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AROMATIC PLANT *MELISSA OFFICINALIS* EXTRACTS SELECTIVITY IN VARIOUS BIOMASS CROP AND LEGUME SPECIES

Panagiotis Kanatas¹, Ioannis Gazoulis², Ioanna Kakabouki², Panayiota Papastylianou²

¹Agricultural Cooperative of Mesolonghi-Nafpaktia, 30200 Mesolonghi, Greece ²Agricultural University of Athens, 11855, Athens, Greece

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Vastutav autor: Corresponding author: E-mail: pakanatas@gmail.	Panagiotis Kanatas com

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ABSTRACT. Allelopathic effects of various plants can be exploited for use against weeds; however, the selectivity in different crops is also important. In the current study, the effects of lemon balm (Melissa officinalis) allelochemicals on seed germination and seedling emergence of three biomass crops and three legume species were evaluated. Seed germination of rapeseed was reduced by 19, 30, 56, and 80% in the concentrations of 1, 2, 5 and 10%, respectively, as compared to the control group, whereas sweet sorghum seeds showed a more intermediate response and sunflower germination was affected only by the highest concentration. Seed germination of common bean was by 25, 34 and 60% lower at 1, 2 and 5% extract concentrations, respectively, in comparison to the control whereas up to 85% reduction of seed germination was recorded in 10% concentration. Peanut seed germination percentage ranged between 72 and 47% of control in 5 and 10% concentrations, respectively, while soybean germination was least affected from M. officinalis leaf extracts since it was reduced by only 25 and 41% in 5 and 10% concentrations, respectively, as compared to the control. Seedling emergence of rapeseed was reduced by 14, 25, 46, and 79% in the concentrations of 1, 2, 5 and 10%, respectively, as compared to the control whereas lemon balm extracts showed increased selectivity on the sunflower. Soybean emergence was reduced by only 27 and 46% in 5 and 10% concentrations, respectively, in comparison to the control whereas common bean's seedling emergence was reduced up to 35% even in 2% concentration. Allelopathic response index values confirmed that sunflower and rapeseed were the least and most sensitive biomass crops to lemon balm allelochemicals, respectively, whereas sweet sorghum showed an intermediate response. Increased was the selectivity of the aqueous leaf extracts on soybean, whereas seed germination and seedling emergence of peanut were more affected and common bean was the most sensitive crop. Further research is needed to investigate the selectivity of M. officinalis and other aromatic plants' allelochemicals on various crops and under different soil and climatic conditions to optimize their efficacy as tools of more eco-friendly weed management strategies.

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Introduction

Weed management is a crucial issue for overall crop growth and productivity. It has been documented that weed infestation is the reason for a 5% loss to agricultural production in most developed countries, a 10% loss in less developed countries and a 25% loss in the least developed countries (Oerke, Dehne, 2004). The extended use of plant protection products is often related to serious environmental issues and therefore, the promotion of ecologically-friendly practices is imperative.

Allelopathy is a noticeable phenomenon through which some plants exude organic compounds known as allelochemicals and affect seed germination, growth and survival, establishment and reproduction of other



plants (Kanatas, 2020). Allelochemicals consist of a variety of organic compounds present in different plant parts such as simple polysaccharides, amino acids, organic acids and phenolic compounds. Phenolic compounds are of major importance in reducing seed germination growth and establishment of surrounding plants (Fan *et al.*, 1997; Inderjit, Duke, 2003; Wang *et al.*, 2014). There is a growing consideration regarding the potential use of allelochemicals in terms of weed management during the last two decades (Singh *et al.*, 2005; El-Rokiek, Eid, 2009; Puig *et al.*, 2013; Travlos *et al.*, 2018).

Allelopathy is something common in various aromatic plants and therefore important research has been conducted (Azirak, Karaman, 2008; Economou *et al.*, 2011). *Melissa officinalis*, a perennial aromatic plant, is already known to contain several allelochemicals with recent studies carried out in Greece and elsewhere revealing the effects of lemon balm on various grass and broadleaf weed species (Kato-Noguchi, 2001; Kanatas, 2020).

The selectivity of either natural or synthetic herbicides can be defined as the differential response level among species after the application of a particular molecule (de Oliveira, Inoue, 2001). Selectivity is mentioned as the capacity of a particular natural or synthetic herbicide to eliminate weeds in a crop, without affecting the yield or quality of the final product (Negrisoli et al., 2004). Selectivity is directly related to the fact that plant species respond differently to the same natural or synthetic herbicide (Devine et al., 1993). Although the herbicidal effects of the allelochemicals as derived from the tissues of aromatic plants is well established, there is not much evidence regarding the selectivity of such allelochemicals on crops and the response of the crops to their application. After the evaluation of the allelopathic potential of *M. officinalis* against different weeds (Kanatas, 2020), the objective of the current study was to evaluate the selectivity of lemon balm aqueous leaf extracts in three biomass and three leguminous crop species and find out which were the most and the least affected from the allelochemicals' application in four different concentrations.

Materials and methods

M. officinalis plants were collected at flower stage (2018), from a field located in Etloakarnania prefecture in Greece (20° 53′54″ E, 38° 53′38″ N) as previously described by Kanatas (2020). Leaves were washed, cut, ground and mixed with distilled water. After the overnight maintenance at room temperature, the mixture was filtered and diluted with distilled water, to have different aqueous extracts (1, 2, 5 and 10%). Five replicates for each aqueous extract were used, whereas the untreated seeds were used as the control group for each experiment as described by Kanatas (2020). Twenty seeds of three biomass crop species (sunflower, sweet sorghum and rapeseed), as well as three leguminous crop species (soybean, peanut and common bean) (Table 1), were placed in Petri dishes on the surface of

two Whatman No. 1 sheets of filter paper. An untreated control group was moisturized only with distilled water. The emergence of the radicle one week after incubation was considered to be germination (Bewley, Black, 1994).

Table 1. List of the crops tested in the present study

		-
Crop	Species	Family
Sunflower	Helianthus annuus L.	Asteraceae
Sweet sorghum	Sorghum bicolor (L.) Moench.	Poaceae
Rapeseed	Brassica napus L.	Brassicaceae
Soybean	Glycine max L.	Fabaceae
Peanut	Arachis hypogaea L.	Fabaceae
Common bean	Phaseolus vulgaris L.	Fabaceae

Allelopathic response index (RI) was also calculated as suggested by Williamson and Richardson (1988):

$$RI = 1 - \left(\frac{C}{T}\right) (when T > C)$$
(1)

and

$$RI = \left(\frac{C}{T}\right) - 1 \text{ (when } T < C\text{)}, \qquad (2)$$

where T and C were the germination percentages (%) of the treated and the untreated seeds, respectively.

To evaluate the effects of the extracts on the first growth of the plants and consequently crop establishment, the emergence was also measured outdoors in two pot experiments. Twenty pregerminated seeds of each treatment having a radicle of 1-2 cm length were sown at 1-3 cm depth in four pots filled with 3 l of a sandy clay loam (SCL) soil of pH = 7.1 and organic matter of 1.2%.

Regarding the statistical analysis, ANOVA was performed for all data and differences between means were compared using Fisher's Protected LSD test (P < 0.05). The Statsoft software package (Statsoft, Inc. 2300 East 14th Street, Tulsa, OK 74104, USA) was used.

Results

The results of the in vitro experiments revealed that M. officinalis aqueous leaf extracts showed their maximum allopathic effects on all the biomass and leguminous crops studied when applied in the concentrations of 5 and 10% whereas sensitive crops were affected even in lower concentrations. For example, seed germination of rapeseed was reduced by 19, 30, 56, and 80% in the concentrations of 1, 2, 5 and 10%, respectively, as compared to germination percentage recorded for the untreated seeds. Also, sweet sorghum showed an intermediate response to M. officinalis aqueous leaf extracts since seed germination was not affected in the low concentrations but in 5 and 10%, germination percentage was decreased by 31-55% in comparison to the untreated check. It is worth mentioning that lemon balm extracts showed increased selectivity on sunflower given that seed germination reduction did not surpass the level of 53% compared to the control even in the case where the greatest concentration was applied (Table 2).

 Table 2. M. officinalis extracts' effect on seed germination (%)

 of three biomass crop species

Species	Control	Control Concentration of M. officinalis				
		1%	2%	5%	10%	
Sweet sorghum	93ª	84 ^a	82ª	64 ^b	42°	
Sunflower	97 ^a	95ª	98 ^a	75 ^a	59 ^b	
Rapeseed	91ª	74 ^b	64 ^b	40 ^c	18 ^d	

Different lower case letters in the same row denote statistically significant differences (P < 0.05) between the means

Regarding leguminous crops, the common bean was the most sensitive to the allopathic extracts of lemon balm. In particular, 25, 34 and 60% fewer seeds did germinate in 1, 2 and 5% extract concentrations, respectively, in comparison to the control whereas up to 85% reduction of seed germination was recorded in 10% concentration. Peanut seed germination percentage ranged between 72 and 47% of control in 5 and 10% concentrations, respectively, while it was not influenced by the low concentrations. Soybean germination was least affected by *M. officinalis* leaf extracts. For instance, it was reduced by only 25 and 41% in 5 and 10% concentrations, respectively, as compared to the control (Table 3).

Table 3. *M. officinalis* extracts' effect on seed germination (%)

 of three legumes species

Crop	Control	Concentrati	on of M. of	<i>ficinalis</i> le	eaf extract
		1%	2%	5%	10%
Soybean	94ª	94 ^a	88 ^b	71 ^b	55°
Peanut	92ª	90 ^a	89 ^a	66 ^b	43°
Common bean	93ª	70 ^b	61 ^b	37°	14 ^d

Different lower case letters in the same row denote statistically significant differences (P < 0.05) between the means

For the biomass crops studied, it was noticed that the majority of the values of the RI were negative apart from the case where sunflower was treated with the aqueous leaf extracts of lemon balm in 1 and 2% concentration. This outcome indicates the increased selectivity of lemon balm extracts on this crop. Moreover, the sunflower was slightly affected even from the highest concentration of extract (10%). Regarding sweet sorghum, its RI value ranged between -0.004 and -0.019 in 1 and 2% extract concentration. Treatment with a 5% concentration increased RI over the level of 80% as compared to lower concentrations whereas the response of sweet sorghum was even greater in the highest concentration. The results of the in vitro experiments clarified that rapeseed was the most sensitive biomass crop to lemon balm extracts since its allelopathic response index value was -0.144 in the concentration of 1% whereas, in 2% concentration, RI value was increased by 35% as compared to the value mentioned above (Table 4).

 Table 4. M. officinalis extracts' effect on the allelopathic response index (RI) of three biomass crop species

Crop	Control	Concentration of <i>M. officinalis</i> leaf extract				
		1%	2%	5%	10%	
Sweet sorghum	_	-0.004^{a}	-0.019^{a}	-0.098^{b}	-0.204 ^c	
Sunflower	-	0.045 ^a	0.007^{a}	-0.069^{b}	-0.192°	
Rapeseed	_	-0.144^{a}	-0.222 ^b	-0.329°	-0.468^{d}	

Different lower case letters in the same row denote statistically significant differences (P < 0.05) between the means

The estimated RI values of the leguminous crops showed that soybean was the species least affected by the application of lemon balm allelochemicals. In particular, its RI was positive (0.017) in 1% concentration whereas it was -0.221 in the highest concentration of the aqueous extracts and this value was lower than the corresponding estimated for either peanut or common bean. Peanut showed an intermediate response in the application of *M. officinalis* leaf extracts. It was noticed that the RI value estimated in 5% concentration was by 64 and 93% greater than the corresponding values estimated in 1 and 2% concentrations, respectively. Regarding common bean, it was validated that this crop was by far the most sensitive of the three legumes studied. This outcome is derived from RI values which ranged between -0.158 and -0.344 in extract concentrations between 1 and 5% whereas, in the highest concentration, RI value surpassed the value of -0.5. In 10% concentration, the response of common bean to allelochemicals was by 32, 53 and 69% greater than the response observed in 5, 2 and 1% concentrations, respectively (Table 5).

 Table 5. M. officinalis extracts' effect on the allelopathic response index (RI) of three legume species

Crop	Control	Concentration of M. officinalis leaf extract				
		1%	2%	5%	10%	
Soybean	_	0.017 ^a	-0.028^{a}	-0.103 ^b	-0.221°	
Peanut	_	-0.011^{a}	-0.059^{a}	-0.166^{b}	-0.277°	
Common bean	_	-0.158^{a}	-0.236 ^b	-0.344°	-0.505^{d}	

Different lower case letters in the same row denote statistically significant differences (P < 0.05) between the means

The results of the current study indicated that seedling emergence of rapeseed was reduced by 14, 26, 53, and 79% in the concentrations of 1, 2, 5 and 10%, respectively, as compared to the emergence percentage recorded for the untreated check. Moreover, *M. officinalis* aqueous leaf extracts put an intermediate effect on sweet sorghum seedling emergence since it as much in the low concentrations of the allelochemical extracts. However, in 5 and 10% concentrations, seedling emergence was reduced by 30–55% as compared to the control. Lemon balm extracts showed great selectivity on sunflower given that seedling emergence of this crop was affected from the allelochemicals' application only in the highest concentration where the reduction percentage was only 36% (Table 6).

Table 6. *M. officinalis* extracts' effect on the emergence (%) ofthree biomass crop species

Crop	Control	Concentra	tion of M.	officinalis	leaf extract
		1%	2%	5%	10%
Sweet sorghum	87ª	85ª	80 ^a	61 ^b	39°
Sunflower	90 ^a	88 ^a	84 ^a	71 ^b	58°
Rapeseed	86 ^a	74 ^b	64 ^b	40 ^c	18 ^d

Different lower case letters in the same row denote statistically significant differences (P < 0.05) between the means

From the leguminous crop species studied, the common bean was the most sensitive to the leaf extracts of *M. officinalis*. In particular, 26, 37 and 64% fewer seedlings emerged in 1, 2 and 5% concentrations, respectively, as compared to the control whereas up to 89% reduction of seed germination was recorded in 10% concentration. Peanut seedling emergence ranged between 50 and 31% of control in 5 and 10% concentrations, respectively, while it was slightly affected by the low concentrations of 1 and 2%. Soybean emergence was least affected by lemon balm allelochemicals. In particular, it was reduced by only 27 and 47% in 5 and 10% concentrations, respectively, in comparison to the untreated check (Table 7).

 Table 7. M. officinalis extracts' effect on the emergence (%) of three legume species

Crop	Control	Concentration of M. officinalis leaf extract					
		1%	2%	5%	10%		
Soybean	88 ^a	80 ^a	81 ^a	64 ^b	47°		
Peanut	85ª	72ª	74 ^a	50 ^b	31°		
Common bean	89 ^a	66 ^b	56 ^b	32°	10 ^d		

Different lower case letters in the same row denote statistically significant differences (P < 0.05) between the means

Discussion

The growth-inhibiting effects of lemon balm aqueous leaf extracts on the different crops showed variation dependent on species level. From the biomass crops evaluated, rapeseed was the most sensitive whereas from the leguminous crops common bean was most affected by the application of the allelochemicals. Such findings highlight the susceptibility of some crops in the allelochemical compounds derived from other plant species. This outcome is validated by the corresponding of Souto et al. (2001) who noticed that soil bioassays revealed the allelopathic effects of Acacia spp. Allelochemicals on the growth of lettuce (Lactuca sativa L.) and white clover (Trifolium repens L.) under the soil and climatic conditions of the Mediterranean region. Also, the findings of another study have established that aqueous leaf extracts derived from the tissues of allelopathic species can reduce seed germination of important forage and pasture crops up to 80% (Hussain et al., 2011). Csiszár (2009) noticed that seed germination and root length of white mustard (Sinapis alba L.) was reduced at either the 0.01 or the 0.001 probability levels, respectively, due to the application of allelochemical extracts as derived from invasive plant species notorious for their allelopathic potential. Sweet sorghum and peanut had an intermediate response to lemon balm allelochemicals since they were slightly affected by the low concentration but more influenced by the allelochemicals' application in the concentrations of 5 and 10%. This outcome is in a common direction with the study of Uddin et al. (2010) where lettuce and cucumber (Cucumis sativus L.) were slightly affected by the application of sorgoleone. The same authors also noticed that major food crops such as wheat, barley, rice, corn and soybean were tolerant to sorghum root allelochemical extracts whereas other crop species showed a slight susceptibility (Uddin et al. 2010). This is validated from the results of the present study where soybean's seed germination ability, seedling emergence was slightly affected by *M. officinalis* aqueous leaf extracts. Furthermore, similar observations were made regarding sunflowers' tolerance to the allelochemicals applied as it is indicated from the values of the RI estimated in the different concentrations. Regarding RI, the observed variability among either the biomass or the leguminous crops has also been demonstrated in the recent study of Scavo *et al.* (2018).

The facts that growth inhibition was very low for two crops and intermediate for others means that M. officinalis leaf extracts contained allelopathic compounds and that their phytotoxicity remained lower in the case of the least sensitive crop species. Hill et al. (2007) showed that although germination and radicle growth of various weed species was significantly reduced by methanol and ethyl acetate extracts of hairy vetch extracts, corn, tomato and cucumber were not affected from the presence of the allelochemicals. The same authors also mentioned that corn and cucumber radicle elongation was stimulated at low concentration of the extracts (Hill et al., 2007). Also, Uddin and Pyon (2010) demonstrated that rotation crop residues can have intense herbicidal effects on weeds without inhibiting the growth of crop species which was found in our study. Furthermore, the same scientists observed no growth inhibition in plants like red pepper and lettuce at any incorporation rates, but tomato, cucumber and corn were slightly inhibited in a few cases at their highest incorporation levels (Uddin, Pyon, 2010). The observed differences could be probably explained by the different concentration of the allelochemicals' in the several extracts. The positive values of RI, as well as the stimulation of seed germination observed for sunflower and soybean at the rates of 1% and 2%, are in full accordance with findings reported by other researchers (Travlos, Paspatis, 2008; Economou et al., 2011). The results of the *in vitro* experiments carried out during the present study indicated that the phytotoxicity of lemon balm allelochemicals was a matter of concentration. There is much evidence in direct conformity with this outcome observed from the current study. For example, radicle length of perennial grass weed species Eragrostis curvula (Schrad.) Nees was by 44 and 74% reduced when treated with 10 and 20 g l^{-1} of aqueous leave extracts as compared to the control treatment in the study of Fatunbi et al. (2009). Furthermore, there is evidence that flower methanol extracts derived from highly allelopathic plants can reduce Hordeum murinum (L.) germination by 56-87% when applied at the concentrations of 5 and 10 g l^{-1} as compared to control treatments whereas the corresponding effect of methanol leaf extracts on this monocotyledonous species' seed germination can reach the level of 75% (Abd El Gawad et al., 2015).

As mentioned above, there is much evidence derived from the literature regarding the herbicidal effects of several aromatic plants on various grass and broadleaf weed species' seed germination, seedling emergence and growth. For instance, oregano can be used as a source of natural herbicides (Azirak, Karaman, 2008; Economou et al., 2011). Azizi and Fuji (2006) noted that the undiluted hydro-alcoholic extracts of both perforate St John's-wort (Hypericum perforatum L.) and common sage (Salvia officinalis L.) did put a strong inhibitory effect on seed germination percentage for Amaranthus retroflexus L. The herbicidal effects of lemon balm on either broadleaves or grasses have been evaluated and highlighted (Kato-Noguchi, 2001; Kanatas, 2020). However, apart from the herbicidal potential of *M. officinalis* extracts, there is a growing concern regarding the selectivity of such allelochemicals in crops and crop response to their application has not been widely studied. Under that concept, the present study is of major importance.

Conclusions

In the present research, the effects of aqueous extracts of *M. officinalis* on various biomass and leguminous crops were evaluated. From the study on seed germination and seedling emergence percentages, it can be concluded that sunflower and rapeseed were the least and most sensitive biomass crops to lemon balm allelochemicals, respectively, whereas sweet sorghum showed an intermediate response. Regarding leguminous crops, increased was the selectivity of the aqueous leaf extracts on soybean whereas seed germination and seedling emergence of peanut were more affected and common bean was the most sensitive crop. These results can be further explained from the estimated values of RI for each crop in each concentration. RI values were negative, apart from the cases where sunflower and soybean were exhibited to the low concentrations of the allelochemicals. In any case, the impact of the M. officinalis extracts on the crops was concentration-dependent and of course, the effect was species-specific. In particular, the higher the concent-'ration, the greater the inhibiting effects of the allelochemicals on each crop. Due to these species-specific effects, the use of M. officinalis extracts for weed management is suggested only on particular crops and not in all species. Further research is needed to investigate the selectivity of *M. officinalis* and other aromatic plants' allelochemicals on various crops and under different soil and climatic conditions to optimize their efficacy as tools of more eco-friendly weed management strategies.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

PK, IG, IK and PP contributed equally to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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