

EOs from Mediterranean Aromatic Plants

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Herbicidal potential of *Thymbra capitata* (L.) Cav., *Mentha × piperita* L. and *Santolina chamaecyparissus* L. essential oils (EOs) at different concentrations on *Avena fatua* L., *Echinochloa crus-galli* (L.) P. Beauv, *Portulaca oleracea* L. and *Amaranthus retroflexus* L., and on soil microorganisms was tested. EOs demonstrated herbicidal activity, increasing their toxicity with the dose. *T. capitata* was the most effective against all weeds at the maximum dose. *P. oleracea* was the most resistant weed. Soil microorganisms, after a transient upheaval period induced by the addition of EOs, recovered their initial function and biomass. *T. capitata* EO at the highest dose did not allow soil microorganisms to recover their initial functionality. EOs exhibited great potential as natural herbicides but the optimum dose of application must be identified to control weeds and not negatively affect soil microorganisms.

weed control

natural herbicides

soil microorganisms

1. Introduction

One of the main challenges of the Agriculture of the 21st century is to increase crop production in a sustainable way, e.g., minimizing the use of pesticides ^[1]. The widespread use of synthetic chemicals may lead to the accumulation of toxic residues in agricultural products and result in soil and groundwater pollution, development of weed resistance, and adverse effects on human and animal health ^{[2][3]}. Furthermore, synthetic chemicals can be immobilized in soil by adsorption or binding to colloids ^[4], affecting both soil organic matter turnover and microbial community composition ^{[5][6][7]}. One potential fulfillment to the demand of alternative natural and safe products is the exploitation of renewable resources, such as medicinal and aromatic plants known for their allelopathic properties ^{[8][9]}.

Plant secondary metabolites, such as essential oils (EOs), include allelochemicals compounds which have been proved to inhibit seed germination and seedling growth ^[9]. EOs are suitable for sustainable and organic agriculture because of their rapid volatilization and degradation in the environment ^[10]. Their effectiveness in controlling weeds lies in the joint action of an array of different held compounds, whose quantity and persistence in the environment may be low to inhibit seed germination and plant growth ^{[11][12]}. However, when the concentration of one active compound within a given EO is very high, this active compound alone could be effective ^{[13][14][15]}. In addition, since EOs usually have various modes of action, it is more complicated for weeds to easily develop resistance against them ^{[16][17]}. In fact, EOs can suppress the weed growth by affecting biochemical and physiological processes such as reducing cell survival, chlorophyll and RNA contents; acid soluble carbohydrates; and water-soluble carbohydrates ^{[18][19]}. Despite their allelopathic potential, many EOs are classified as “Generally Recognized as Safe” (GRAS) by the US Food and Drug Administration ^[20].

2. EOs Extracted from Lamiaceae

EOs extracted from Lamiaceae have demonstrated to be effective in inhibiting seed germination in vitro assays [21]. The most important species in this family, in terms of high economical value due to the great production of EOs, are *Mentha × piperita* L. (Peppermint) and *Thymbra capitata* L. (Cav) (synonym *Thymus capitatus* (L.) Hoffmanns. & Link) (Thyme) [22][23]. *Mentha × piperita* L. is a cultivated natural hybrid of *Mentha aquatica* L. (water mint) and *Mentha spicata* L. (spearmint), both native species of the Mediterranean region. *M. Piperita* is cultivated worldwide because its EO has antioxidant and antimicrobial activities and is used as eco-friendly pesticide [24][25]. Likewise, the phytotoxic activity of *M. piperita* EO has been demonstrated in several studies [26][27].

The species of *Thymus* genus, native of Southern Europe, North Africa and Asia [28][29], are largely used as medicinal plants [30]. Due to the presence of polyphenols, *T. capitata* EO is used in food preservation [31] and it has been demonstrated that it possesses antioxidant properties [32]. Moreover, the antimicrobial [33][34] and herbicidal activities [15][35][36] of *T. capitata* EO have been verified. *Santolina chamaecyparissus* L. (cotton lavender) is an aromatic plant belonging to Asteraceae family. Its analgesic, bactericidal, fungicidal, vermifuge, and vulnerary properties have been described [37]. Furthermore, its herbicidal activity is well documented [38]. Although, as aforementioned, some studies have shown that EOs extracted from *M. piperita*, *T. capitata* and *S. chamaecyparissus* may inhibit seed germination and weed growth; the majority of them have been conducted in in vitro conditions and against few weed species. Therefore, their selectivity towards some of the most widespread and troublesome weeds has yet to be investigated. On the other hand, few studies deal with the effects of such EOs on soil microorganisms. Vokou and Liotiri [39] found that EOs extracted from five aromatic plants, not including those tested in this study, increased microbial respiration. Similarly, also EOs extracted from *Lavandula stoechas* L. increased microbial respiration as a result of bacteria growth stimulation [40]. Such results, however, contrast with those of Khare et al. [41] who reported a decrease of microbial biomass and activity. Such few studies with even conflicting results demonstrated that, if EOs were deemed to be used in the field for an integrated pest management, further studies would need to better elucidate their effects on soil microorganisms as playing pivotal roles in the soil organic matter turnover and nutrient cycling. In addition, not all EOs exert the same effect on weeds at a given concentration [35][42].

3. Findings

Several studies have been carried out on the phytotoxic activity of EOs against weeds and on their potential use as natural herbicides. The majority of these works have been performed in vitro experiments and not in microcosms that try to mimic the natural conditions. Moreover, in vitro approaches seeds and/or seedlings are directly exposed to the EOs in sterile conditions, i.e., strongly reducing and/or retarding EOs transformation/degradation normally mediated by soil microorganisms. To our knowledge, this is the first time that the effects of essential oils from *T. capitata*, *M. piperita* and *S. chamaecyparissus* against targeted weeds and soil microorganisms have been studied with a more practical approach, i.e., in vivo conditions, monitoring their effects in order to know their real potential as an alternative to synthetic chemicals, within a strategy of Integrated Weed Management and analyzing the benefits or disadvantages derived from their employment.

Results clearly demonstrated that tested EOs, to a different extent, were significantly effective against weeds, killing them completely or reducing significantly their growth parameters. Among them, *T. capitata* was the most effective, followed by *M. piperita*. Both EOs showed a broad spectrum of activity, with *T. capitata* at the highest doses applied ($12 \mu\text{L mL}^{-1}$) killing plants of all weed species (100 efficacy), except for *P. oleracea* (90 efficacy). *M. piperita* at the highest dose ($20 \mu\text{L mL}^{-1}$) controlled completely (100 efficacy) *A. retroflexus* and *A. fatua* plants but showed 90 and 40 efficacy on *P. oleracea* and *E. crus-galli*, respectively. Although *S. chamaecyparissus* EO was less active compared with the other EOs, it displayed a very remarkable selective activity, being highly effective against *A. retroflexus* (90 efficacy at the highest dose, $20 \mu\text{L mL}^{-1}$). It could be interesting to study it more profoundly as a selective herbicide, while *T. capitata* and *M. piperita* could have a wider action, exhibiting excellent potential for the development of broad-spectrum herbicides. A good natural herbicide, besides being effective, at the same time should not have side effects on soil microorganisms. Here, results clearly demonstrated that, except for *T. capitata* EO at the highest concentration, which significantly increased the specific respiration rate, the other EOs generally stimulated soil biochemical properties, or their effect on them was transient. Furthermore, even when changes in the main microbial groups persisted, soil microbial activity was not irredeemably affected, suggesting that essential oils did not compromise the functional redundancy.

Since EOs are able to decrease the weed growth parameters by reducing their fitness and competitiveness, another advantage in using these EOs, from a conservationist point of view in agro-ecosystems, could be that to maintain a high biodiversity by not completely eradicating the weeds, instead giving the crop an opportunity to outcompete them.

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