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THE AROMATIC PLANT *MELISSA OFFICINALIS* AND EFFECTS OF ITS AQUEOUS EXTRACTS ON SUMMER ANNUAL AND INVASIVE WEED SPECIES

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ABSTRACT. The effects of aqueous extracts of the aromatic plant *Melissa officinalis* were studied on the seed germination and early seedlings growth of ten summer annual species in Petri dish bioassays and pot experiments. The *in vitro* experiments on the aqueous extracts from *M. officinalis* on seed germination shows that the extracts of 5 and 10% were the most active inhibitors for all the studied weed species. Seed germination reduction by the aqueous extracts was up to 54% of the untreated seed lot for each species. On the contrary, the concentration of 1% resulted in germination reduction ranging from 1 to 11%, while in some of the weed species (*P. minor*, *S. nigrum*, *P. angulata* and *C. albida*) the effect was rather stimulatory. This finding is in full agreement with “novel weapons hypothesis” and supports that native plants compared with invasive (like *C. albida* and *P. angulata*) are affected more due to the absence of tolerance or resistance to the allelochemicals. The allelopathy RI of aqueous extracts of lemon balm was negative in most cases, while in the case of 10% concentration, emergence was reduced by 58, 54, 48, 46 and 43% for *X. strumarium*, *C. album*, *S. faberi*, *C. canadensis* and *C. bonariensis*, respectively. The allelopathic activity of *M. officinalis* could be exploited in future studies, to identify and isolate the allelochemicals, as models for future herbicides for integrated weed management.

Introduction

Weeds are considered to be among the most important constraints for crop production, with average yield crop reduction being higher than 30% (Oerke, 2006; Travlos, 2012). Chemical weed control remains one of the most widely used, effective and cheap methods of weed management. Unfortunately, this overreliance on herbicides has led to serious problems, such as herbicide resistance development and environmental issues, with negative impacts and side effects (Jabran *et al.*, 2015). Consequently, ecologically-based strategies for weed management are necessary.

Allelopathy is the phenomenon of interaction between plant species, with many of them possessing inhibitory or stimulatory effects on the growth of neighbouring or successional plants by releasing chemicals and several secondary metabolites into the soil (Rice, 1984; Seigler, 1996). The potential use of allelochemicals or even allelopathic crops for weed management is not something new (Putnam, Duke, 1978); however, lately, the interest is rapidly increasing (Foy, 2001; Travlos, Paspatis, 2008; Mirmostafaee *et al.*, 2020). Many allelochemicals from various plant species have been identified, including phenolics, terpenes, benzoxazinoids, sorogoleones, glucosinolates and others (Niinemets *et al.*, 2014; Jabran, 2017). They can be found in different plant tissues and are biodegradable (Bhowmik, Inderjit, 2003; Rasulov *et al.*, 2019). Besides, they can be selective and also be the basis for a new mode of actions (Macias *et al.*, 2007; Dayan *et al.*, 2012).

Several aromatic and medicinal plants have been evaluated in terms of their allelopathic potential due to the volatile compounds which are important secondary metabolites. For instance, many *Origanum* species can be used as biopesticides due to their strong allelopathic effects (Azirak, Karaman, 2008; Economou *et al.*, 2011). Lemon balm (*Melissa officinalis* L.) is a perennial aromatic plant and its essential oil contains polyphenols, tannins, flavonoids and other substances (Scholey *et al.*, 2014). To date, there is some information regarding the potential allelopathic effects of *M. officinalis* on weeds and other plants; however, very few data are available on the comparative study of the same extracts on a wide range of weeds (Kato-Noguchi, 2001; Geimadil *et al.*, 2015)

For this reason, this research aimed to study the allelopathic potential of *M. officinalis* on seed germination and plant emergence of ten common summer annual weeds, with two of them considered as invasive for Mediterranean regions.

Material and Methods

Fresh material was sampled from an *M. officinalis* crop at flower stage (2018), from a field located in western Greece (20º 53′54″ E, 38º 53′38″ N). 1 kg of leaves was washed and ground. Afterwards, an equal quantity was mixed with 1 l of distilled water as proposed by Norsworthy (2003). Next day the mixture was filtered through filter paper to minimize the solid fraction. From this solution and after dilution with distilled water, four different concentrations (1, 2, 5 and 10%) of aqueous extracts were obtained. The prepared aqueous extracts were stored in a refrigerator (4 °C) for further use.

There were used five replicates (glass Petri dishes) for each aqueous extract (treatment), while untreated weed seeds were used as a control for each experiment (only diluted water was added). Twenty seeds of ten different weed species (Table 1) were placed between two Whatman No. 1 paper filter disks and 5 ml of distilled water was added. Seeds were considered germinated at the emergence of the radicle (Bewley, Black, 1994) at 7 days after placement. It has to be noted that mature seeds of the tested weed species have been collected during previous summer (2017) from fields of several annual and perennial crops in the area of western Greece. After their collection, seeds were cleaned, stored in paper bags, selected to have similar size and colour and put in a refrigerator at 5 °C until germination and emergence studies.

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

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

and

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

where T and C represent seed germination (%) for the treated plants and the untreated control, respectively.

Besides, the emergence of the weed seedlings was also recorded in two pot experiments in a glasshouse. Minimum/maximum air temperature and relative humidity were: 25/40 °C and 30/60%, respectively and the plants were subjected to a natural day length ranging between 12–14 h during the experiments. Ten pregerminated seeds of each treatment having a radicle of 1–2 cm length were sown at 1–2 cm depth in five plastic pots filled with 2 l of a sandy clay loam (SCL) soil (pH = 7.1) and the several aqueous extracts were added. The only exceptions to that were the *Conyza* species, the seeds of which were placed on soil surface due to their small size.

Analysis of variance was performed for all data and differences were tested at the 5% level of significance using the Fisher's Protected LSD test. Statsoft software package (Statsoft, Inc. 2300 East 14th Street, Tulsa, OK 74104, USA) was used.

Table 1. Weeds species included in the present study

Common name	Scientific name	Family	Biological cycle
Small canarygrass	*Phalaris minor*	Poaceae	Summer annual
Giant foxtail	*Setaria faberi*		
Cutleaf groundcherry*	*Physalis angulata*	Solanaceae	
Black nightshade	*Solanum nigrum*		
Lambsquarters	*Chenopodium album*	Amaranthaceae	
Prostrate pigweed	*Amaranthus blitoides*		
Common cocklebur	*Xanthium strumarium*	Asteraceae	
Tall fleabane*	*Conyza albida*		
Flaxleaf fleabane	*Conyza bonariensis*		
Horseweed	*Conyza canadensis*		

* considered as invasive for Mediterranean regions

Results

The *in vitro* experiments on the aqueous extracts from *M. officinalis* on seed germination shows that the extracts of 5 and 10% were the most active inhibitors for all the studied weed species. In particular, seed germination reduction by the aqueous extracts of 10% was ranged between 21 and 54% of the untreated seed lot for each species (Table 2). On the contrary, the concentration of 1% resulted in germination reduction ranging from 1 to 11%, while in some of the weed species (*P. minor*, *S. nigrum*, *P. angulata* and *C. albida*) the effect was rather stimulatory (Table 2).

Table 2. Effects of aqueous extracts of *M. officinalis* on seed germination (%) of the tested species.

Weed species	Untreated	1%	2%	5%	10%
Phalaris minor	84^{a}	85^{a}	82^{a}	72^{b}	64^{b}
Solanum nigrum	38^{a}	40^{a}	37^{a}	29^{ab}	23^{b}
Chenopodium album	62^{a}	55^{a}	52^{ab}	46^{bc}	38^{c}
Setaria faberi	75^{a}	72^{a}	62^{b}	64^{ab}	48^{c}
Physalis angulata	94^{a}	95^{a}	88^{ab}	81^{b}	74^{c}
Amaranthus blitoides	92^{a}	91^{a}	85^{ab}	79^{b}	63^{c}
Xanthium strumarium	56^{a}	52^{a}	47^{a}	38^{b}	26^{c}
Conyza albida	96^{a}	97^{a}	89^{a}	76^{b}	62^{c}
Conyza bonariensis	100^{a}	95^{a}	88^{ab}	76^{c}	59^{d}
Conyza canadensis	100^{a}	96^{a}	85^{b}	72^{c}	57^{d}

Means followed by the same superscript letter within a row are not significantly different at 5 % Fisher's least significant difference test.

Table 3. Effects of aqueous extracts of *M. officinalis* on the allelopathic response index (RI) of the tested species

Weed species	Untreated	1%	2%	5%	10%
Phalaris minor	–	0.012[a]	-0.024[a]	-0.143[b]	-0.238[b]
Solanum nigrum	–	0.050[a]	–0.026[b]	–0.237[c]	–0.395[d]
Chenopodium album	–	–0.113[a]	–0.161[a]	–0.258[b]	–0.387[c]
Setaria faberi	–	–0.040[a]	–0.173[b]	–0.147[b]	–0.360[c]
Physalis angulata	–	0.011[a]	–0.064[a]	–0.138[b]	–0.213[c]
Amaranthus blitoides	–	–0.011[a]	–0.076[ab]	–0.141[b]	–0.315[c]
Xanthium strumarium	–	–0.071[a]	–0.161[b]	–0.321[c]	–0.536[d]
Conyza albida	–	0.010[a]	–0.073[a]	–0.208[b]	–0.354[c]
Conyza bonariensis	–	–0.050[a]	–0.120[a]	–0.240[b]	–0.410[c]
Conyza canadensis	–	–0.040[a]	–0.150[a]	–0.280[b]	–0.430[c]

Means followed by the same superscript letter within a row are not significantly different at 5% Fisher's least significant difference test.

The allelopathy RI of aqueous extracts of lemon balm was negative in all weed species except *P. minor*, *S. nigrum*, *P. angulata* and *C. albida* in the concentration of 1% (Table 3). The major negative effects were observed in the higher concentration (10%) for *X. strumarium*, *C. bonariensis* and *C. canadensis*.

Seedling emergence of the several weeds was significantly and negatively affected by the addition of *M. officinalis* extracts in concentrations higher than 1% (Table 4). In the case of aqueous extracts of 1%, seedling emergence of *S. nigrum*, *C. album*, *P. angulata*, *A. blitoides* and *C. albida* was slightly increased but without any significant changes. On the other hand, in the case of 10% concentration, emergence was reduced by 58, 54, 48, 46 and 43% for *X. strumarium*, *C. album*, *S. faberi*, *C. canadensis* and *C. bonariensis*, respectively.

Table 4. Effects of aqueous extracts of *M. officinalis* on seedling emergence of the tested species

Weed species	Untreated	1%	2%	5%	10%
Phalaris minor	74[a]	72[a]	63[b]	61[b]	51[c]
Solanum nigrum	26[ab]	28[a]	32[a]	21[b]	18[b]
Chenopodium album	41[a]	43[a]	32[b]	28[b]	19[c]
Setaria faberi	67[a]	66[a]	53[b]	48[b]	35[c]
Physalis angulata	86[a]	87[a]	78[a]	68[b]	65[b]
Amaranthus blitoides	85[a]	88[a]	74[b]	68[b]	57[c]
Xanthium strumarium	52[a]	49[a]	44[a]	34[b]	22[c]
Conyza albida	92[a]	93[a]	86[a]	74[b]	58[c]
Conyza bonariensis	96[a]	95[a]	85[b]	72[c]	55[d]
Conyza canadensis	100[a]	95[a]	82[b]	68[c]	54[d]

Means followed by the same superscript letter within a row are not significantly different at 5% Fisher's least significant difference test.

Discussion

Allelopathy is one of the main forces in the development of plant communities and spatial patterns (Rice, 1984). The effects of several aromatic plants on weed species' germination and growth have been extensively studied (Economou *et al.*, 2011; Kashkooli, Saharkhiz, 2014). To date, the effects of lemon balm on weeds have been evaluated in a few studies. For instance, Geimadil *et al.* (2015) found an inhibitory effect of aqueous extracts of *M. officinalis* on seed germination of *Sinapis arvensis*. Moreover and Kato-Noguchi (2001) showed that water-soluble fractions obtained from aqueous acetone extracts of lemon balm inhibited the seed germination and plant growth of weeds like *Digitaria sanguinalis* and *Lolium multiflorum*.

Another interesting finding is related to the different germinability of the untreated seeds for the several weeds and it could be attributed to the potential dormancy for some of them and the enormous variability and plasticity of the weeds. Furthermore, stimulation of seed germination at low rates of aqueous extracts is in agreement with previous studies on several aromatic plants and weeds (Travlos, Paspatis, 2008; Economou *et al.*, 2011). According to Ambika (2013), a compound may cause inhibition at high concentration, stimulation at low concentration, or no significant effect in intermediate rates.

Regarding RI, the observed variability among weeds has been previously reported by Scavo *et al.* (2018) and can be partially attributed to the different combination and concentration of allelochemicals' profile in each aqueous extract.

Concerning the different responses observed between the weed species, this is also something common in previous studies. For instance, Azizi and Fuji (2006) have found that the extracts of *Hypericum perforatum* and *Salvia officinalis* had a significant inhibitory effect on seed germination for *A. retroflexus*, but not for *P. oleracea*. Another interesting finding is related to the response of two invasive plants on aqueous extracts of low concentrations. In particular, aqueous extracts of 1% have not affected or slightly stimulated seed germination and seedling emergence of *the species P. angulata* and *C. albida*. These two species are considered as invasive for the Meditteranean and other regions (Travlos, 2012). Therefore, our results suggest that agro-ecologically speaking, their observed 'resistance' to allelopathy may correlate with their invasiveness and dispersal dynamics or with an allelopathic potential of the particular species as previously indicated (Economou *et al.*, 2002). Indeed, allelopathy is a possible explanation for the mechanism of success of many invasive plants (Hierro, Callaway, 2003), while the "novel weapons hypothesis" supports that native plants are affected due to the absence of tolerance or resistance to the chemicals (Callaway, Ridenour, 2004).

In agronomy and weed science, herbicides are the focus of many studies (Foy, 2001). However, further research is necessary to identify novel ecologically-based methods of weed management. Finding new effective allelopathic species and evaluating them against a wide range of agronomically important weed species can be crucial in this regard. Consequently, results on the potential effect of aromatic plants on seedling emergence could be of great value. For the case of *M. officinalis* it could be said that the findings on the reduction of seedling emergence up to 58% for several weeds could be a clear indication and a basis for the further exploitation under field conditions.

Conclusions

The allelopathic effects of aqueous extracts of *M. officinalis* on ten common summer annual weeds in Greece were evaluated in the present study. Concentration was partially responsible for the observed differences, even if different response was recorded among the studied weed species. Seed germination reduction by the aqueous extracts was up to 54% of the untreated seed lot for each species. Seed germination and seedling emergence reduction by the aqueous extracts were up to 54 and 58% of the untreated seed lot for each species. Moreover, aqueous extracts of 1% have not affected or slightly stimulated seed germination and seedling emergence of the invasive species *P. angulata* and *C. albida*, indicating the potential role of allelopathy on plant invasion process. These results are very promising to further exploit aromatic plants in terms of their allelopathy and their potential role in ecologically based weed management.

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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 contributed 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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