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Mentha sylvestris: A Potential Allelopathic Medicinal Plant

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Abstract

Mentha sylvestris L. (Lamiaceae), a medicinal herb is well known for many pharmacological and toxicological properties, but very few are known yet about its allelopathic property. Hence, to investigate the allelopathy of *M. sylvestris* L., the aqueous methanol extracts of this plant at four different concentrations were examined on the seedling growth of cress (*Lepidum sativum* L.); lettuce (*Lactuca sativa* L.); alfalfa (*Medicago sativa* L.); rapeseed (*Brassica napus* L.); timothy (*Phleum pratense* L.); crabgrass (*Digitaria sanguinalis* L. scop.); barnyard grass (*Echinochloa crus-galli* L.) and Italian ryegrass (*Lolium multiflorum* Lam.). The hypocotyl/coleoptile growth of all test species was significantly inhibited by the plant extracts at concentrations ≥30 mg dry weight equivalent extract/mL except crabgrass, barnyard grass and Italian ryegrass. Conversely, the root growth of all but rapeseed, timothy and crabgrass was significantly inhibited at all the tested concentrations. The inhibitory activity of the extracts was concentration and species dependent, and the root growth of all test plant species was more sensitive to the extracts than the hypocotyls/coleoptiles. Considering I_{50} value the hypocotyl growth of lettuce and the root growth of timothy were the most sensitive to the extracts, whereas both the coleoptile and root growth of barnyard grass were the least sensitive. Results of this study suggest that *M. sylvestris* have allelopathic property and may possess allelochemicals. Therefore, this plant could be used as a potential candidate for isolation and identification of allelochemicals, which may promote the development of new natural herbicides for sustainable weed management strategies. © 2013 Friends Science Publishers

Keywords: Allelochemicals; Seedling growth; Weed management; Sustainable agriculture; Natural herbicide

Introduction

Weed management is an important and challenging task in sustainable agriculture as weeds cause substantial loss in crop yields and quality (Beveridge and Naylor, 1999; Davies and Welsh, 2002). On an average approximately 9.7% of total crop yields is lost every year by the effects of 1800 kind of weeds worldwide (Li *et al.*, 2003). Conventional agriculture is mainly relying on synthetic herbicides, perhaps due to their easy accessibility and more rapid out return. But over reliance on synthetic herbicides may cause herbicide-resistant weed biotypes (Vyvyan, 2002; Kabir *et al.*, 2010), degrade the soil and water quality (Pell *et al.*, 1998; Aktar *et al.*, 2009), affect other non-target organisms (Batish *et al.*, 2002) and also creates severe environmental pollution. These hurtful effects of synthetic herbicide are shifting the attention of researchers to develop environment friendly, cost-effective and relatively safe weed management technology based on natural products (Copping and Duke, 2007; Duke *et al.*, 2010).

The term ‘allelopathy’ is used to define the harmful or beneficial effects of one plant on another through the release of allelochemicals (Molísch, 1937). A number of processes have involved in releasing allelochemicals from the plants such as volatilization from the leaves (Oleszek, 1987), leaching from the above ground parts by precipitation (Overland, 1966), decomposition of leaf litter or sloughed root tissues (Hedge and Miller, 1990), microbial transformation from the decayed leaf, stem, leaf litter or roots (Chick and Kielbaso, 1998), exudates from the root (Tang and Young, 1982), pollen of some crop plants (Ortega *et al.*, 1988) or other processes in both natural and agricultural systems. Many efforts have been made by the researchers to isolate and identify those allelochemicals from different plant species, because of their herbicidal activity, and trying to apply them as a means for sustainable and eco-friendly weed management strategies (Khanh *et al.*, 2005; Farooq *et al.*, 2011). Fujii *et al.* (2003) reported that screening of allelopathic plants from medicinal plants is easier than that from other plants. It may be possible that medicinal plants contain more bioactive compounds than other plants. Isolation and characterization of unknown allelochemicals from allelopathic medicinal plants may provide the chemical basis for the development of new natural herbicides.

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Mentha is a small but one of the most important genus of Lamiaceae family comprising 19 species with 13 natural hybrids (Kumar *et al.*, 2011). Since the ancient time the genus has been well-known to the researchers due to its myriad of medicinal properties (Flückiger, 1879; Blumenthal, 1998). For example, the plants are used for the treatment of wounds, swollen glands, cough, cold, fever, asthma, indigestion, influenza, vomiting, gastro-intestinal disorder (Grieve, 1931; Zhao, 2013). *Mentha* is also well known for its essential oil menthol, a chemical constituent widely used in pharmaceutical, flavoring and cosmetic industries (Perveen *et al.*, 2010). Beside the medicinal properties, its oil has insecticidal, antibacterial, antifungal, anti-cancer activity (Worwood, 1993; Lee *et al.*, 2001; Bakkali *et al.*, 2008; Tyagi and Malik, 2010a, b). Among the species of the genus, *M. sylvestris* L. (synonyms of *M. spicata* L.), a fast growing, perennial, rhizomatous herb is native to north-eastern Africa, western Asia and south-eastern Europe (Wunderlin and Hansen, 2008; USDA, 2013). The plant is the most commonly cultivated and widely used as a constituent of various drugs as well as in aromatherapy (Khan *et al.*, 2011).

Nonetheless, allelopathic activity of *Mentha*, especially of *M. sylvestris*, has rarely been studied. Therefore, the current research was undertaken to investigate the allelopathic properties of *M. sylvestris* on different test plant species in both dicotyledonous and monocotyledonous group of plants. The research findings would be helpful to isolate and identify the allelochemicals and will foster the development of new natural herbicides for sustainable weed management strategies.

Materials and Methods

Plant Materials

Whole plants (leaves, stem and roots) of *M. sylvestris* were collected between March-April, 2012 from Bangladesh. After collection, plants were washed with tap water to remove the soil and other debris followed by sun drying. The dried plants were then kept in a refrigerator at 2ºC temperature until extraction.

Test Plant Species

Eight test plant species, cress (*Lepidum sativum* L.); lettuce (*Lactuca sativa* L.); alfalfa (*Medicago sativa* L.); rapeseed (*Brassica napus L.*); timothy (*Phleum pratense* L.); crabgrass (*Digitaria sanguinalis* L. scop.); barnyard grass (*Echinochloa crus-galli* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) were selected for the current research. Among these, the first four are dicotyledonous and the rest are monocotyledonous plants species. Cress, alfalfa, lettuce, rapeseed and timothy were chosen due to their known seedling growth, whereas crabgrass, barnyard grass and Italian ryegrass were chosen because they are most common weeds worldwide (Islam and Kato-Noguchi, 2013).

Extraction Procedure

Whole dried plants of *M. sylvestris* (30 g) were cut into small pieces and extracted with 300 mL of 70% (v/v) aqueous methanol for 48 h. After filtration using one layer of filter paper (No. 2; Advantec® Toyo Roshi Kaisha, Ltd., Tokyo, Japan), the residue was re-extracted with 300 mL methanol for 24 h and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40ºC.

Bioassay

An aliquot of the extract (final assay concentration was 3, 10, 30 and 100 mg dry weight [DW] equivalent extract/mL) was evaporated to dryness at 40ºC in *vacuo* by rotary evaporator, dissolved in methanol and added to a sheet of filter paper (No. 2) in a 28 mm Petri dish. The methanol was evaporated in a draft chamber then the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 (polyoxyethylene sorbitan monolaurate; Nacalai Tesque, Inc., Kyoto, Japan) which was used for surfactant and did not cause any toxic effects. Ten seeds of cress, lettuce, alfalfa, rapeseed or 10 germinated seeds of timothy (germinated in the darkness at 25ºC for 72 h after overnight soaking), crabgrass (germinated in the darkness at 25ºC for 120 h after 24 h incubation in the light chamber at 25ºC), barnyard grass or Italian ryegrass (germinated in the darkness at 25ºC for 24 h after overnight soaking) were arranged on the filter paper in Petri-dishes. The hypocotyl/coleoptile and root lengths of the seedlings were measured at 48 h after incubation in darkness at 25ºC. Control seeds were also sown on the filter paper moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 without plant extracts. The inhibition percentage was calculated using the following equation as prescribed by Islam and Kato-Noguchi (2012):

$$Inhibition(\%) = [1 - \frac{length\ with\ aqueous methanol extract}{length\ of\ control}] \times 100$$

Statistical Analysis

The bioassay was conducted in a completely randomized design with three replications and repeated twice. Experimental data were analyzed using predictive analytics software (PASW) statistics 17.0 (SPSS Inc., Chicago, Illinois, USA). All measured variables were subjected to two-way analysis of variance (ANOVA) and the differences between the means were compared using the least significant difference (LSD) at 5% level of probability. The concentration required for 50% growth inhibition (defined as I_{50}) of the test species in the assay was calculated from the regression equation of the concentration response curves, using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California, USA).

Results

The effects of aqueous methanol extracts of *M. sylvestris* on the hypocotyl/coleoptile and root growth of both dicotyledonous (cress, lettuce, alfalfa and rapeseed) and monocotyledonous (timothy, crabgrass, barnyard grass and Italian ryegrass) plant species were determined (Fig. 1 and 2). The effectiveness of the extracts was different among the test species, and the inhibitory activity of the extracts was concentration dependent. The two way analysis of variance (ANOVA) indicates that the effects of four different concentrations, test plant species and their interaction has a significant ($p<0.01$) effect on the seedling growth of both dicotyledonous and monocotyledonous test plant species (Table 1 and 2).

Effects of Plant Extracts on the Hypocotyl/Coleoptile Growth of Test Species

The hypocotyl growth (dicotyledonous plants) was more susceptible to the extracts of *M. sylvestris* compare to the coleoptiles (monocotyledonous plants). The hypocotyl growth of cress was significantly inhibited by all concentrations of the extracts, and that of lettuce, alfalfa and rapeseed was inhibited at concentrations ≥ 10, 30 and 30 mg DW equivalent extract/mL, respectively. Lower concentrations below the threshold of the growth inhibition showed stimulatory activity on the respective test plant species. At 100 mg DW equivalent extract/mL, the inhibition percent of the hypocotyl growth of cress, lettuce, alfalfa and rapeseed were 86, 90, 92 and 79, respectively (Fig. 1). On the other hand, the coleoptiles growth of timothy were significantly inhibited at concentrations ≥ 30 mg DW equivalent extract/mL, and that of crabgrass, barnyard grass and Italian ryegrass were inhibited only at 100 mg DW equivalent extract/mL. The inhibition percent of the coleoptile growth of timothy, crabgrass, barnyard grass and Italian ryegrass at 100 mg DW equivalent extract/mL were 55, 1, 23 and 35, respectively (Fig. 1). The I_{50} value for the hypocotyls/coleoptiles of test species was in between 29.2 to 108.7 mg DW equivalent extract/mL. The hypocotyl growth of lettuce seedling was most sensitive to the extracts, whereas the coleoptile growth of barnyard grass was least sensitive (Table 3).

Effects of Plant Extracts on the Root Growth of Test Species

The root growth of both dicotyledonous and monocotyledonous plant species, were significantly inhibited at concentrations ≥10 mg DW equivalent extract/mL except rapeseed (Fig. 2). At concentration 30 mg DW equivalent extract/mL, the root growth inhibition percent of cress, lettuce, alfalfa, rapeseed, timothy, crabgrass, barnyard grass and Italian ryegrass seedlings was 40, 62, 55, 39, 92, 56, 13 and 68, respectively (Fig. 2).

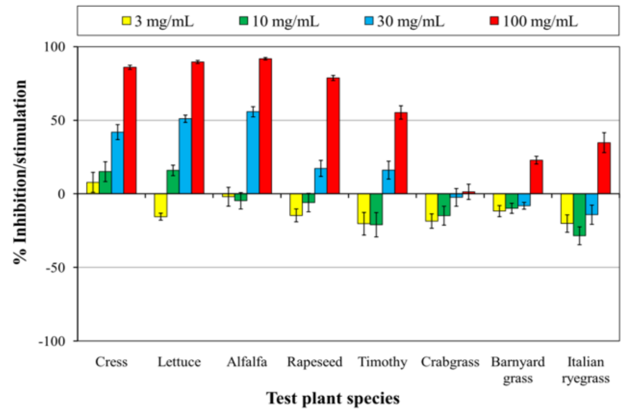

Fig. 1: Effects of aqueous methanol extract of *M. sylvestris* on hypocotyl/coleoptile growth of the test plant species. Concentrations of tested samples corresponded to the extract obtained from 3, 10, 30 and 100 mg dry weight of *M. sylvestris*. Means±SE from three independent experiments with 10 seedlings for each determination are shown. The negative (–) value in the Y axis indicates stimulation and positive (+) value indicates inhibition of the hypocotyl/coleoptile growth of eight test plant species by the aqueous methanol extracts of *M. sylvestris*

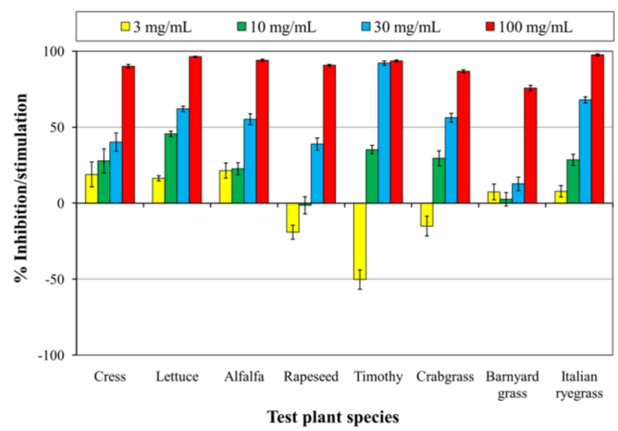

Fig. 2: Effects of aqueous methanol extract of *M. sylvestris* on root growth of the test plant species. Concentrations of tested samples corresponded to the extract obtained from 3, 10, 30 and 100 mg dry weight of *M. sylvestris*. Means±SE from three independent experiments with 10 seedlings for each determination are shown. The negative (–) value in the Y axis indicates stimulation and positive (+) value indicates inhibition of the root growth of eight test plant species by the aqueous methanol extracts of *M. sylvestris*

The inhibition percent was 90, 96, 94, 91, 94, 87, 76 and 98, respectively when the test species were applied to 100 mg DW equivalent extract/mL. On the contrary, the root growth of rapeseed, timothy and crabgrass were stimulated by the extracts at 3 mg DW equivalent extract/mL (Fig. 2).

Table 1: Two-way analysis of variance (ANOVA) of the percent hypocotyl/coleoptile growth inhibition of eight test plant species by the four concentrations of *M. sylvestris* plant extract

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1.205E6	31	38883.8	12.6	0.000	0.297
Intercept	206300.8	1	206300.8	67.1	0.000	0.067
Test plant	326819.3	7	46688.5	15.2	0.000	0.103
Concentration	726251.9	3	242084.0	78.7	0.000	0.203
Test plant × Concentration	152325.1	21	7253.6	2.4	0.001	0.051
Error	2854454.7	928	3075.9			
Total	4266151.8	960				
Corrected Total	4059851.0	959				

a. R Squared = 0.297 (Adjusted R Squared = 0.273)

Table 2: Two-way analysis of variance (ANOVA) of the percent root growth inhibition of eight test plant species by the four concentrations of *M. sylvestris* plant extract

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	1.440E6	31	46441.9	12.4	0.000	0.293
Intercept	1656756.4	1	1656756.4	443.3	0.000	0.323
Test plant	97488.3	7	13926.9	3.7	0.001	0.027
Concentration	1132488.5	3	377496.2	101.0	0.000	0.246
Test plant × Concentration	209720.7	21	9986.7	2.7	0.000	0.057
Error	3468649.1	928	3737.8			
Total	6565103.1	960				
Corrected Total	4908346.6	959				

a. R Squared = 0.293 (Adjusted R Squared = 0.270)

Table 3: I_{50} values of the aqueous methanol extract of *M. sylvestris* plant on hypocotyl/coleoptile and root growth of the eight test plant species

Test plant species	I_{50} (mg dry weight equivalent extract/mL)	
	Hypocotyl/Coleoptile	Root
Cress	34.5	27.6
Lettuce	29.2	13.7
Alfalfa	29.4	22.1
Rapeseed	58.4	36.3
Italian ryegrass	Not converged	17.8
Barnyard grass	108.7	63.7
Crabgrass	Not converged	24.2
Timothy	88.2	10.9

Note: The values were determine by a logistic regression analysis after bioassays

The I_{50} value for the root of test species was in between 10.9 to 63.7 mg DW equivalent extract/mL. The root growth of timothy was most sensitive to the plant extracts, and that of barnyard grass was least sensitive (Table 3).

Discussion

The aqueous methanol extracts of *M. sylvestris* had inhibitory effects on the seedling growth of both dicotyledonous (cress, lettuce, alfalfa and rapeseed) and monocotyledonous (Italian ryegrass, barnyard grass, crabgrass and timothy) plant species. These results indicate that the extracts of *M. sylvestris* had an allelopathic property on a wide range of plant species and may possess allelochemicals, which are responsible for their growth retarding activity. The inhibitory activity of the extracts was concentration dependent. The concentration-dependent inhibitory activity of the allelopathic plant extracts (allelochemicals) was also reported by Einhellig and Souza (1992), Chung *et al.* (2001), Bogatek *et al.* (2006), Mahmood *et al.* (2010), Kato-Noguchi *et al.* (2012).

M. sylvestris plant extracts were more effective in inhibiting the hypocotyl/coleoptile and root growth of dicotyledonous plant species (cress, lettuce, alfalfa and rapeseed) than that of monocotyledonous (Italian ryegrass, barnyard grass, crabgrass and timothy). The root growth of test plants was also more sensitive to the extracts than the hypocotyls/coleoptiles. The different effectiveness of *M. sylvestris* plant extracts may be mainly due to the difference in physiological and biochemical properties of each test plant species (Kobayashi, 2004). Such species-dependent responses to allelochemicals could influence the plant species composition of natural ecosystems (Imatomi *et al.*, 2013). The prominent root growth inhibition over hypocotyls/coleoptiles could be due to: (i) the more intensive contact in between the roots and plant extracts and subsequently with allelochemicals (Qasem, 1995; Tefera, 2002), (ii) roots which are the first organ to absorb allelochemicals from the environment (Turk and Tawaha, 2002), (iii) the reduced rate of cell division in presence of allelochemicals, which might inhibit gibberellin and/or indoleacetic acid function (Tomaszewski and Thimann, 1966), (iv) the hypocotyls/coleoptiles growth of seedling largely depends on cell expansion which is relatively insensitive to the allelochemicals, whereas root growth requires not only cell expansion, but also cell proliferation, which is sensitive to the allelochemicals and thus exerts higher root growth inhibition than the hypocotyls/coleoptiles (Nishida *et al.* 2005). The length of seedlings and/or their roots are the most commonly used parameters to assess the allelopathic effects of any plant extracts on the development and/or growth of the test species (Inderjit and Duke, 2003).

On the other hand, the stimulatory activity on the hypocotyls/coleoptiles growth of lettuce, alfalfa, rapeseed, timothy, crabgrass, barnyard grass and Italian ryegrass, and the root growth of rapeseed, timothy and crabgrass by the *M. sylvestris* plant extract at concentrations $\leq$ 30 mg DW equivalent extract/mL are in agreement with the previous findings of Rice (1984), Lovett *et al.* (1989), Liu and Chen (2011), Islam and Kato-Noguchi (2012). They reported that allelochemicals can stimulate the seedling growth at very low concentrations but could inhibit the same at higher concentrations. The stimulatory activity of any compound at lower concentrations is called hormesis (Southam and Erlich, 1943). Duke *et al.* (2006) gave a plausible explanation of hormesis; the availability of some chemicals at lower doses could affect the plant hormones and are responsible for shoot or root elongation, while they might have growth retarding activity at higher doses due to the same or another mechanism of actions.

In conclusion, The aqueous methanol extracts of *M. sylvestris* showed growth inhibitory activity with dicotyledonous and monocotyledonous plants. The inhibitory activity was concentration and species dependent. These results suggest that the plant have strong allelopathic potential and may possess allelochemicals. Therefore, this plant could be used as an important candidate for isolation and identification of allelochemicals, which could foster the development of bio-degradable, environment friendly natural herbicides for sustainable weed management strategies. Nevertheless, the species-dependent responses of that allelochemicals could also be helpful to design selective herbicides.

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