

Effect of Aqueous Extracts of Peppermint (*Mentha × piperita* L.) on the Germination and the Growth of Selected Vegetable and Cereal Seeds

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Abstract

Peppermint (*Mentha × piperita* L.) is grown in the gardens for its attractive look and multilateral use in the kitchen and medicine. The grown plant spreads very easily producing stolons and could effectively compete with other plant species. For the purpose of this study, the effect of aqueous extracts from dry peppermint leaves was examined on the germination, growth, fresh and dry weight, and electrolytes leakage of 10 species commonly grown in different regions of the world: *Beta vulgaris* L., *Cucumis sativus* L., *Lactuca sativa* L., *Lupinus luteus* L., *Phaseolus vulgaris* L., *Raphanus sativus* L. var. *radicula* Pers., *Sinapis alba* L., *Lycopersicon esculentum* Mill., *Triticum aestivum* L., and *Zea mays* L. Several concentrations of the aqueous extracts from peppermint leaves were prepared: 1, 3, 5, 10, and 15%. The seeds germination decreased with increasing concentration of aqueous extracts from peppermint leaves. The seed germination was completely inhibited on Petri dishes with 10 and 15% extracts. The tomato seeds were the most sensitive, they germinated on the distilled water and 1% extract only. The most resistant were the bean seeds that had the highest germination capacity. In general, the growth of seedlings of analyzed species was inhibited by the aqueous extracts from peppermint leaves. However, it was noticed the stimulating effect on the seedling growth of bean and maize on the 1% extract. The aqueous extracts from peppermint leaves caused the electrolyte leakage in all examined species and it raised with increasing extract concentration at the concentration higher than 3%.

Keywords: allelopathy; aqueous extracts; cereals; germination capacity; peppermint; vegetables

Introduction

One of the obstacles to the development of organic farming is competition between plants being the consequence of interaction known as allelopathy (Kieć and Wiczorek, 2009; Sekutowski, 2010). The significant part of studies on this phenomenon relates to the weed - crop relationship, which illustrates synergistic or antagonistic interactions (de Albuquerque *et al.*, 2011). These interactions are associated with the production of specific chemical compounds in plants called allelopathins (Jezińska-Domaradzka, 2007; Jezińska-Domaradzka and Kuźniewski, 2007). These compounds belong to the group

of plant secondary metabolites. They are released from plants into the environment in many ways, e.g., as volatile substances from aboveground parts, leaching from the leaves, excretion from the roots or during the decomposition. In general, leaves are believed to produce and contain the highest level of allelopathins. The lowest level of the secondary metabolites was found in seeds and fruits (Rice, 1984). Allelochemical substances act through direct interference with the physiological functions of 'receiver', such as seed germination, root growth, shoot growth, stem growth, symbiotic effectiveness or act indirectly through additive or synergistic impact along with pathological infections, insect injury and/or environmental stress (Dahiya *et al.*, 2017).

Numerous experiments demonstrated the inhibitory effect of extracts made from native weed species on germination and early growth of cereals (Ciesielska and Borkowska, 2010; Kraska and Kwiecinska-Poppe, 2007; Marczevska-Kolasa et al., 2017), germination of legumes, e.g. beans (*Phaseolus vulgaris* L.), and other vegetables, as pumpkin (*Cucurbita pepo* L.) or tomato (*Lycopersicon esculentum* Mill.) (Kadioglu et al., 2005). Allelopathic effects of extracts were also documented on cultivated plants, e.g., *Sorghum vulgare* L., buckwheat (*Fagopyrum esculentum* Moench), common sunflower (*Helianthus annuus* L.) (Macias et al., 2002; Tsuzuki and Dong, 2003), lemon balm (*Melissa officinalis* L.), white mulberry (*Morus alba* L.) (Możdzen and Repka, 2014), lavender (*Lavandula angustifolia* Mill.) (Teaca et al., 2008), or peppermint (*Mentha × piperita* L.) (Skrzypek et al., 2015a, b, 2016). However, the allelopathic potential of many of these crop plants is still not sufficiently known, such an example is peppermint.

Peppermint (*Mentha × piperita* L., Lamiaceae) is a spontaneous hybrid of *M. aquatica* L. and *M. spicata* L. em. L. It probably emerged in England and was cultivated from the 18th century (Szweykowska and Szweykowski, 2003). Currently, mint is planted all over the world and used as a flavoring in the kitchen, food industry, as well as a source of essential oils widely used in pharmacy (especially aromatherapy) and cosmetology (Rita and Animesh, 2011; Bufalo et al., 2015). The most frequently, leaves (*Folium Menthae piperitae*) and mint herb (*Herba Menthae piperitae*) are used due to the highest content of biologically active substances in these organs. Its main chemical compounds are essential oils (mainly peppermint oil containing, among others, menthol), flavonoids, glycosides, acids (e.g. ascorbic acid) and mineral compounds (Czikow and Laptiew, 1983; Mystkowska et al., 2016).

The aim of the present study was to examine the allelopathic influence of *Mentha × piperita* extracts on seed germination (i), growth (ii), biomass (iii), and electrolyte leakage (iv) of 10 vegetable and cereals taxa commonly grown in different regions of the world: common beetroot *Beta vulgaris* L., cucumber *Cucumis sativus* L., lettuce *Lactuca sativa* L., yellow lupine *Lupinus luteus* L., common bean *Phaseolus vulgaris* L., radish *Raphanus sativus* L. var. *radicula* Pers., light mustard *Sinapis alba* L., common tomato *Lycopersicon esculentum* Mill., common wheat *Triticum aestivum* L., and common maize *Zea mays* L.

Materials and Methods

Plant material

Seeds of the studied vegetables (*Beta vulgaris*, *Cucumis sativus*, *Lactuca sativa*, *Lupinus luteus*, *Phaseolus vulgaris*, *Raphanus sativus* var. *radicula*, *Sinapis alba*, *Lycopersicon esculentum*), and cereal species (*Triticum aestivum*, *Zea mays*) were obtained from the gardening store Polan sp. z.o.o (Poland), and dry peppermint leaves (*Folium Menthae piperitae*) from Flos company (Morsko, Poland).

Prepared extract

Aqueous extracts of peppermint leaves were made at concentrations of 1, 3, 5, 10, and 15%, weighing the appropriate amount of dry leaves per 100 ml of distilled

water. After 24 h extraction, the extracts were strained using a vacuum pump on a Büchner funnel with Whatman filter paper. The extracts prepared in this way were stored at a temperature of 8 °C (in the refrigerator) for the duration of the entire experiment.

Seed germination

Seeds of vegetables and cereals, washed in running and distilled water, were placed on sterile Petri dishes lined with a triple layer of filter paper (105 °C /2 h). It was used 100 morphologically identical seeds of every species per one Petri dish per every extract and control (saturated with distilled water). The plant material was placed in the dark at a constant temperature (25 °C) and humidity condition (90%). The number of germinated seeds was counted every 24 h over 7 days. As germinated seeds were considered ones with sprouts of length equal to the 2 mm. Total germination was checked according to the formula $GT = (Nt \times 100) / N$, where GT – total germination, Nt – proportion of germinated seeds at each treatment for the last time, N – number of seeds used in the bioassay.

Length of seedlings, fresh and dry weight, electrolyte leakage

After 7 days, using the caliper (Topex 31C615, Poland), the length of the seedlings was measured and next, the fresh weight (Medicat 1600 C, Poland) and dry weight (dryer Termaks 8430, Poland) were determined. The degree of the destabilization of cell membranes was determined by measuring the electrolytes leakage using the method described in Skrzypek et al. (2015a).

Statistical analysis

The experiment was repeated five times. The differences between the test and control samples were determined by Duncan's parametric test, with $p \leq 0.05$. The calculations were performed using Statistica for Windows 13.0 software.

Results

Seed germination

The germination capacity of the tested seeds decreased with the increase of the concentration of aqueous extracts of *Mentha × piperita* leaves. In most species, total inhibition of seed germination was observed on Petri dishes saturated with 15% extract. Similar germination model was observed for *Beta vulgaris*, *Raphanus sativus* var. *radicula*, *Sinapis alba*: the number of germinating seeds decreased rapidly already at 1% of the extract. The most sensitive were *Lycopersicon esculentum* seeds, which germinated only on the control sample and on Petri dishes saturated with 1% extracts of mint leaves. Total inhibition of seed germination was found for *Lactuca sativa* on plates with extracts at 10% and 15%. *Phaseolus vulgaris* seeds were the most resistant and they showed the highest germination capacity (with extracts from 1 to 10% more than 90% of seeds germinated, the germination was totally inhibited at 15% concentration only). *Zea mays* and *Cucumis sativus* were also resistant to extracts. They responded to the extracts by reducing germination, but even at the highest 15% concentration of mint leaves, more than 30% of their seeds germinated (Fig. 1).

Length of seedlings

P. vulgaris and *Z. mays* were the only species that showed the stimulating effect of the mint extract on the seedlings growth: it was observed with 1% aqueous extracts of mint leaves. *L. esculentum* seedlings were the most sensitive: already at 3% of the extract, the elongation growth was completely inhibited. In the case of *Beta vulgaris*, *Raphanus sativus* var. *radicula*, and *Sinapis alba* extracts of mint leaves delayed the elongation irrespective of extract concentration. The smallest inhibiting effect was documented for *P. vulgaris* seedlings; only the 15% extract inhibited their growth (Table 1).

Fresh and dry weight

Analyzing the values of fresh and dry weight of seedlings, it was found that mint extracts at concentrations of 1%, 3%, and 5% only slightly inhibited the mass growth. The most inhibiting effect was connected with the highest concentrated extracts – 10 and 15%, which clearly limited the growth of seedlings mass, relative to the control (Table 2).

Electrolyte leakage

With increasing concentrations of mint extracts, an increase in electrolyte flow through the cell membranes of seedlings was observed, as compared to the control sample. Extracts with concentrations of 1 and 3% did not cause

statistically significant changes in the destabilization of cell membranes. *L. esculentum* seedlings were the only exception, in whom the extract with a concentration of 3% caused a 4-fold increase in electrolyte leakage from the cells, as compared to the control. Extracts of 5% and higher affected seedlings of all studied species, an increase of electrolyte leakage was demonstrated. The largest over 90% destabilization of the ionic economy in the cells of all tested seedlings was found at the 15% concentration of the extract (Table 3).

Discussion

In the soil environment, the allelopathic effect depends on the concentration of active compounds in the soil rhizosphere and their interaction with soil contents, organic matter and factors that alter the physicochemical and biotic properties of soils (Blum and Shafer, 1988). Increased evapotranspiration and lower soil moisture may result in reduced phytotoxicity of allelopathic compounds in the soil substratum. The level of toxins in the soil depends also on the soil type, its aeration, temperature and microbial activity.

For example, loamy soils are poorly permeable and toxins do not diffuse easily in them. In contrast, sandy soils are well-permeable and have a tendency for maximum leaching and easy diffusing, including allelochemicals. Plants sensitive to toxins may be more vulnerable when planted in heavy soils (Dahiya et al., 2017). Taking into consideration the relationship between phytotoxins and soil properties, it could be important to control the presence of plant species that coexist and compete with crop plants.

The germination of seeds is influenced by many external factors, including water availability, soil pH, temperature or light. The internal factors also play a key role, such as the state of the embryo, the level of hormones and enzymes activated in the semen (Koger et al., 2004). Appropriate germination of seeds results in the appearance of hypocotyl and root. The root is the first part of the seedling, which under certain conditions is responsible for chemical changes that have a subsequent effect on the growth of the hypocotyl, as a result of the absorption of water and minerals. If the seedling root is in the suitable conditions for growth, it gets the substances needed for the development. Otherwise, the surrounding environment causes disturbances in the absorption capacity of water and

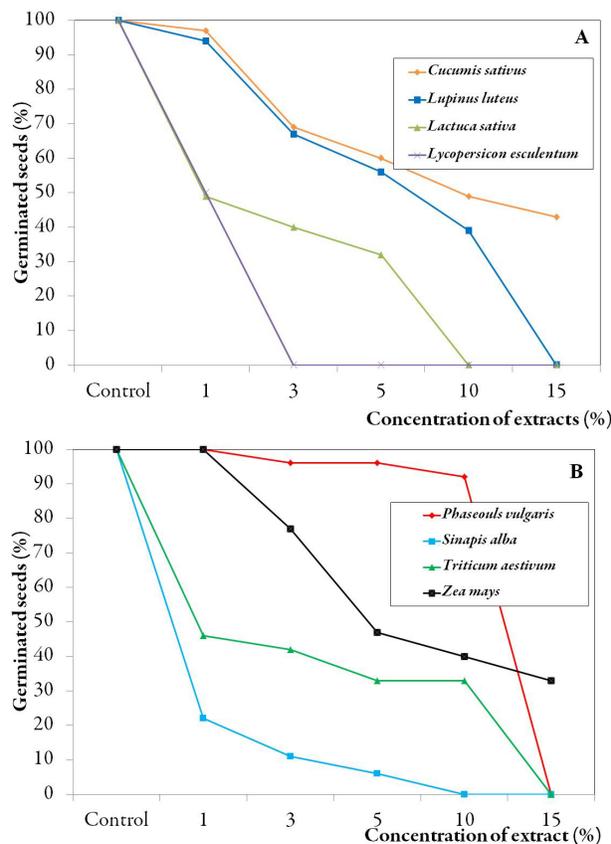


Fig. 1. The germination capacity of seeds of selected cultivated plants species on substrates with aqueous extracts of peppermint leaves (*Mentha x piperita* L.), at concentrations of 1, 3, 5, 10 and 15%

Table 1. The length of seedlings of the studied cultivated species, which germinated on substrates with aqueous extracts of peppermint leaves (*Mentha x piperita* L.), at concentrations of 1, 3, 5, 10 and 15%

No.	Seedlings	Concentration extract (%)					
		0	1	3	5	10	15
1.	<i>Beta vulgaris</i>	3.4	2.1	1.5*	0.9*	0.2*	0.0*
2.	<i>Cucumis sativus</i>	3.6	3.4	2.4	2.0	1.4*	0.0*
3.	<i>Lactuca sativa</i>	1.4	0.6*	0.6*	0.5*	0.0*	0.0*
4.	<i>Lupinus luteus</i>	3.6	3.4	2.4	2.0*	1.4*	0.0*
5.	<i>Phaseolus vulgaris</i>	2.5	2.8	2.4	2.4	2.3	0.0*
6.	<i>Raphanus sativus</i> v. <i>radicula</i>	3.5	1.7*	1.0*	0.7*	0.5*	0.0*
7.	<i>Sinapis alba</i>	3.6	0.8*	0.4*	0.2*	0.0*	0.0*
8.	<i>Lycopersicon esculentum</i>	0.6	0.3	0.0*	0.0*	0.0*	0.0*
9.	<i>Triticum aestivum</i>	2.4	1.1	1.0	0.8*	0.8*	0.0*
10.	<i>Zea mays</i>	4.3	4.5	3.3	2.0*	1.7*	1.4*

Note: values (± SD) marked with (*) differ significantly according to Duncan's test at p < 0.05. 0 - control

Table 2. The fresh (A) and dry (B) weight (g) of the studied cultivated species seedlings, which germinated on substrates with aqueous extracts of peppermint leaves (*Mentha × piperita* L.), at concentrations of 1, 3, 5, 10 and 15%

No.	Seedlings	Concentration of extract (%)											
		Control		1		3		5		10		15	
		A	B	A	B	A	B	A	B	A	B	A	B
1.	<i>Beta vulgaris</i>	0.54	0.25	0.41	0.16	0.30	0.11	0.29	0.10	0.15*	0.06*	0.12*	0.04*
2.	<i>Cucumis sativus</i>	1.48	0.65	1.42	0.63	1.44	0.66	1.21	0.67	1.12	0.69	1.11*	0.69
3.	<i>Lactuca sativa</i>	0.12	0.02	0.09	0.03	0.07	0.03	0.08	0.02	0.05*	0.02	0.06*	0.02
4.	<i>Lupinus luteus</i>	10.58	2.63	8.70	2.82	8.71	3.03*	8.51	2.81	8.31	3.00	6.70*	3.00
5.	<i>Phaseolus vulgaris</i>	14.08	4.18	10.29	3.29	11.53	3.71	11.45	3.85	9.30*	3.32	8.46*	4.26
6.	<i>Raphanus sativus</i> var. <i>radicula</i>	0.81	0.28	0.81	0.24	0.65	0.25	0.58	0.27	0.53*	0.26	0.49*	0.26
7.	<i>Sinapis alba</i>	0.63	0.15	0.39	0.14	0.42	0.18	0.41	0.18	0.35*	0.17	0.38*	0.19
8.	<i>Lycopersicon esculentum</i>	0.19	0.06	0.22	0.07	0.17	0.06	0.18	0.07	0.20	0.07	0.19	0.07
9.	<i>Triticum aestivum</i>	2.75	0.96	1.64	0.91	1.59	0.92	1.60	0.96	1.56	0.96	1.64	1.03
10.	<i>Zea mays</i>	9.27	3.32	8.50	3.26	7.61	3.33	8.67	3.28	7.35	3.29	5.89*	3.50

Note: values (\pm SD) marked (*) within the line differ significantly according to Duncan's test, at $p < 0.05$

Table 3. The percentage of electrolyte leakage from the cell membranes of the studied cultivated species seedlings, which germinated on substrates with aqueous extracts of peppermint leaves (*Mentha × piperita* L.), at concentrations of 1, 3, 5, 10 and 15%

No.	Seedlings	Concentration of extract (%)					
		Control	1	3	5	10	15
		1.	<i>Beta vulgaris</i>	15.21	29.10	41.45	56.99*
2.	<i>Cucumis sativus</i>	29.39	35.40	51.89	60.29*	71.56*	79.29*
3.	<i>Lactuca sativa</i>	19.12	39.18	42.79	44.89*	70.47*	93.99*
4.	<i>Lupinus luteus</i>	30.11	32.00	40.56	50.28*	75.36*	98.39*
5.	<i>Phaseolus vulgaris</i>	30.13	40.01	48.25	59.89*	67.12*	75.89*
6.	<i>Raphanus sativus</i> var. <i>radicula</i>	36.15	47.19	59.21	67.89*	82.26*	94.69*
7.	<i>Sinapis alba</i>	24.25	39.23	56.06	70.59*	88.89*	97.29*
8.	<i>Lycopersicon esculentum</i>	12.19	29.39	55.89	76.36*	80.43*	94.89*
9.	<i>Triticum aestivum</i>	22.89	36.00	39.02	49.29*	56.49*	79.26*
10.	<i>Zea mays</i>	36.23	35.09	49.25	64.89*	79.56*	85.27*

Note: values (\pm SD) marked (*) within the line differ significantly according to Duncan's test, at $p < 0.05$

minerals, leading to the abnormal growth of the entire seedling (Pula *et al.*, 2016). During this experiment, it was found that aqueous extracts of *Mentha × piperita* leaves, regardless of concentration, clearly limited seed germination. In relation to the control, *Phaseolus vulgaris* seeds showed the highest germination capacity, they were the most resistant to the allelopathins of mint. On the other hand, the most sensitive were seeds of *Lycopersicon esculentum*, which germinated only on the substrate with 1% mint extracts and in the control (Fig. 1). Chemical interactions between weeds and crops revealed that substances released from weed remnants affect the germination of neighboring crops (Ridenour and Callaway, 2001). At the same time, the germination efficiency of seeds depends on their size and weight. Large-scale seeds contain more nutrients than small seeds. Therefore, large seeds are capable of faster germination and growth (Nik *et al.*, 2011; Sadeghi *et al.*, 2011; Amin and Brinis, 2013). In the case of seedling elongation, 1% extracts from *M. × piperita* stimulated the growth of seedlings of *P. vulgaris* and *Z. mays*, relative to the control. A similar tendency of growth was observed by Skrzypek *et al.* (2015b) in the study of the influence of aqueous extracts of mint leaves on the germination of common sunflower seeds. In this study, in the remaining vegetables and cereals, each of the mint extracts inhibited seedling elongation (Table 1).

Plant phytotoxicity may result from the synergistic reaction of the chemicals produced by them (Saharkhiz *et al.*, 2010). Often, it is the result of the simultaneous action of several compounds and usually affects those compounds whose chemical properties are divergent (Kaur *et al.*, 2010). In the experiment conducted here, the values of fresh and dry weight of seedlings significantly decreased with increasing concentrations of mint extracts, in comparison with the control. The smallest biomass increase was found in seedlings grown on Petri dishes saturated with extracts with the highest concentrations of allelopathins (Table 2). The chemical composition of secondary metabolites, such as allelopathins, depends largely on the plant taxon, its age, origin and environmental factors (Lawrence, 2007; Newerli-Guz, 2016). Biologically active substances produced by plants demonstrate various properties. Some of them are selective for other species, others are not. Some have the ability to inhibit completely and others to slow growth slightly (Dudai *et al.*, 1999; Campiglia *et al.*, 2007; Ayoub *et al.*, 2018). An important role is also played by the plant growth hormones indoleacetic acid (IAA) and gibberellins (GA) that regulate cell extension in plants. IAA is present in both active and inactive forms and is inactivated by IAA-oxidase. IAA-oxidase is inhibited by various allelochemicals (Rice, 1984), other inhibitors block GA and induce extension growth.

It has been demonstrated that the cell membranes of the examined species were destabilized with increasing concentration of mint extracts, relative to the control condition (Table 3). Such seedling reactions can be explained by the presence of biologically active compounds in "donor" plants – peppermint, which act on the components of the recipient cell membranes – analyzed cultivated species (Harper and Balke, 1981). These substances cause, among others, enzyme dysfunction and disturbances of mineral and water absorption, which result in changes in the growth and development of plants (Siyar et al., 2017). The ability to bind many natural plant products with membranes results not only in conformational changes in ion channels and proteins but also increases or decreases ion flux. It was documented that monoterpenoids have a toxic effect on seed germination (Rice, 1984) and interact at the cell level with cytosolic Ca^{2+} , probably through an intracellular Ca^{2+} store release and Ca^{2+} channel blocking (Takeuchi et al., 1994; Sanders and Bethke, 2000). According to Griffin et al. (2000), the relative hydrophobicity of most monoterpenoids can accumulate in biological membranes. Additionally, some allelopathins can indirectly contribute to cell death by producing reactive oxygen that causes lipid peroxidation and disintegrates membranes (Golisz et al., 2008; Mutlu et al., 2011).

In agro-ecosystems, weeds compete with arable crops for environmental resources, reducing yields and reducing the quality of crops. As a result, they contribute to financial losses (Lipinska, 2006). The dynamically developing agriculture is still looking for new ways to increase crop production efficiency. However, due to the increase of the environmental pollution, organic farming is becoming more and more popular. Knowledge of the mutual interactions between plant species is one of the important factors that could be conducive to the development of organic farming. It allows to avoid or to reduce the use of different types of pesticides and to obtain better, more fertile, healthy, and tasty crops. Well-chosen plant species will not only protect each other against pests but also better flowering and better use of nutrients contained in the soil. The potential for using allelopathins as natural herbicides or components allowing to reduce the use of artificial chemicals increases year by year (Bhadoria, 2011; Khalaj et al., 2013).

Although many issues related to allelopathy have not yet been resolved, the use of allelopathic compounds for weed suppression remains an "open path" to reduce the use of herbicides. Regardless of whether by the development of natural herbicides from isolated allelopathins or through the use of plants with allelopathic potential, allelopathy will most likely be a way of ensuring sustainable agricultural systems in the future.

Conclusions

Reduced germination [i], inhibition of seedling elongation [ii], reducing in weight [iii], and increase in electrolyte leakage [iv] in response to aqueous peppermint extracts confirm that the leaves of this species contain biologically active allelopathic compounds. Most likely, the high phytotoxic activity of mint extracts results from the presence of a high concentration of chemical substances well soluble in water, which, under suitable soil conditions, are readily available to plants growing in the closest vicinity.

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