showed that it is reasonable to use MV for at least 20–25 min to control Japanese knotweed. Applying MV to Japanese knotweed for shorter times is not effective, as the plants regenerate from underground rhizomes within a period of up to one month. MV does not negatively affect the ecotoxicity of the tested soil. The use of MV significantly reduces the content of resveratrol in knotweed rhizomes.

Declarations

Data availability statement: The datasets used during the current study are available from the corresponding or first author on reasonable request.

Author contributions: K.S., B.G., S.T. and A.S. designed the experiments; K.S., S.T. and B.G. performed the field research; A.B. performed the ecotoxicity tests; FM and DP analyzed resveratrol in rhizomes, B.G., A.S., A.B. and D.P. analysed data and wrote the manuscript, S.T. and F.M. reviewed the manuscript.

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Figures

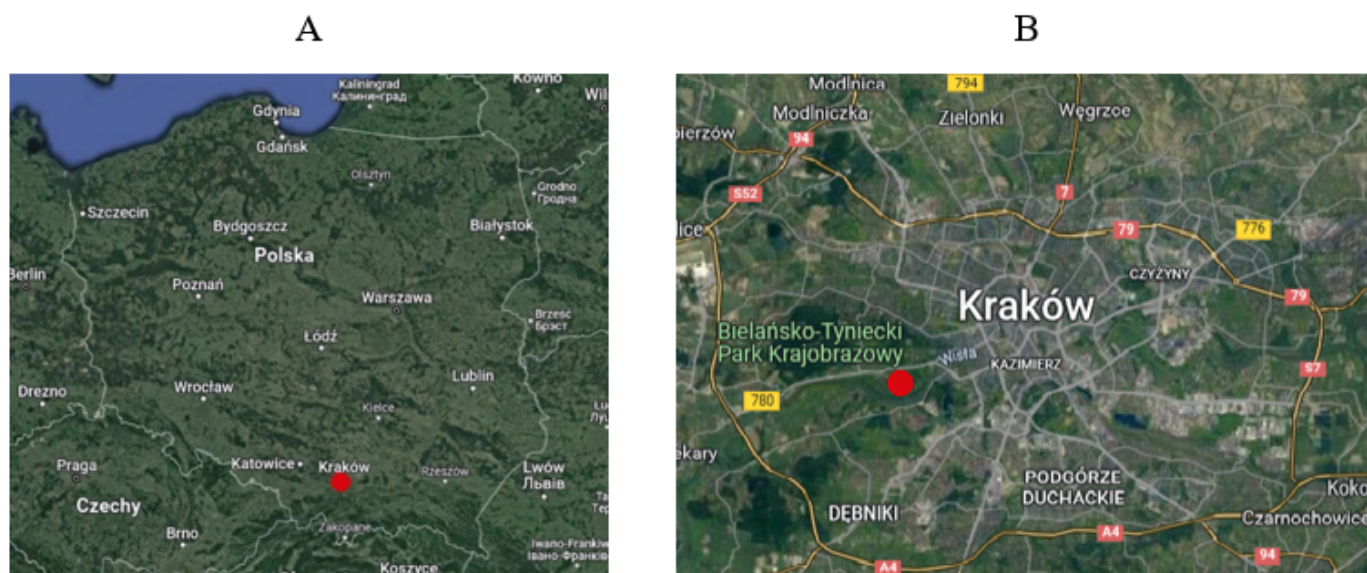


Figure 1

Location of study site in Poland (A) and Kraków (B).

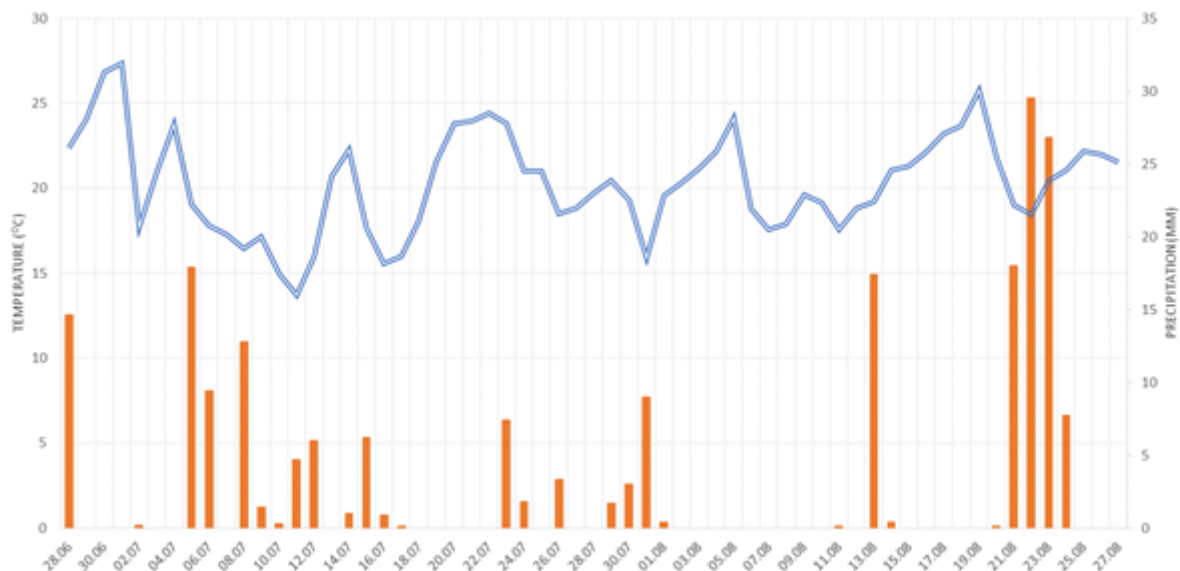


Figure 2

The average daily air temperatures in C (blue line) and daily precipitation sum in mm (orange bars) from June 28 to August 27 2023, from the meteorological station in Balice.

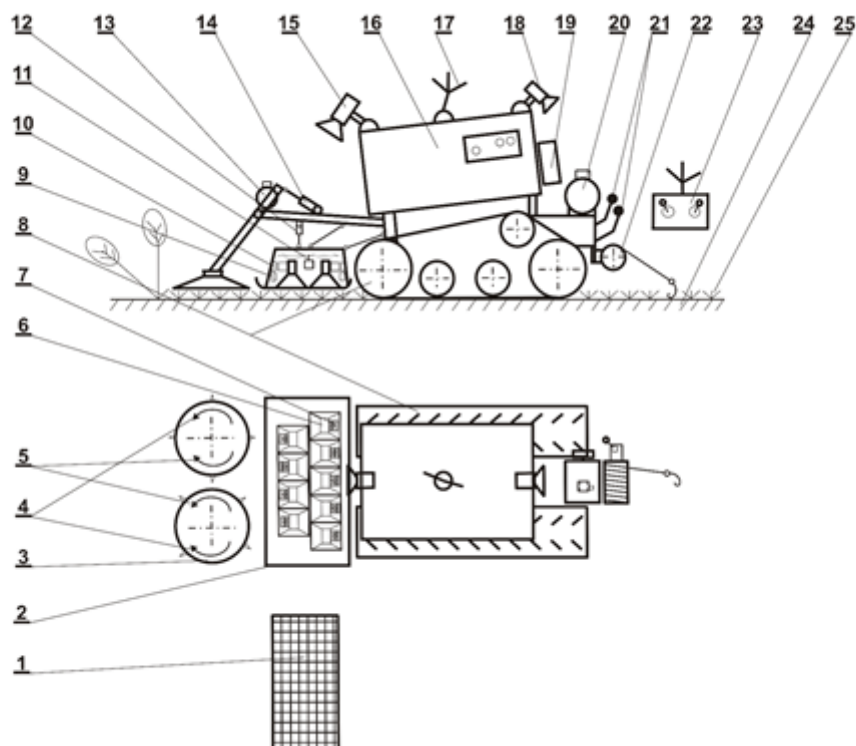


Figure 3

Scheme of a self-propelled HOGWEED device for microwave destruction of invasive plants. 1 - air filter, 2 - microwave emitter, 3 - rotary mower, 4-5 - direction of rotation depending on the plant development phase,

6 - microwave antenna, 7 - magnetron, 8 - track system, 9 - capacitor, 10 - transformer, 11 - cooling fan, 12 - microwave emitter actuator, 13 - mower drive motor, 14 - mower actuator, 15 - camera 1, 16 - power generator, 17 - remote control antenna, 18 - camera 2, 19 - control system, 20 - drive electric motor, 21 - gear levers, 22 - cable winch, 23 - remote control system, 24 - ground, 25 - plants.

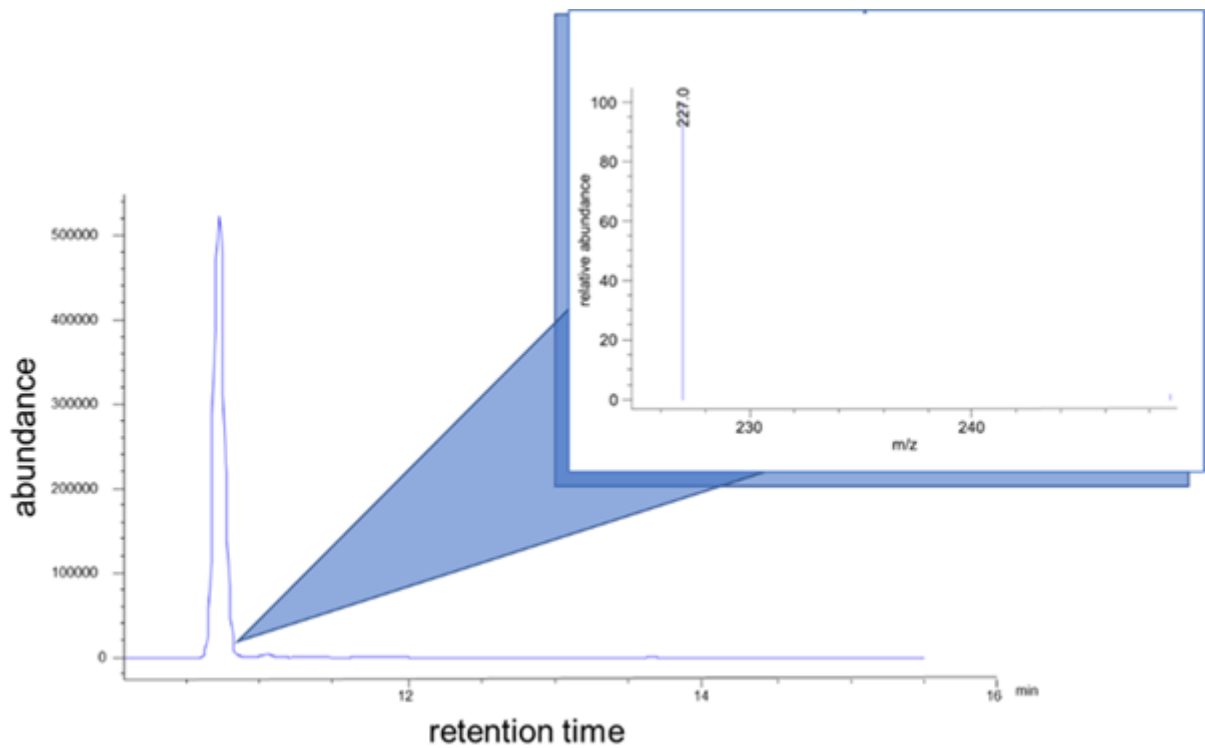


Figure 4

Extracted ion chromatogram (EIC) 227 with a relative mass spectrum of the trans-resveratrol peak.

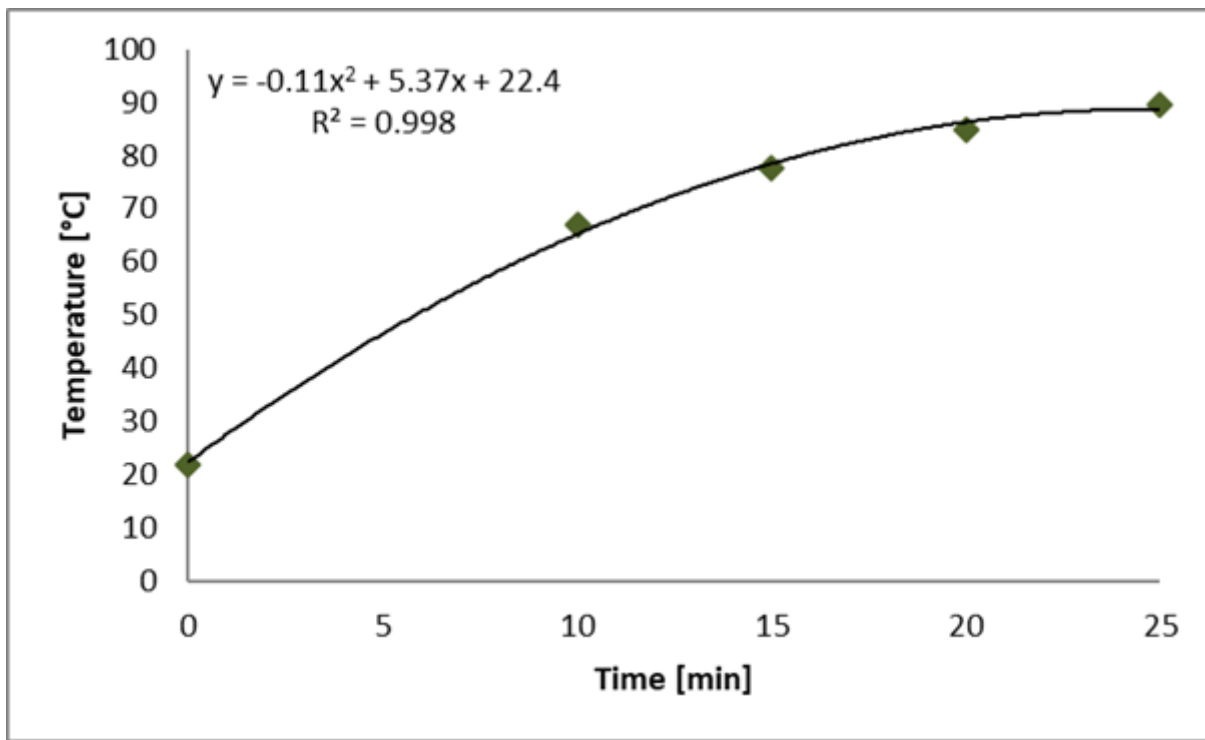


Figure 5

The curve and regression equation for the temperature of cut knotweed shoots and surrounding soil following radiation with microwaves for 5.0, 7.5, 10.0, 15.0, 20.0, and 25.0 min in field conditions.

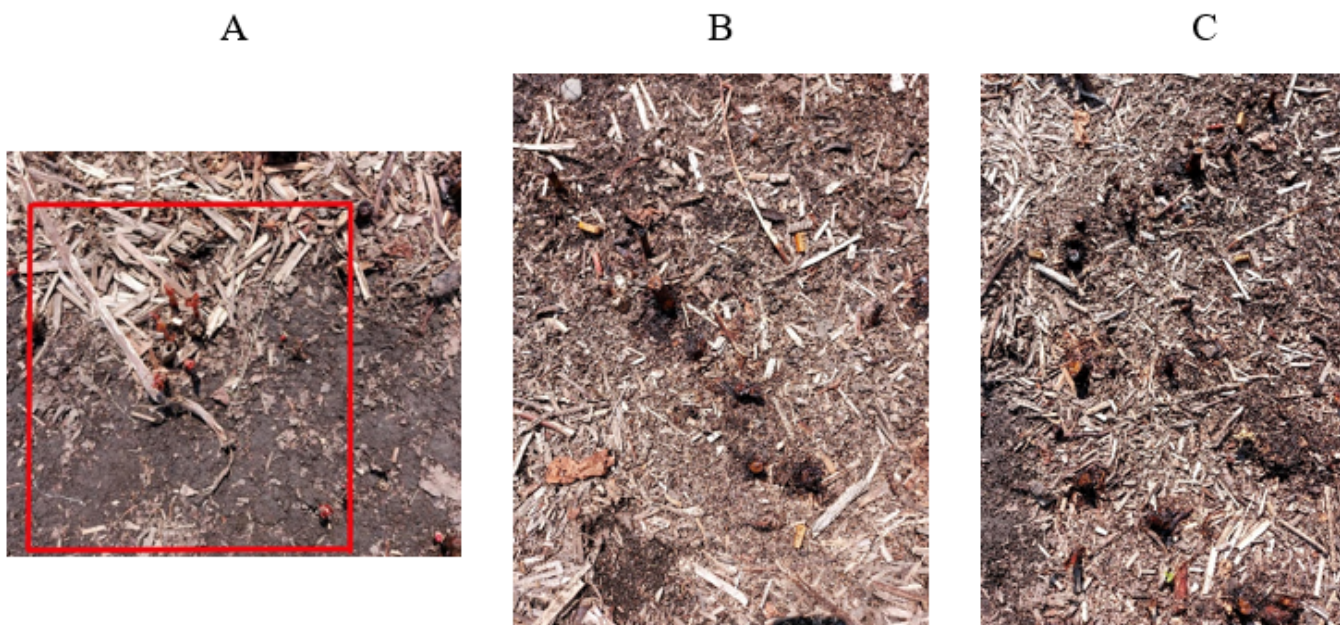


Figure 6

Japanese knotweed 10 days after microwave radiation. Radiation times: A - 5.0 mins., B - 20.0 mins., C - 25.0 mins. In Photo 4 A, red square indicate new, re-growing shoots of Japanese knotweed.

A



B



Figure 7

Japanese knotweed at 56 days after microwave radiation. Radiation times: A - 20.0 mins., B - 25.0 mins. Empty spots indicate that no new shoots appeared.

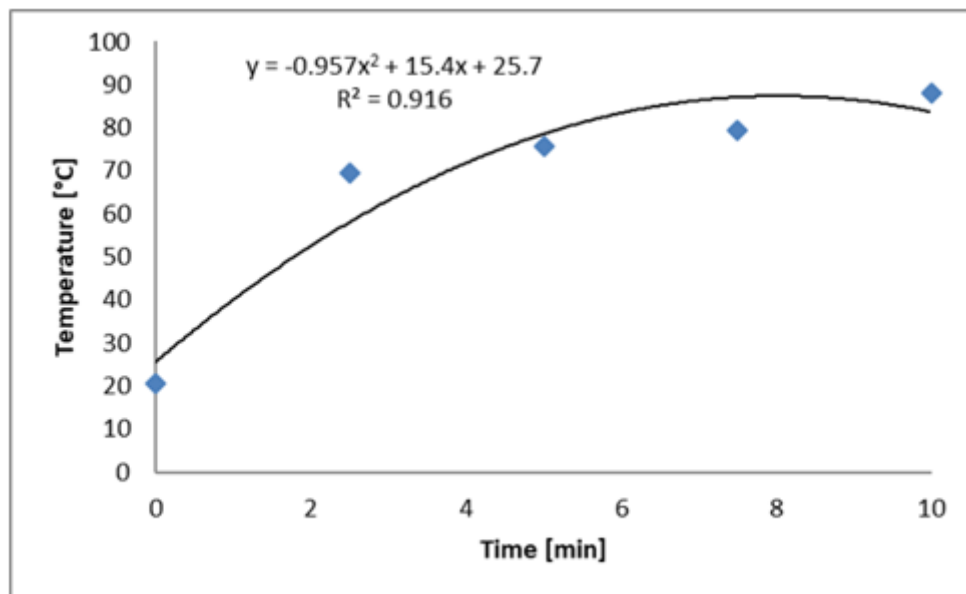


Figure 8

Regression curve for an air temperature of knotweed rhizomes microwaved in ambient conditions for 2.5, 5.0, 7.5, and 10.0 minutes.

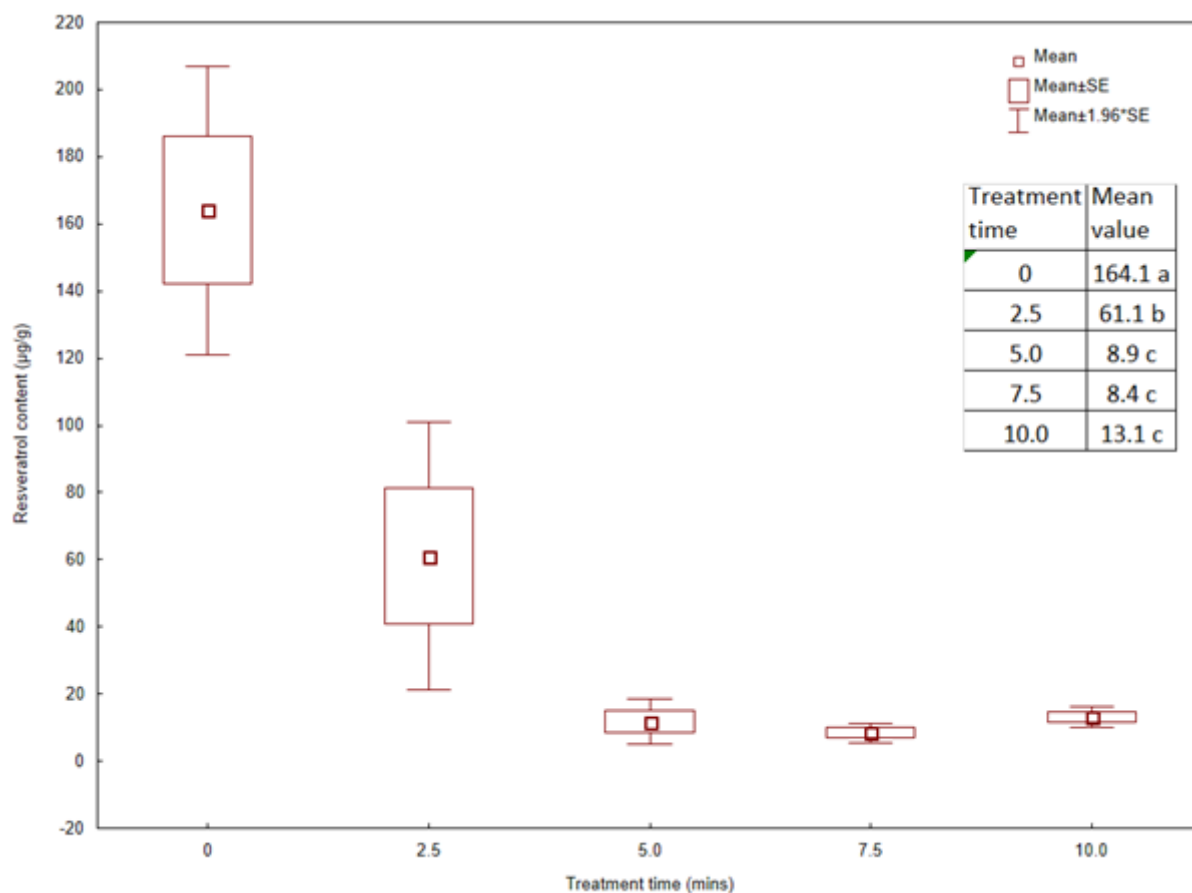


Figure 9

Resveratrol content in *Reynoutria japonica* extracts ($\mu\text{g/g}$) by HPLC-DAD-ESI-MS (n=6). Treatment time refers to the time-length of microwave radiation in min. Means were compared by one-way ANOVA, $F=24.3$; $p<0.001$. Different letters by the mean values in the table refer to significant differences according to the LSD test.