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Review article

REVIEWS ON PHYTOTOXIC EFFECTS OF ESSENTIAL OILS AND THEIR INDIVIDUAL COMPONENTS: NEWS APPROACH FOR WEEDS MANAGEMENT.Ismail Amri ^{a,b}, Lamia Hamrouni ^b, Mohsen Hanana^c and Bassem Jamoussi ^d.^aDépartement de Biologie, Faculté des Sciences de Bizerte. Zarzouna, 7021 Bizerte, Tunisie.^bLaboratoire d'Ecologie Forestière, Institut National de Recherches en Génie Rural, Eaux et Forêts. BP 10, 2080 Ariana, Tunisie.^cLaboratoire de Physiologie Moléculaire des Plantes, Centre de Biotechnologie de Borj-Cédria. BP 901, 2050 Hammam-lif, Tunisie.^dLaboratoire de Chimie, Institut Supérieur d'Education et de Formation Continue. 43 Rue de la Liberté, 2019 Le Bardo, Tunisie.

ABSTRACT: Currently, the use of synthetic chemicals to control weeds raises several concerns related to environment and human health. An alternative is to use natural products that possess good efficacy and are environmentally friendly. Among those, essential oils have been extensively tested to assess their herbicidal properties as valuable natural resource. The essential oils whose phytotoxic activities have been demonstrated, as well as the importance of the synergistic effects among their components are the main focus of this review. Essential oils are volatile mixtures of hydrocarbons with a diversity of functional groups (ketones, ether, ester, alcohol, phenol, aldehyde ...) and their herbicidal activity has been linked to the presence of monoterpenes and sesquiterpenes. However, in some cases, these chemicals can work synergistically, improving their effectiveness. Among the plant families with promising essential oils used as herbicide, Lamiaceae, Myrtaceae, Asteraceae and Anacardiaceae are the most cited. Individual compounds present in these mixtures with high activity include α -pinene, limonene, 1,8-cineole, carvacrol, camphor and thymol. Finally, although from an economical point of view synthetic chemicals are still more frequently used as herbicide than essential oils, these natural products have the potential to provide efficient and safer herbicide for humans and the environment.

Keys words: essential oils, terpenes, phytotoxicity, allelopathy, terpenes, chemotypes.

INTRODUCTION

The usually complex carbon skeleton of natural products derived from secondary metabolism is the result of natural selection of molecules that provided some protection against specific biotic challenges. Nature has, in a sense, performed a "high throughput" screen over long period of time to select particularly suitable biologically active compounds. The "high throughput" refers not to the rapidity of the selection, but rather to the innumerable permutations of relatively complex structures that have been made. Structural diversity that resulted from allelopathic interactions particularly between plant and biotic and abiotic challenges, has been, and still remains, an invaluable source of lead compounds in developing novel agrochemical products. Allelopathy is the science that studies processes in which biologically active compounds from plants and microorganisms are involved, affecting growth and development of biological systems (Fig.1)(Qiming et al., 2006; Singh et al., 2003). These studies of allelopathic interactions between plants are one of the most interesting strategies for herbicide discovery. The use of secondary metabolites implicated in allelopathic interactions as sources for news agrochemical models could satisfy the requirements for crop protection and weeds management (Singh et al., 2003; Dayan et al., 2009; Dudai et al., 1999). Most plants exhibit allelopathic effects on seed germination, growth and development of other plants by releasing allelochemicals into the soil, either as exudates from living organs or by decomposition of plant residues.

Allelochemicals are present in almost all plants and their tissues such as leaves, stems, roots, flowers, seeds, bark, and buds (Scrivanti et al., 2010). Allelochemicals alter a variety of physiological processes, they have significant effects on cell division and differentiation, ion and water uptake, phytohormone metabolism, respiration, photosynthesis and enzyme function. It is quite possible that allelochemicals may produce more than one effect on the cellular processes responsible for reduced plant growth and in the inhibition of seed germination (Koitabashi et al., 1997; Nishida et al., 2005; Weir et al., 2004; Abraham et al., 2003; Yang et al., 2008). On the other hand, plants have several strategies to avoid, detoxify and repair the damage caused by reactive oxygen species that the increase of proline content and the antioxidative enzyme activities such as superoxide dismutase, catalase and a variety of peroxidases (Weir et al., 2004; Abraham et al., 2003; Yang et al., 2008).

Essential oils and weeds management:

Weeds are defined as plants with no real use; these plants can grow in different habitats, especially cultivated fields. The presence of weeds in crop fields is generally unwanted by farmers for a number of reasons; Firstly, it reduces crop production by competing with the desired plants for the resources that a plant typically needs such as soil nutrients, water and space for growth and most important, they are considered in most cases as host plants for pests (Buririo et al. 2003; Kolahi et al., 2009). According to an estimate conducted in the United States by Pimentel et al. (2001), weeds cause crop loss which reaches 12% and costing to nearly US\$ 33 billion to control them. Furthermore, the increasing herbicidal resistance of weeds has resulted in a dramatic increase in the use of herbicides. Now days, scientists have focused on the increase of pesticide residues in food. This has encouraged researchers to look for new alternative pesticides. Terpenes represent the largest and most diverse group of secondary metabolites, and the most abundant constituents of essential oils. Over 25,000 individual isoprenoid compounds have been characterized (Gershenzon et al., 2007), but only a small fraction has been investigated to understand their functional perspective. For many years during the last century, terpenes were described as products of detoxification or waste products resulting from the primary metabolism. This concept was changed after 1970s, when several terpenes showed potential as toxins, repellents or attractants to other organisms, showing the importance of their ecological roles in antagonistic or mutualistic interactions among organisms. Although some terpene compounds show primary roles in plant metabolism, such as carotenoids in photosynthesis, ubiquinone in respiration, or the hormones abscisic acid, gibberellins or cytokinins in growth and development, most of terpenes have ecological roles being harmful or beneficial for the metabolism of other organisms (Gershenzon et al., 2007; Mizutani et al., 1997; Knudsen et al., 2006). More than 1700 volatile components of flower perfume have been isolated in about 1000 plant species, which are divided into three groups: terpenoids, aliphatic compounds and phenyl propanoids. The high variety of combinations of these components can favor plants to produce specific scents to attract pollinators, and make it easier for insects to locate host plants and floral rewards. This mutualism is rather precise, because each participant depends on the other one (Laothawornkitkul et al., 2008). It can also be the otherwise case, where volatile compounds act as repellents to insects.

Finally, mono- and sesquiterpenes are known to affect physiological processes in weeds, as photosynthesis, chlorophyll synthesis and cellular disruption, which can implicate the accumulation of lipid globules in the cytoplasm or reduction in organelles (De Feo et al., 2002). Recently they have been considerable interest in biologically active compounds from plants as source of bio-pesticides. Essential oils from aromatic plants are examples of compounds with potential to control pests; they are becoming more popular because many synthetic drugs are connected with unpleasant side effects. Volatile oils also represent an interesting alternative due to emerging resistance pests against synthetic agents. (Ghasemi et al., 2007; Koudouet al., 2008; Hegazy et al., 2007). In this context, the use of terpenoids implicated in allelopathic interactions as sources for new agrochemical models could satisfy the requirements for crop protection and weeds management (Singh et al., 2003). The phytotoxic potential of essential oils and their pure components have been also studied (Tab.1), in fact, earlier studies have documented that a lot of analyses have referenced that volatile oils or their constituents inhibit and delay seed germination and inhibit seedling growth of many weeds and cultivated crops (Singh et al., 2006; Amri et al., 2012).

The phytotoxic effects of essential oils have increased the interest in exploring volatile oil from aromatic plants for potential weed management (Singh et al., 2003; Dayan et al., 2009). These studies are important in view of the environmental and human health concerns of chemical weed control, and increasing resistance in weeds caused by synthetic herbicides; thus, there is a need to search for environmentally safer compounds. Important benefits of natural-based herbicides products, such as the absence of “unnatural” ring structures and the presence of few heavy atoms, are their short environmental half-lives and their tendency to affect novel target sites. This latter fact is particularly important since the need for new modes of action is pressing. Agrochemical companies are actively seeking novel mechanisms of action for which they can develop new chemistry.

Most studied compounds:

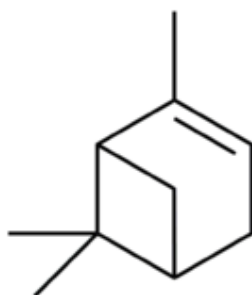
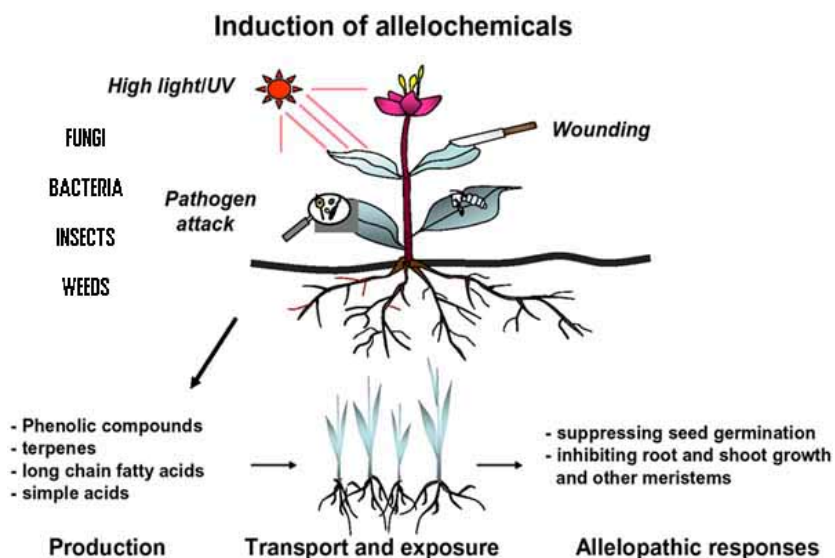
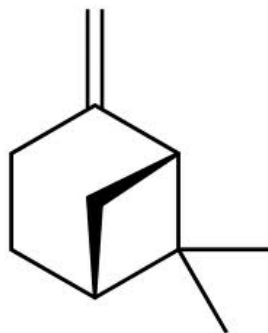
Phytotoxic effects of α and β -pinene:

α and β -pinene (Fig.2, 3) were two alkenes, bicyclic, hydrocarbonated monoterpenes and they contain a reactive four membered ring. They are found in the oils of many species of many coniferous trees, notably *Pinus* sp, *Cupressus* sp, *Pistacia* sp, they are also found in the essential oil of rosemary and myrtle. The interaction of α -pinene with plants of several species is a highly probable event in nature. It is now recognized that this component has an important ecological role on the allelopathic interactions between plants, so, it can be considered as a potential candidate for the development of new herbicides. In fact, Abraham et al. (2000), have shown that α -pinene inhibits seed germination and primary roots growth in maize, in 2003. The same authors have demonstrated that α -pinene, limonene, 1,8-cineole and camphor affect the respiratory activity of mitochondria of maize and soybean hypocotyl axes and α -pinene has been shown to be the most active among the all tested monoterpenes (Abraham et al., 2003a), in another report, Abraham et al., have demonstrated that α -pinene acts on energy metabolism of isolated mitochondria from maize by uncoupling of oxidative phosphorylation, inhibition of electron transfer and mitochondrial ATP production (Abraham et al., 2003b). In addition, an increase in malondialdehyde levels in maize roots has been reported (Scrivanti et al., 2003). De Feo et al., (2002), have tested the herbicidal activity of 10 volatile compounds from *Ruta graveolens* essential oils; they demonstrated that α -pinene significantly inhibits the germination and radical elongation of radish after 120 hours sowing and they indicated that α -pinene was the most potent inhibitor of germination at light and darkness. In another study initiated by De Martino et al., (2010) on the herbicidal activity of twenty seven monoterpenes such as α -pinene and its isomer β -pinene against germination and radical elongation of *Raphanus sativus* and *Lepidium sativum*, results obtained from this study revealed significant phytotoxic properties of these two compounds in comparison with all tested components. Pinenes were also found to reduce chlorophyll content in *Oryza sativa* coleoptiles, cell respiration, enzymatic activity of proteases, α and β -amylases, and root and coleoptiles length. Parallel, peroxidases and polyphenol oxidases activities increased in a dose-dependent way as a defence mechanism (Chowhan et al. 2011). Singh et al. (2006), have shown that α -pinene inhibits the germination and seedling growth of *Cassia occidentalis*, *Amaranthus viridis* and *Triticum aestivum* and the exposure of *Cassia occidentalis* roots to α -pinene enhanced solute leakage, and increased levels of malondialdehyde, proline and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress. On the other hand, activities of the antioxidant enzymes superoxide dismutase, catalase, guaiacol peroxidase, ascorbate reductase and glutathione reductase were significantly elevated, thereby indicating the enhanced generation of reactive oxygen species upon α -pinene exposure as shown in (Fig. 4).

Phytotoxic effects of 1,8-cineole:

Monoterpenes cineoles have been suspected of phytotoxic activity since the 1960s. 1,8-cineole (Fig.5) has been identified as one of the most potent allelochemicals released by many species like *Eucalyptus* sp. Laboratory studies support the field data by showing that 1,8-cineole, and its natural analogue 1,4-cineole, both suppress the growth of several weeds (Romagni et al., 2000a). Singh et al., (2002), have shown that 1,8-cineole inhibits the germination, speed of germination, seedling growth, chlorophyll content and respiratory activity of *Ageratum conyzoides*. De Feo et al., (2002), have tested the herbicidal activity of 10 volatile compounds from *Ruta graveolens* essential oils; they demonstrated that 1,8-cineole significantly inhibits the germination and radical elongation of radish at light and darkness.

A study conducted by De Martino et al., (2010) on the herbicidal activity of twenty seven monoterpenes such as 1,8-cineole against germination and radical elongation of *Raphanus sativus* and *Lipidium sativum*, results obtained from this study revealed significant phytotoxic properties of this compounds in comparison with all tested components.

Figure 2: α -pineneFigure 3: β -pinene

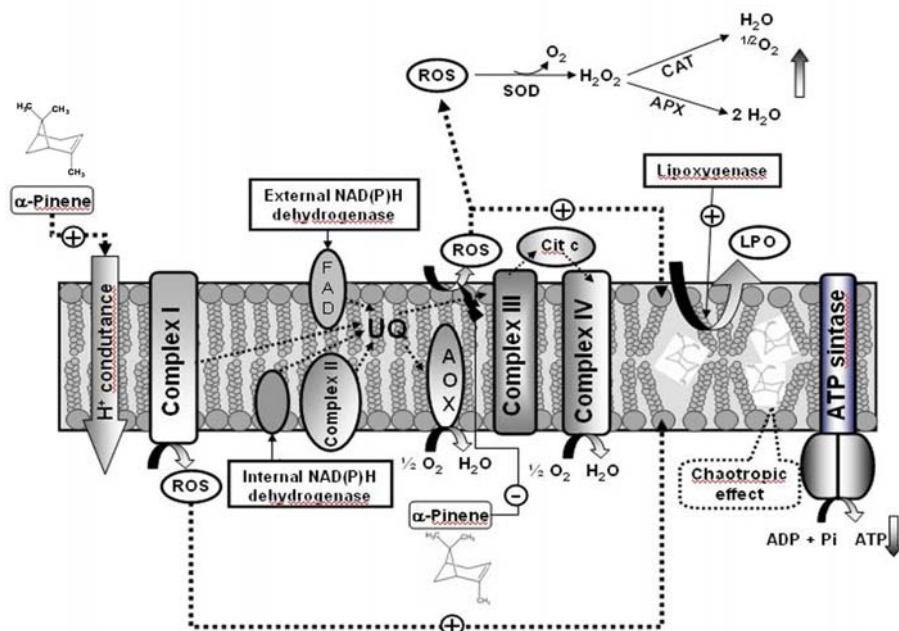


Figure 4: Proposed mechanism of action for the monoterpene α -pinene in the inner mitochondrial membrane.

The commercial herbicide cinnethylin is a 2-benzyl ether-substituted analogue of the natural product 1,4-cineole used for monocot weed control (Fig.6). Ell Deek and Hess (1986) have published that cinnethylin prevents entry of meristematic cells into mitosis. Cinnethylin has been examined for effects on polyamine synthesis, but no dramatic effects were noted (Di Thomas and Duke, 1991). Romagni et al., (2000b) indicated that cinnethylin and its analogue 1,4-cineole and 1,8-cineole share a same mechanism of action causing inhibition of asparagine synthetase. The benzyl ether moiety of cinnethylin must be cleaved to generate toxophore (most likely *cis*-2-hydroxy-1,4-cineole) to inhibit asparagine synthetase. Whereas the presence of the hydroxyl group probably enhances the level of interaction with the enzyme by decreasing the volatility, increasing the hydrophobicity of the inhibitor, this bulky apparently hinders the biological activity of the monoterpene center. The stereospecificity observed between the *cis* and *trans* analogs suggests that the hydroxyl group is also involved in the specific interaction between ligand and the active site.

Phytotoxic effects of monoterpenes phenylpropanoids: carvacrol and thymol

Carvacrol and thymol (Fig.7,8) are two monoterpenoid phenols which are frequently present on essential oils obtained from many species belonging *Lamiaceae* family like *Thymus* sp and *Origanum* sp.

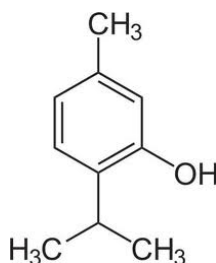


Figure 5: Thymol

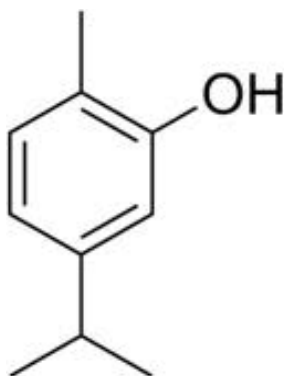


Figure 6: Carvacrol

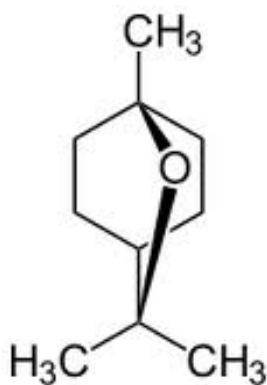


Figure 7: 1,8-cineole

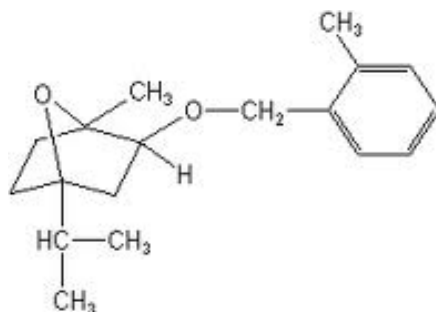


Figure 8: Cinmethylin

Many plants essential oils rich in carvacrol and or thymol have been reported to possess high herbicidal effects against the germination and seedling growth of weeds and cultivated crops (table 1).

Phytotoxic effects of pure thymol and carvacrol have been reported to inhibit the germination and seedling growth of: *Amaranthus retroflexus* and *Chenopodium album* and their effects were higher than the commercial herbicide 2,4.D isooctyl ester. (Kordali et al., 2008). Azirak (2008), have shown the herbicidal effects of carvacrol and thymol against the germination and radical elongation of *Raphanus sativus* and *Lepidium sativum*.

Azirak (2008), have evaluated the potential phytotoxicity of carvacrol and thymol against the germination and seedling growth of six weeds: *Sinapis arvensis*, *Amaranthus retroflexus*, *Centaurea salsotitalis*, *Raphanus raphanistrum*, *Rumex nepalensis*, *Alcea pallida* and *Sonchus oleraceus*. Obtained results show high phytotoxic effects against all tested weeds, only *Alcea pallida* showed resistances against these two compounds.

Table 1: Essential oils tested for their phytotoxic potential

Species	Families	Main constituents %	References
<i>Nepeta meyeri</i> Benth.	Lamiaceae	4aa,7a,7ab-Nepetalactone (83.40)	(Mutlu et al., 2011)
<i>Satureja montana</i> L.	Lamiaceae	δ -terpinene, <i>p</i> -cymene, carvacrol, 1,8-cineole	(Angelini et al., 2003; Grosso et al., 2010)
<i>Nepeta nuda</i> L. subsp. <i>albiflora</i>	Lamiaceae	β -bisabolene (11.8), pulegone (10.8), (<i>E,Z</i>)-nepetalactone (8.0), (<i>E</i>)- β -farnesene (7.1) and caryophyllene oxide (6.9)	(Macini et al., 2009a)
<i>Nepeta curviflora</i> Boiss.	Lamiaceae	β -caryophyllene (41.6), caryophyllene oxide (9.5), (<i>E</i>)- β -farnesene (6.2) and (<i>Z</i>)- β -farnesene (4.8)	
<i>Thymus vulgaris</i> L.	Lamiaceae	Thymol, <i>p</i> -cymene, δ -terpinene	(Almeida et al., 2010 ; Angelini et al., 2003 ; Grosso et al., 2010)
<i>Callicarpa americana</i> L.	Lamiaceae	Humulene epoxide II (13.9), α -humulene (10.1), 7-epi- α -eudesmol (9.5)	(Tellez et al., 2000)
<i>Rosmarinus officinalis</i> L.	Lamiaceae	α -pinene, 1,8-cineole, borneol	(Angelini et al., 2003)
<i>Origanum acutidens</i>	Lamiaceae	Carvacrol (87.0), <i>p</i> -cymene (2.0)	Kordali et al., 2008
<i>Lavandula</i> sp, <i>mentha piperita</i> , <i>cinnamomum zeylanicum</i> L.	Lamiaceae	Nv	(Cavailieri et al., 2010)
<i>Hyssopus officinalis</i>	Lamiaceae	iso- pinocamphone (29.1), (<i>Z</i>)- pinocamphone (11.2), β -pinene (18.2)	Almeida et al., 2010
<i>Mentha piperita</i>	Lamiaceae	menthol (44.99), menthone (20.78), menthofuran (4.32), 1,8-cineole (6.53), menthyl acetate (4.07)	Mucciarelli et al., 2001
<i>Cirsium creticum</i>	Asteraceae	4-ethyl guaiacol (15), hexadecanoic acid (10.6), (<i>E</i>)- β -damascenone (7.8)	(Formisano et al., 2007)
<i>Carduus nutans</i>	Asteraceae	Hexadecanoic acid (18.6), hexahydrofarnesylacetone (7.8)	(Formisano et al., 2007)
<i>Teucrium arduini</i>	Lamiaceae	(<i>Z</i>)- Caryophyllene (10) caryophyllene oxide(7.7) spathunelol (5.8)	De Martino et al., 2010
<i>Teucrium maghrebinum</i>	Lamiaceae	germacrene D (14.3) delta-cadinene (7.5) γ -cadinene(13.5)	
<i>Teucrium polium</i> ssp. <i>capitatum</i>	Lamiaceae	Carvacrol (9.6) (<i>Z</i>)-caryophyllene(10.1)	
<i>Teucrium montbretii</i> ssp. <i>heliotropiifolium</i>	Lamiaceae	carvacrol (13.5) caryophyllene oxide (8.8) (<i>Z</i>)-caryophyllene(8.2)	

<i>Lavandua stoechas</i> , <i>Lavandula angustifolia</i> , <i>mentha spicata</i> <i>Origanum onites</i> , <i>Origanum vulgare</i> , <i>Salvia fruticosa</i> <i>Salvia pomifera</i>	Lamiaceae	Nv	(Epameinondas et al., 2008)
<i>Lavandula angustifolia</i>	Lamiaceae	Linalol (23.1), borneol (6.3)	(Almeida et al., 2010)
<i>Majorana hortensis</i>	Lamiaceae	Linalol, 1.8-cineole, β -phellandrene, α -pinene	
<i>Melissa officinalis</i>	Lamiaceae	Citronellol, <i>iso</i> -menthone, carvacrol	
<i>Origanum vulgare</i>	Lamiaceae	<i>p</i> -cymene, carvacrol	
<i>Ocimum basilicum</i>	Lamiaceae	<i>iso</i> -pinocamphone, carvone	
<i>Salvia officinalis</i>	Lamiaceae	<i>trans</i> -thujone, camphor	
<i>Thymus vulgaris</i>	Lamiaceae	<i>p</i> -cymene, myrtenyl acetate	
<i>Salvia africana</i>	Lamiaceae	<i>p</i> -cymene (21.2), γ -terpinene (15.5), <i>t</i> -cadinol (13.6), α -eudesmol (10.7)	(De Martino et al., 2010)
<i>Salvia elegans</i>	Lamiaceae	<i>cis</i> -thujone (38.7), γ -cadinene (11.5)	
<i>Salvia greggii</i>	Lamiaceae	<i>cis</i> -thujone (43.4), γ -cadinene (14)	
<i>Salvia mellifera</i>	Lamiaceae	1,8-cineole (39.8), camphor (12.2)	
<i>Salvia munzii</i>	Lamiaceae	<i>cis</i> -thujone (33.3), camphor (27.2), γ -cadinene (8.9)	
<i>Salvia hierosolymitana</i> Boiss.	Lamiaceae	carbonylic compounds,	(Macini et al., 2009b)
<i>Salvia multicaulis</i> Vahl. var. <i>simplicifolia</i> Boiss.	Lamiaceae	monoterpenes and sesquiterpenes hydrocarbons	
<i>Eucalyptus citrodora</i>	Myrtaceae	N.V.	(Paudel et al., 2008)
<i>Eucalyptus camaldulensis</i>	Myrtaceae	<i>p</i> -cymene, cryptone, spathulenol	(Verdeguer et al., 2009)
<i>Tagetes minuta</i>	Asteraceae	Limonene (66.3), (<i>E</i>)-ocimenone (19.1)	(Scrivanti et al., 2003)
<i>Ageratum conyzoides</i>	Asteraceae	precocene II, precocene I, β -caryophyllene	(Kong et al., 2006)
<i>Achillea gypsicola</i> Hub-Mor.	Asteraceae	Camphor, 1,8-cineole,	(Kordali et al., 2009)
<i>Achillea biebersteinii</i> Afan.	Asteraceae	Camphor, 1,8-cineole, Borneol, piperitone,	
<i>Tanacetum chiliophyllum</i>	Asteraceae	1.8-cineole, camphor	(Salmaci et al., 2007)
<i>Tanacetum aucheraunm</i>	Asteraceae	1.8-cineole, camphor, borneol	
<i>Erioccephalus africanus</i>	Asteraceae	Artemisia ketone, Intermedeol	(Verdeguer et al., 2009)

<i>Artemisia scoparia</i> Waldst et Kit.	Asteraceae	Acenaphthene, β -Myrcene, limonene, <i>p</i> -cymene	(Kaur et al., 2010 ; Singh et al., 2009)
<i>Artemisia capillaris</i>	Asteraceae	Acenaphthylene (37.91), (Z)-caryophyllene (8.84), β -pinene (12.08), 4-carene (10.61)	(Won et al., 2009)
<i>Artemisia iwayomogi</i>	Asteraceae	Germacrene D (32.15), borneol (21.45), camphor (20.45)	
<i>Artemisia scoparia</i>	Asteraceae	β -myrcene (29.27), (+)-limonene (13.3), (Z)- β -ocimene (13.37), and γ -terpinene (9.51).	(kaur et al., 2010 ; Singh et al., 2009;)
<i>Santolina chamaecypariss-us</i> L.	Asteraceae	<i>p</i> -cymene , γ -terpinene, thymol, carvacrol	(Grosso et al., 2010)
<i>Chrysanthemoid-es monilifera</i> spp. <i>Rotundata</i>	Asteraceae	β -maaliene, α -isocomene, β -isocomene, δ -cadinene, 5-hydroxycalamenene	(Ens et al., 2008)
<i>Coriandrum sativum</i> L.	Apiaceae	Linalool, γ -terpinene, camphor	(Grosso et al., 2010)
<i>Pimpinella anisum</i>	Apiaceae	<i>cis</i> -anthenol	(Almeida et al., 2010)
<i>Foeniculum vulgare</i>	Apiaceae	fenchone, <i>cis</i> -anthenol	
<i>Carum carvi</i>	Apiaceae	estragole, limonene	
<i>Hypericum perforatum</i>	Clusiaceae	Germacrene D (17.1), (Z)- caryophyllene (12.3) γ -muurolene (11.1%).	(Marandino et al., 2011)
<i>Hypericum hircinum</i>		<i>cis</i> - β -guajene (27.5), γ -selinene (11.4), <i>n</i> -nonane(10.2)	
<i>Hypericum perforatum</i> ,		α -pinene (25.3%), thymol (15.8), <i>tau</i> -cadinol (11.5).	
<i>Helichrysum italicum</i> (Roth) Don ssp. <i>italicum</i>	Asteraceae	<i>iso</i> -italicene epoxide (16.8), β -costol (7.5), (Z)- α - <i>trans</i> -bergamotol (4.7), Hexadecene (9.8)	(Mancini et al., 2011)
<i>Lantana camara</i>	Verbenaceae	δ -Muurolene, α -curcumene, δ -Curcumene	(Verdeguer et al., 2009)
<i>Verbena officinalis</i>	Verbenaceae	<i>iso</i> -bornyl acetate, (<i>E</i>)-citral	(Almeida et al., 2010)
<i>Schinus areira</i>	Anacardiaceae	α -pinene (85.3), camphene (10.8)	(Scrivanti et al., 2003)
<i>Schinus molle</i> leaves	Anacardiaceae	Limonene + β -phellandrene (65.4), α -phellandrene (20.1)	(Zahed et al., 2010)
<i>Schinus molle</i> fruits	Anacardiaceae	Limonene + β -phellandrene (35.9), α -phellandrene (24.3)	
<i>Pistacia vera</i>	Anacardiaceae	α -terpinene (32.44), α -pinene (16.07), limonene (25.1)	(Amri et al., 2012a)
<i>Pistacia terebenthis</i>	Anacardiaceae	α -terpinene (41.34), α -pinene (19.21)	
<i>Juniperus phoniceae</i>	Cupressaceae	α -pinene (49.38), α -terpinene (8.26)	
<i>Pinus patula</i>	Pinaceae	α -pinene (35.24), β -phellandrene (19.51).	(Amri et al., 2011b)
<i>Pinus pinea</i> Lin	Pinaceae	limonene (54.1%), α -pinene (7.7), β -pinene (3.4).	(Amri et al., 2012b)

<i>Juniperus oxycedrus</i>	Cupressaceae	α -pinene (42), manoyl oxide (9 %), (Z)-caryophyllene (6 %)	(Amri et al., 2011a)
<i>Drimys winterii</i>	Winteraceae	terpinen-4-ol, elemol, δ , β -eudesmol,	(Verdeguer et al., 2011)
<i>Peumus boldus</i> Mol.	Monimiaceae	p-cymene, 1,8-cineole, ascaridole	
<i>Ruta graveolens</i> L.	Rutaceae	undecan-2-one, Nonan-2-one	(De Feo et al., 2002)
<i>Antirrhinum majus</i>	Scrophulariaceae	myrcene, (E)- β -ocimene, methyl benzoate	(Horiuchi et al., 2007)
<i>Cinnamomum camphora</i>	Lauraceae		(Paudel et al., 2008)
<i>Cymbopogon citratus</i>	Poaceae	Citral (76%)	(Paudel et al., 2008 ; Poonpaiboonpipat et al., 2013)
<i>Hibiscus cannabinus</i> L.	Malvaceae	(E)-phytol (28.16), (Z)-phytol (8.02)	(Kobaisy et al., 2001)
<i>Piper hispidinervium</i> C.DC.	Piperaceae	Monoterpenes and sesquiterpenes	(Souza Filho et al., 2009)
<i>Pogostemon heyneanus</i> (benth)	Lamiaceae	Oxygenated monoterpenes	
<i>Conyza albida</i> willd.ex	Compositae	cis-lachnophyllum (30.0)	(Tzakou et al., 2004)

Table 2: Some essential oil components known to have phytotoxic effects

Compounds	Nature (Adams ; 2007)	Functions (Adams ; 2007)	Formula (Adams ; 2007)	References
α -pinene	MH	Hydrocarbure	C ₁₀ H ₁₆	(Abraham et al., 2000, 2003a,b; De Feo et al. 2002 ; De Martino et al., 2010 ; Bulut et al., 2006 ; Kordali et al. 2006 ; Singh et al., 2006 ; Scrivanti et al., 2003 ; Vokou et al., 2003)
Limonene	MH	Hydrocarbure	C ₁₀ H ₁₆	(Abraham el al., 2000 ; Azirak et al., 2008 ; De Feo et al. 2002 ; De Martino et al., 2010 ; Vaid et al., 2011 ; Vokou et al., 2003)
1,8-cineole	MO	Ether	C ₁₀ H ₁₈ O	(Abraham el al., 2000, 20003a ; De Feo et al., 2002 ; De Martino et al., 2010 ; Koitabashi et al., 1997 ; Romangzi et al., 2000 a,b ; Singh et al., 2002 ; Vokou et al., 2003 ; Zunino and Zygadlo, 2005)
Thymol	MO	Phenol	C ₁₀ H ₁₄ O	(Angelini et al., 2003 ; Azirak et al., 2008 ; De Martino et al., 2010 ; Kordali et al., 2008 ; Vokou et al., 2003 ; Zunino and Zygadlo, 2005)
Carvacrol	MO	Phenol	C ₁₀ H ₁₄ O	
Eugenol	MO	Phenol	C ₁₀ H ₁₂ O ₂	(Bainard et al., 2006 ; Vaid et al., 2010)
Borneol	MO	Alcohol	C ₁₀ H ₁₈ O	(Angelini et al., 2003 ; De Martino et al., 2010 ; Vokou et al., 2003)
Carvone	MO	Ketone	C ₁₀ H ₁₄ O	(Azirak et al., 2008 ; De Martino et al., 2010 ; Hartmans et al. 1995; Hartmans et al., 2008 ; Vokou et al., 2003)
(Z)-caryophyllene	SH	Hydrocarbure	C ₁₅ H ₂₄	(Wang et al., 2009)
Citronellol	MO	Alcohol	C ₁₀ H ₂₀ O	(De Martino et al., 2010 ; Singh et al., 2002 ; Vokou et al., 2003)

p-cymene	MH	Hydrocarbure	C ₁₀ H ₁₄	(De Martino et al., 2010 ; Kordali et al., 2008 ; Vokou et al., 2003)
Camphor	MO	Ketone	C ₁₀ H ₁₆ O	(Abraham el al., 2000, 20003a ; De Martino et al., 2010 ; Vokou et al., 2003 ; Zunino and Zygadlo, 2005)
α-terpineol		Alcohol	C ₁₀ H ₁₈ O	De Martino et al., 2010
γ-terpinene		Hydrocarbure	C ₁₀ H ₁₆	
Estragole	MO	Methoxy	C ₁₀ H ₁₂ O	
Citral	MO	Aldehyde	C ₁₀ H ₁₆ O	(Chaimovitsh et al., 2011 ; De Martino et al., 2010)
Geraniol	MO	Alcohol	C ₁₀ H ₁₈ O	(De Martino et al., 2010 ; Vokou et al., 2003 ; Zunino and Zygadlo, 2005)
Geranyl acetate	MO	Ester	C ₁₂ H ₂₀ O ₂	(De Martino et al., 2010 ; Barney et al., 2005 ; Vokou et al., 2003)
Linalool	MO	Alcohol	C ₁₀ H ₁₈ O	
Linalyl acetate	MO	Ester	C ₁₂ H ₂₀ O ₂	
Camphene	MH	Hydrocarbure	C ₁₀ H ₁₆	
α-phellandrene	MH	Hydrocarbure	C ₁₀ H ₁₆	
α-β-thujone	MO	Ketone	C ₁₀ H ₁₆ O	
β-myrcene	MH	Hydrocarbure	C ₁₀ H ₁₆	
α-terpinene		Hydrocarbure	C ₁₀ H ₁₆	(De Martino et al., 2010 ; Bulut et al., 2006 ; Kordali et al., 2006 ; Vokou et al., 2003)
β-pinene		Hydrocarbure	C ₁₀ H ₁₆	(Chowhan et al., 2012 ; De Martino et al., 2010 ; Kordali et al., 2006 ; Vokou et al., 2003)
Decan-2-one	NT	Ketone	C ₁₀ H ₂₀ O	(De Feo et al., 2002)
Methyl salicylate	NT	Ester	C ₈ H ₈ O ₃	
Nonan-2-ol	NT	Alcohol	C ₉ H ₂₀ O	
Nonan-2-one	NT	Ketone	C ₉ H ₁₈ O	
Octanoic acid	NT	Acid	C ₈ H ₁₆ O ₂	
Tridecan-2-one	NT	Ketone	C ₁₃ H ₂₆ O	
Undecan-2-one	NT	Ketone	C ₁₁ H ₂₂ O	
Valeric acid	NT	Acid	C ₅ H ₁₀ O ₂	
Menthol	OM	Ketone	C ₁₀ H ₂₀ O	(De martino et al., 2010 ; Mucciarelli et al. 2001; Vokou et al., 2003 ; Zunino and Zygadlo, 2005)
Menthone	OM	Ketone	C ₁₀ H ₁₈ O	
Pulegone	OM	Alcohol	C ₁₀ H ₁₆ O	(Maffei et al., 2001; Mucciarelli et al. 2001 ; Vokou et al., 2003)
Citronellal	OM	Aldehyde	C ₁₀ H ₁₈ O	(Singh et al., 2002; Vokou et al., 2003)
Myrtenal	OM	Aldehyde	C ₁₀ H ₁₄ O	(Vokou et al., 2003)
Limonene oxide	OM	Ether	C ₁₀ H ₁₆ O	
Terpen-4-ol	OM	Alcohol	C ₁₀ H ₁₈ O	
neo-menthol	OM	Alcohol	C ₁₀ H ₂₀ O	
iso-menthol	OM	Alcohol	C ₁₀ H ₂₀ O	
iso-pulegol	OM	Alcohol	C ₁₀ H ₁₈ O	
Dihydrocarveol	OM	Alcohol	C ₁₀ H ₁₈ O	
Carveol	OM	Alcohol	C ₁₀ H ₁₆ O	
iso-borneol	OM	Alcohol	C ₁₀ H ₁₈ O	
Menthyl acetate	OM	Acetate	C ₁₂ H ₂₂ O ₂	
iso-pulegyl acetate	OM	Acetate	C ₁₂ H ₂₀ O ₂	
Carvyl acetate	OM	Acetate	C ₁₂ H ₁₈ O ₂	
Bornyl acetate	OM	Acetate	C ₁₂ H ₂₀ O ₂	
Fenchone	OM	Ketone	C ₁₀ H ₁₆ O	
Dihydrocarvone	OM	Ketone	C ₁₀ H ₁₆ O	
2-carene	MH	Hydrocarbure	C ₁₀ H ₁₆	
3-carene	MH	Hydrocarbure	C ₁₀ H ₁₆	

MH : monoterpenes hydrocarbons ; OM : Oxygenated monoterpenes ; SH : Sesquiterpenes hydrocarbons ; NT : Non terpenic compounds

Table 3: Pearson correlations among essential oils doses, roots growth and relative electrolyte leakage (REL)

	Doses	Roots growth	REL
Doses	1	-0,916**	0,910**
Roots growth		1	-0,811**
REL			1

** Significant correlations at $p < 0.01$.

Mechanisms of essential oil effects on germination and seedling growth inhibition **Essential oils and their pure components induces loss of membrane integrity by generation of reactive oxygen species**

Monoterpenes are broadly lipophilic compounds, it has been demonstrated that most of their biological activities are mediated through direct interactions with the lipid bilayer portions of biological membranes, changing their lipid packing densities and the fluidity and/or physical arrangements of the phospholipids in the membranes (Witzke et al., 2010). In recent study, we have shown that essential oils of *P. terebinthus*, *P. vera* and *J. phoniceae* caused significant loss of membrane integrity from on the roots *S. arvensis*, *T. campestre*, *L. rigidum* and *P. canariensis* as measured by increased relative electrolyte leakage. This indicates that tested oils disrupt membrane permeability resulting in solute leakage. Obtained results indicated that relative electrolyte leakage was variable depending on weed species; generally, dicots were more sensitive than monocots and depending to the oil species. The increase of relative electrolyte leakage was concentration-dependent (Pearson's correlations between doses and REL are shown in table 3). It was observed that at the dose of 1 µl/ml, all essential oils induced a relative electrolyte leakage ranged between (19.81 and 37.17%) and increased with increasing dose to be ranged between 38.8 and 65.7% at the dose of 3 µl/ml. On the other hand, inhibition of roots growth and increasing of REL were significantly correlated (-0,811 at $p < 0.01$) as presented in (Tab. 3) (Amri et al., 2012). Singh et al. (2009), Artemisia oil and β -myrcene caused a significant ion leakage from roots of *C. rotundus* as indicated by increased electrical conductivity of the bathing medium. The ion leakage increased with time up to 20 h in the dark and then for another 8 h in light, which indicates cellular membrane disruption resulting in excessive solute leakage. The effects of β -pinene on membrane integrity of *Phalaris minor* were studied by Chowhan et al. (2012) and it have shown that β -Pinene (≥ 0.04 mg/ml) enhanced electrolyte leakage by 23–80%, malondialdehyde content by 15–67%, hydrogen peroxide content by 9–39%, and lipoxygenases activity by 38–83% over that in the control. It indicated membrane peroxidation and loss of membrane integrity that could be the primary target of β -pinene. Even the enhanced (9–62%) activity of protecting enzymes, peroxidases, was not able to protect the membranes from β -pinene (0.04-0.20 mg/ml)-induced toxicity. In fact, the studies that were conducted on artificial monolayer membranes by Turina et al. (2003, 2006), obtained results demonstrated that the monoterpenes camphor, cineole, thymol, menthol and geraniol penetrate into the dipalmitoyl-phosphatidylcholine monolayer at the air-water interface. They affect the topology of phosphatidylcholine vesicles, increasing their surface curvatures, through their localisations at the polar head group regions of the membranes, particularly the oxygenated constituents. Witzke et al. (2010), studied the interactions of four structurally related monoterpenes, limonene, perillyl alcohol, perillaldehyde and deprotonated perillic acid, with model lipid bilayers, using molecular dynamics simulations and isothermal titration calorimetry. They found that limonene, perillyl alcohol and perillaldehyde have an ordering effect on the lipid bilayer and that they can diffuse across the membrane. Deprotonated perillic acid has a disordering and membrane thinning effect. Essential oils of *N. meyeri* significantly increased H_2O_2 levels of the seven weed species and increased lipid peroxidation activity of all tested weed plants (Mutlu et al., 2010). The volatile oil from *Tagetes minuta* and *Schinus areira* and its monoterpenes (ocimene, ocimenone, α -pinene, 1,8-cineole, thymol, geraniol, menthol, and camphor) have been reported to inhibit maize growth by causing lipid peroxidation and enhanced MDA content due to lipid peroxidation indicates an induction of ROS-generated oxidative stress (Scrivanti et al., 2003; Zunino and Zygadlo 2004). Observed membrane damage upon exposure to Artemisia oil or β -myrcene was confirmed further by a significant decline in conjugated dienes content. In fact, upon exposure to ≥ 0.14 mg/ml of Artemisia oil or β -myrcene, the conjugated dienes content in roots of *C. occidentalis* decreased by 21–59% and 9–13%, respectively which indicate a ROS-induced damage to biological membranes (Singh et al., 2009).

Twoorkoski et al. (2002) reported that essential oils from *S. hortensis*, *T. vulgaris*, *S. aromaticum* and *C. zeylanicum* (1% v/v) cause electrolyte leakage and cell death in *Taraxacum officinalis* leaf discs. In the leaf tissues of *E. crus-galli* and *C. occidentalis* that were sprayed with *Artemisia* oil, Kauret al. (2010), observed a dose-dependent increase in electrolyte leakage. Besides changes in membrane properties, Zunino and Zygadlo (2004), demonstrated changes in fatty acid composition and lipid peroxidation in roots from maize seedlings (*Zea mays*) that were grown in monoterpene-saturated headspaces. They examined the effects of 1,8-cineole, geraniol, thymol, menthol, and camphor at concentrations that caused 80% inhibition on root growth (21.7, 2.0, 1.9, and 7.4 mg/l, respectively). The monoterpenes 1,8 cineole, geraniol, thymol, menthol and camphor caused variable changes in the different unsaturated and saturated fatty acids, but they generally tended to increase the ratios of unsaturated to saturated fatty acyls. Another study examined the effects of the same monoterpenes on the concentrations of sterol esters, free sterols and phospholipid fatty acids (Zunino and Zygadlo, 2005). At 96 hours of treatment, each monoterpene caused quantitative and qualitative modifications in the phospholipid fatty acids, the free sterol fraction and saturated sterol ester fatty acids. The alcoholic monoterpenes geraniol and menthol together with camphor increased the percentage of unsaturated phospholipid fatty acids and increased stigmasterol to a greater extent than the nonalcoholic monoterpenes. So, the loss of membrane integrity affects all other physiological and biochemical processes linked to membrane functions such as the penetration of monoterpenes through the cell wall and cell membrane, or causes a leakage of cellular potassium and inhibits glucose-dependent respiration. In addition, monoterpenes are lipophilic, and may induce the expansion of cell membranes, which increases fluidity, destroys the membrane structure and inhibits membrane enzymes (Singh et al., 2009; Zunino and Zygadlo, 2005; Chowhan et al., 2012).

Mitochondrial Respiration

In general, it is difficult to discern whether the effects of monoterpenes described above are due to a primary action or are the consequences of an alternative primary event. Most of the fundamental cellular activities that are modified, including the transport of ions and solutes across membranes, the synthesis of molecules and macromolecules, such as membranes lipids, chlorophyll, proteins and nucleic acids, and cell division, require a source of metabolic energy. A perturbation on mitochondrial ATP production may therefore lead to the impairment of many of these physiological processes, particularly during seed germination and initial seedling growth when ATP production is greatly dependent on the mitochondrial metabolism. Thus, impaired mitochondrial metabolism could be a primary mode of action of some monoterpenes. It is clear that the resumption of mitochondrial respiration occurs very early during imbibition, indicating the presence of functional mitochondria in the dry seeds (Botha et al., 1992; Neuburger et al., 1996). Besides their crucial function of providing ATP supplies, the mitochondria also contribute to pathways that are related to germination and reserve mobilisation (Neuburger et al., 1996). Studies that were conducted by the Rolletschek (2003) group demonstrated that as soon as seeds are imbibed, internal oxygen levels fall to very low levels because of the mitochondrial activities. Muller et al. (1968, 1969) were the first to suggest that monoterpenes act on mitochondrial respiratory activities. They observed that emission of monoterpenes from *Salvia leucophylla* leaves diminished oxygen uptake by the seedlings and excised roots of *Cucumis sativus* as well as inhibited oxygen uptake by mitochondria that were isolated from *Avena fatua*. To examine the mechanisms by which monoterpenes affect mitochondrial respiration, Abraham et al., evaluated the effects of four different monoterpenes on mitochondria that were isolated from the primary roots of two cultivated species, corn (*Zea mays*) and soybean (*Glycine max*) (Abraham et al., 2000, 2003a). They found that the effects of monoterpenes are similar irrespective of the source of the mitochondrial preparation (corn or soybean). This is in correlation with another study in which the responses of mitochondria that were isolated from corn coleoptiles or primary roots to α -pinene were observed to be very similar (Abraham et al., 2003b). Among the monoterpenes that were assayed the most active were found to be α -pinene, followed by limonene, 1,8-cineole and camphor (Abraham et al., 2000a,b). The finding that all assayed monoterpenes activate state IV respiration and decrease the respiratory coefficient ratio suggests that they share a common action, the uncoupling of oxidative phosphorylation.

Proline accumulation:

In general, various types of environmental stresses (including abiotic and xenobiotic) mediate their impact through oxidative stress caused by generation of reactive oxygen species. ROS, such as singlet oxygen ($^1\text{O}_2$), superoxide radicles (O_2^-), hydroxyl radicles ($\text{OH}^\cdot$) and hydrogen peroxide (H_2O_2), are highly reactive and toxic molecules that can cause oxidative damage to membranes, DNA, proteins, photosynthetic pigments and lipids .

Recently, ROS generation and related oxidative stress has been proposed as one of the modes of action of plant growth inhibition by allelochemicals (Weir et al., 2004). To avoid the cellular damage due to reactive oxygen species generation, plants have evolved various defense strategies such as proline accumulation. Like other abiotic environmental stresses such as salt and drought, it has been documented that essential oils and their individual components increases levels of proline, which acts as an electron acceptor and prevents damage to membranes. So the increased proline content in plants tissues may be evaluated as an important response against the increasingly oxidative stress caused by essential oils. Treatment with α -pinene significantly increased the amount of endogenous proline in root tissue of *C. occidentalis*. Proline content increased by nearly 1.3-fold at 2.5 mM α -pinene concentration compared with the control, whereas at 5 mM concentration the increase was nearly 1.9-fold. Proline acts as an electron acceptor and prevents damage to membranes. It also provides protection against ROS-induced disruption of photosystems (Singh et al., 2006).

In a recent study, we assessed the effect of essential oil of *Juniperus phoeniceae* on roots proline content of *S. arvensis*, *T. campestre*, *L. rigidum* and *P. canariensis*. According obtained results, essential oil of juniper induced a significant accumulation of proline in the root tissues of all weeds. The increase of proline content was concentration-dependent. At the dose of 1 μ l/ml, the increase compared with the control was nearly 2.23, 1.76, 1.52 and 1.16 fold and it further increased to nearly 3.35, 4.04, 3.91 and 4.22 fold at the dose of 3 μ l/ml, respectively on the roots of *S. arvensis*, *T. campestre*, *L. rigidum* and *P. canariensis*.

Inhibition of photosynthesis

There are several studies about the effects of essential oils and their individual monoterpenes on photosynthetic efficiency of plant. Generally, there is reduction in the chlorophyll concentrations of leaves treated with essential oils or monoterpenes. Kaur et al. (2010) observed that the application of *Artemisia* oils on 6-week-old weed plants caused losses in chlorophyll concentrations in the leaves and injuries, ranging from chlorosis to necrosis. Singh et al. (2002) also found reduced chlorophyll concentrations in mature *C. occidentalis* and *E. crus-galli* plants that were sprayed with 2.5% to 7.5% of eucalypt oils. Citronellol, citronellal, cineole and linalool also reduced chlorophyll concentrations in the leaves of *C. occidentalis* (Singh et al., 2002). Romagni et al. (2000), measured photosynthetic efficiency in the weed monocot *E. crus-galli* and weed dicot *C. obtusifolia* that had been treated with 1,4- and 1,8-cineole at concentrations ranging from 10 to 1000 μ g per gram of sand. 1,4-cineole caused decreased photosynthetic yields in both species. Klinger et al. (1991) showed that α -pinene in squash causes the *in vitro* degradation of envelope membranes and complete inhibition of electron transport in photosystem II, which occurs on the chloroplast membranes (*Cucurbita pepo*).

Inhibition of DNA synthesis and mitosis

It has been suggested that the inhibition of DNA synthesis may be a mode of action of monoterpenes in reducing cell proliferation in root apical meristem. Koitabashi et al. (1997) demonstrated that 1,8-cineole drastically reduces the mitotic index of the root apical meristem in *Brassica campestris*. They also verified using immunofluorescence microscopy with antibodies against BrdU that 1,8-cineole inhibits the synthesis of both cell nuclear and organellar DNA synthesis. Vaid et al. (2011), have reported that eugenol decreased seedling length, seedling dry weight and germination of *Cassia occidentalis* and *Bidens pilosa*. Furthermore, it caused reduction in chlorophyll content, photosynthetic efficiency and cellular respiration. It was suggested that it may cause disruption of mitotic activity by microtubule disorganization or alteration of cell wall biosynthesis (Vaid et al., 2011). Nishida et al., (2005) have studied the effects of five monoterpenes on *Brassica campestris*. The IC₅₀ values that were necessary for root growth inhibition after four days of treatment were estimated to be 90, 140, 150, 270 and 570 mM for camphor, 1,8-cineole, α -pinene, β -pinene and camphene, respectively. The measurements of the mitotic indices in the shoot apices and root apical meristems revealed that monoterpenes do not inhibit cellular proliferation in the shoot apex, but all of them decrease both the mitotic indices and DNA synthesis activities in the root apical meristem. The effects of 1,8-cineole on cell proliferation and elongation were also examined in BY-2 suspension-cultures of tobacco (*Nicotiana tabacum*) by Yoshimura et al. (2011). They found similar results as Nishida et al. (2005) and Koitabashi et al. (1997) with regards to the observation that 1,8-cineole preferentially inhibits root growth over hypocotyl growth. However, Yoshimura et al. (2011) observed that 1,8 cineole is more efficient in inhibiting cell elongation and reducing starch concentrations than in affecting cell proliferation, indicating that the effects of 1,8 cineole are not specific to cell proliferation.

CONCLUSION

The development of natural herbicides would help to decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution. In this respect, essential oils, as natural herbicides, present two main characters: the first is their natural origin which means more safety to the people and the environment, and the second is that they have been considered at low risk for resistance development by weeds. It is believed that it is difficult to develop resistance to such a mixture of oil components with apparently different mechanisms of action.

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