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Synopsis

Use of Essential Oils as Natural Herbicides

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Abstract. Essential oils are complex mixtures of volatile compounds, mainly terpenes and phenylpropanoids, particularly abundant in aromatic plants. In the last decades, there are many reports about herbicidal potential of essential oils. An initial step to assess their herbicidal activity is to determine their effect on seed germination and seedling growth. The present review summarizes the methods for assess the effects of essential oils on seed germination and seedling growth. The advantages and disadvantages of using essential oils for weed control are discussed. Also important conclusions regarding their application as natural herbicides are drawn. Essential oils obtained from plant species from the families Myrtaceae, Pinaceae, Lauraceae, Poaceae, Lamiaceae, Asteraceae, Cupressaceae, Apiaceae and etc. are determined as promising for weed control. Based on some essential oils derived from lemongrass, cloves and cinnamon, commercial weed control products have already been developed. The studies in Bulgaria have been mainly focused on the effect of extracts on seed germination. However, it was found that essential oils of Bulgarian species *Origanum vulgare* subsp. *hirtum* (Link) Ietsw, *Thymus longidentatus* (Degen & Urum.) Ronniger*.*, *Thymus moesiacus* Velen, *Micromeria dalmatica* Benth. exhibit strong inhibitory activity on seed germination of weeds. The main components of essential oil identified for each species were respectively carvacrol for *O. vulgare* subsp. *hirtum,* citral isomers: neral and geranial of *T. longedentatus*, α-terpinyl acetate of *T. moesiacus*, piperitone oxide, carvacrol, β-pinene of *M. dalmatica*. The presented data show that the essential oils have strong inhibitory effects on germination and seedling growth of weeds and this makes them a promising source for production of natural herbicides.

Key words: germination inhibition, weed, *Origanum vulgare* subsp. *hirtum*, *Thymus longidentatus*, *Thymus moesiacus*, *Micromeria dalmatica.*

Potential of essential oils as natural herbicide

The use of synthetic herbicides in weed control results in a negative impact on the environment and human health. Moreover, weeds develop resistance to applied herbicides and the need for new molecules with new mechanisms of herbicidal action is of great importance. In the course of evolution, plants interacting with each other as well as with the abiotic and biotic factors of their environment, develop mechanisms of protection and adaptation by synthesizing a variety of secondary metabolites. It has been demonstrated that these compounds although called secondary metabolites have important biological activities and role in the plant survive, that making them a source for developing of natural products for pest control in agriculture. A large group of secondary metabolites, with an important biological role, are the terpenes. Monoterpenes (hydrocarbon and oxygenated

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monoterpens) and sesquiterpenes (hydrocarbon and oxygenated sesquiterpens) are main components of the essential oils. The last represent complex mixture of volatile compounds, particularly abundant in aromatic plants, which except terpenes contained phenylpropanoids belonging to various chemical classes: alcohols, ethers or oxides, aldehydes, ketones, esters, amines, amides, phenols, and heterocycles. Essential oils are localized in the cytoplasm of plant cells, components of various secretory structures – glands, secretory hairs, resin ducts, secretory cavities etc. (DHIFI *et al.*, 2016). The advantage of using essential oil to weed control is that they are environmentally safe and human friendly due to their rapid environmental biodegradation and low toxicity to non-target organisms (MANN & KAUFMAN, 2012). Also essential oils expose new mechanism of herbicidal action and they also can improve pollination (HAZRATI *et al.*, 2017; DAYAN *et al.*, 2012). Inhibitory effects of essential oils also showed species specificity (ÖNEN *et al.*, 2002; UREMIS *et al.*, 2009; ULUKANLI *et al.*, 2014; GRICHI *et al.*, 2016). Beside to the advantages of applying essential oils in crop protection, there are also some disadvantages. Essential oil of one species has great variability in its composition because besides genetic factors, geographic and climatic conditions, harvest stage and extraction methods have important effects. The paradoxically high volatility of essential oils, which makes them safe for the environment, is at the same time a disadvantage. Recent studies have been published that this problem is solved through the application of essential oils by microencapsulation and nanoemulsion (HAZRATI *et al.*, 2017). Essential oils are hydrophobic that is another disadvantage. Usually essential oils extracted from freshly (KAUR *et al.*, 2010) or dried plant material (KORDALI *et al.*, 2009; BENVENUTI *et al.*, 2017) by hydrodistillation, the most common method, but there are other techniques such as liquid carbon dioxide, microwaves and etc. (LAHLOU, 2004; DJILANI & AMADOU, 2012).

An initial step to assess the potential herbicidal activity of an essential oil is to determine its effect on seed germination and seedling growth. The results of studies on the allelopathic and phytotoxic properties of essential oils are a valuable source of information for selecting substances with herbicidal activity (SCOGNAMIGLIO *et al.*, 2013; AMRI *et al.*, 2013a; DAYAN *et al.*, 2009; DHIFI *et al.*, 2016).

Methods for assessing germination inhibition

There are different variants for assessing germination of seeds which vary according to the methods of dissolving of the essential oil, the mode of application and doses. Essential oils are slightly soluble in water but soluble in alcohol, ether, ethylene glycol, non polar or weakly polar solvents. Most often essential oil has been dissolved in emulsifying detergent: 1% water solution of Tween 20 (AMRI *et al.*, 2012a) 0.5 %, 0.2% or 0.1% of Tween 80 (LAHLOU, 2004; ULUKANLI *et al.*, 2014). Also they can be dissolved in water–acetone mixture (99.5:0.5) (DE ALMEIDA *et al.*, 2010; SYNOWIEC *et al.*, 2017) and DMSO–water solution (1%, v/v) (KORDALI *et al.*, 2009). In the most cases, the bioassay tests for seed germination are conducted in Petri dishes where the essential oils are applied as solutions in the concentration range of 0.06-5 µg/mL (DE ALMEIDA *et al.*, 2010; AMRI *et al.*, 2012a; AMRI *et al.*, 2017). Essential oils showing completely inhibitory effect on seed germination in the range 0,5-4 µg/mL or from 0.02 to 17 mg/mL are considered as promising for further research on herbicidal effects (DE ALMEIDA *et al.*, 2010; AMRI *et al.*, 2013b; ANGELINI *et al.*, 2003; ULUKANLI *et al.*, 2014; SYNOWIEC *et al.*, 2016; ULUKANLI *et al.*, 2018). It has been reported that essential oil of *Peumus boldus* Mol. shows complete inhibition on germination of *Portulaca oleracea* L. seeds at concentration 0.5-1 µg/mL (BLÁZQUEZ & CARBÓ, 2015). In other cases essential oils has been applied by using a micropipette on the inner side of a Petri dishes in different concentrations 2-32 µL/Petri dish (ÖNEN *et al.*, 2002; SALAMCI *et al.*, 2007; UREMIS *et al.*, 2009), 10 mg/Petri dishes (KORDALI *et al.*, 2009), 44 μg/Petri dishes (BENVENUTI *et al.*, 2017). Rarely seeds are soaked in a solution of essential oils before conducting a germination test (ANGELINI *et al.*, 2003). Except in Petri dishes, some experiments have been conducted in plastic pots. In these cases, essential oils has been applied to the soil surface as aqueous solutions (5 mL) in different concentrations as example from 5.4, 21.6, 86.4 and 345.6 mg/L as the last concentration corresponds to a concentration in which a commercial herbicide is applied (CAMPIGLIA *et al.*, 2007). KAUR *et al.*, (2010) applied *Artemisa* oil in the concentration range 5-50 μg/g sand to study effect on seed germination of test weeds. In these experiments, the need for microencapsulation of essential oils has been noted. It has been reported that the type of soil is decisive for the inhibitory activity of the essential oils (DUDAI *et al.*, 1999).

Methods to assess inhibition of seedling growth

There are many reports on application of essential oils that reduce significantly seedling growth. Reductions in root and shoot length, plant weight, chlorophyll content, respiratory activity as well as visible injury - chlorosis, necrosis, complete wilting of plants are the most common indicators for estimating impaired growth (UREMIS *et al.*, 2009; KORDALI *et al.*, 2009; KAUR *et al.*, 2010; ULUKANLI *et al.*, 2014; TWORKOSKI, 2002; RASSAEIFAR *et al.*, 2013; RAHIMI *et al.*, 2013). Often essential oils are applied for treatment on post- emergence seedling in the form of a spray at concentrations of 5-10% from red thyme, summer savory, cinnamon and clove oil (TWORKOSKI, 2002), 2%, 4%, and 6% from Artemisia oil (KAUR *et al.*, 2010) 10-1000 mg/L from *Artemisia annua* and *Xanthium strumarium* oil (BENVENUTI *et al.*, 2017), 25-100 mL/L from *Eucalyptus astringens* oil (GRICHI *et al.*, 2016) and 1-5 mL/L of *Satureja hortensis* oil (HAZRATI *et al.*, 2017). HAZRATI *et al.*, (2017) reported complete lethality at 4 mL/L .

Plant species - sources of essential oils for weed control

Summarizing the scientific reports in the literature the following plant species are among the most promising sources of essential oils for weed control: Myrtaceae: *Syzygium aromaticum* (L.) Merrill & Perry, *Eucalyptus citriodora* Hook, *E. astringens* Maiden; Poaceae: *Cymbopogon citratus* (DC.) Stapf, *C. nardus* (L.) Rendle, *C. flexuosus* D.C.; Lamiaceae: *Satureja montana* L., *Thymus vulgaris* L., *Rosmarinus officinalis* L., *Origanum* sp., *Micromeria fruticosa* (L.) Druce, *Mentha piperita* (L.) Huds., *Ocinum basilicum* L., *Mellisa officinalis* L., *Thymbra spicata* L. Pinaceae: *Pinus pinea* L., *Pinus brutia* Tenore, *Pinus nigra* J.F.Arnold, *Pinus halepensis* Mill.; Monimiaceae: *Peumus boldus*; Lauracea: *Cinnamomum zeylanicum* L.; Asteraceae: *Artemisia annua* L., *Tanacetum chiliophyllum* (Fisch. & Mey.) Schultz, *Achillea gypsicola* Hub-Mor., *Achillea biebersteinii* Afan.; Apiaceae: *Carum carvi* L.; Anacardiaceae: *Pistacia vera* L., *Pistacia terebinthus* L.; Cupressaceae: *Juniperus phoniceae* L., *Thuja occidentalis* Rutaceae: *Ruta graveolens* L. (ANGELINI *et al.*, 2003; TWORKOSKI, 2002; SETIA *et al.*, 2007; CAMPIGLIA *et al.*, 2007; SALAMCI *et al.*, 2007; KORDALI *et al.*, 2009; UREMIS *et al.*, 2009; DE ALMEIDA *et al.*, 2010; AMRI *et al.*, 2012a; AMRI *et al.*, 2012b; AMRI *et al.*, 2013b; RASSAEIFAR *et al.*, 2013; PINO *et al.*, 2013; ULUKANLI *et al.*, 2014; BLÁZQUEZ & CARBÓ, 2015; GRICHI *et al.*, 2016; BENVENUTI *et al.*, 2017; AMRI *et al.*, 2017).

Weed and crop species used as test objects

The ability of essential oils to inhibit seed germination was evaluated in many studies. The most commonly used weeds and crops in these experiments are *Amaranthus retroflexus* L., *Chenopodium album* L., *Portulaca oleracea* L., *Trifolium campestre* Schreb., *Lolium rigidum* Gaud., *Centaurea cyans* L. *Cynodon dactylon* (L.) Pers., *Lathyrus annuus* L., *Vicia villosa* Roth., *Capsicum annuum* L., *Sinapis arvensis* L., *Lactuca sativa* L., *Zea mays* L., *Raphanus sativus* L., *Brassica napus* L., *Phaseolus vulgaris*, *Triticum durum*, (RASSAEIFAR *et al.*, 2013; RAHIMI *et al.*, 2013; GRICHI *et al.*, 2016; HANANA *et al.*, 2017). It has been observed that dicots weeds are significantly more sensitive than monocots (AMRI *et al.*, 2012a; b); DAYAN *et al.*, 2009, 2012). SYNOWIEC *et al.*, (2017) suggest that the size of the seeds is important for the sensitivity to essential oils. Small seed are more sensitive than large such as *Zea mays* which appeared the most tolerant. Some authors conclude that weeds are more sensitive to essential oils than crops (GRICHI *et al.*, 2016; SYNOWIEC *et al.*, 2017).

Compounds with significant germination inhibitory activity

Many authors reported that the germination inhibitory activity of the essential oils is attributed to their relatively high content of monoterpenes, especially oxygenated ones (VOKOU *et al.*, 2003; AMRY *et al.*, 2012a; b; DHIFI *et al.*, 2016). Compounds such as carvacrol, thymol (DE ALMEIDA *et al.*, 2010; SYNOWIEC *et al.*,2016; ULUKANLI *et al.*, 2018), α- β- pinene (AMRI *et al.*, 2013b; ANGELINI *et al.*, 2003; SALAMCI *et al.*, 2007), 1,8 cineole, borneol, limonene, camphor (BENVENUTI *et al.*, 2017) have been defined as strong inhibitors of seed germination.

Mechanism of action of essential oils on plant cell

Mechanisms of action on essential oils as inhibitors of germination and growth have been insufficiently studied. It has been reported that essential oils induce oxidative damage in cells which is evidenced by the increased proline production and level of antioxidant enzymes. Also, essential oils damage the cell membrane that cause electrolyte leakage, inhibition of mitochondrial ATP production, cell proliferation, DNA synthesis and mitosis (ABRAHIM *et al.*, 2003; BAKKALI *et al.*, 2008; PERGO & ISHII-IWAMOTO, 2011; AMRI *et al.*, 2013a; RAHIMI *et al.*, 2013; GRICHI *et al.*, 2016). The herbicidal effect of d- limonene – main component of commercial product GreenMatch ™ O has been shown to be due to on the disruption of the leaf cuticle which leads to wilting (KOIVUNEN *et al.*, 2008).

Commercial products containing essential oils used for weed control

On the basis of essential oils, there are created several commercial products used for weed control (QUARLES, 2010; DAYAN *et al.*, 2009). GreenMatch EX® is a non-selective, post-emergent weed killer based on the essential oil of lemongrass (*Cymbopogon flexuosus*) - 50%. The best efficacy is achieved at 10 to 15% dilution rate v/v. Good coverage is required for good weed control. Younger weeds is are more sensitive and the product has a stronger effect on them than older (AVILA-ADAME *et al.*, 2018). GreenMatch ™ O contains 70% d-limonene (monoterpene of orange peels) and provides control of broadleaved and grass weeds at dilution 1:3 or as 18%. Good coverage is required for the best control especially at low temperature and for large weeds. (KOIVUNEN *et al.*, 2008). Matran II contains up to 50% clove oil (*Syzygium aromaticum*) and it is applied as 5-8% solution. Weed Zap ™ contains cinnamon and clove oil up to 30% (DAYAN *et al.*, 2009).

Essential oils from Bulgarian Flora with germination inhibitory activity

To the best of our knowledge, the studies in Bulgaria are mainly focused on the effects of water extracts on seed germination (DRAGOEVA *et al.*, 2010, 2014, 2017a, 2017b; PETROVA *et al.*, 2015; VALCHEVA *et al.*, 2018). Recently, YANKOVA-TSVETKOVA *et al.*, (2018) reported that essential oils of *Origanum vulgare* subsp. *hirtum* (Link) Ietsw, *Thymus longidentatus* (Degen & Urum.) Ronniger., *Thymus moesiacus* Velen, *Micromeria dalmatica* Benth. exhibited strong inhibitory activity on seed germination. The main components of essential oils identified for each species were respectively carvacrol (84,59%) for *O. vulgare* subsp. *hirtum*, citral isomers: neral (27,47%) and geranial (30,25%) of *T. longedentatus*, α-terpinyl acetate (76,24%) of *T. moesiacus*, piperitone oxide (34,66%), β-pinene (14,08%), carvacrol (10,75%), of *M. Dalmatica*.

Conclusion

The data presented in this review show that essential oils have strong inhibitory effects on germination and seedling growth of weeds and can be used manufacturing of products for weed control. There are plants species in the Bulgarian flora that also produce essential oils with potent herbicide activity which can be used for production of natural herbicides.

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