*Available online at http://www.journalijdr.com*

ISSN: 2230-9926

*International Journal of Development Research*
*Vol. 06, Issue, 06, pp. 7988-7994, June, 2016*

## *Full Length Research Article*

# THE ALLELOCHEMICALS OF *EUCALYPTUS MAIDENII* ESSENTIAL OIL AND ITS POTENTIAL AS NATURAL HERBICIDE

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**ARTICLE INFO**

***Article History:***
Received 26th March, 2016
Received in revised form
14thApril, 2016
Accepted 22nd May, 2016
Published online 30th June, 2016

***Key Words:***
Allelopathy,
*Eucalyptus maidenii*,
Essential oil,
Bioherbicide.

**ABSTRACT**

This study aimed to explore the allelopathic potential of *Eucalyptus maidenii* on seed germination, root and shoot length, membrane integrity, malondialdehyde (MDA) and proline content of *Triticum durum, Vicia faba, Phaseolus vulgaris, Sinapis arvensis, Erica vesicaria* and *Scorpiurus muricatus* and its application as naturally occurring herbicide in the field. Allelopathic compounds were identified using chromatography mass analysis. Dose-response studies were carried under laboratory and greenhouse conditions. Germination, emergence and seedling growth of test species were significantly inhibited in a dose-response bioassay. In a greenhouse, observation of leaf wilt symptoms was noted at 6h after treatment. Chlorophyll content was decreased with increasing of concentrations indicating that essential oil affects the photosynthetic activity. In addition, *E.maidenii* essential oil induces an electrolyte leakage indicating membrane damage and loss of integrity and enhanced the level of proline suggesting induction of oxidative stress. The test plants responded differently to *E.maidenii* essential oil exhibiting a differential species-specificity. Indeed, the weeds were affected more strongly than the crops. Developing the natural herbicide to replace the traditional agrochemicals becomes an important subject seeing that the use of synthetic agrochemicals has caused serious environment pollution due to their poor biodegradability. These results indicated that *E.maidenii* essential oil exhibited potential as natural herbicides and provide helpful information to develop new and potent bioactive chemicals from natural products.



## INTRODUCTION

The intensive use of synthetic agrochemicals is of increasing concern due to contamination of environment and has enhanced the resistance in weeds to herbicides. Therefore, scientists have focused on searching for plant compounds to develop bioherbicides as alternative strategy for weed control (Cantrell *et al.,* 2012; Dayan *et al*., 2009). Allelopathy has been defined as any direct or indirect effects of one plant, including micro-organisms, on another through the release of chemical compounds into the environment. It may also include the subsequent influence on growth and development of nearby plants through both inhibitory and stimulatory biochemical interactions. These chemicals inhibit the growth of a species at a certain concentration and may also stimulate the growth of same species or another at lower concentration (Rice, 1974, 1984; Tang, 1986).

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The use of allelopathy in agriculture has become one strategy for weed management (Bhadoria *et al.*, 2011). Most plants exhibit allelopathic effects on seed germination and development of other plants by releasing secondary metabolites into the soil, either as exudates from living organs or by plant residues decomposition (Scrivanti *et al.*, 2010). After a variety of physiological processes, allelochemicals causes significant changes on cell division and differentiation, ion and water absorption, phytohormone metabolism, photosynthesis, respiratory activity and enzyme function. In general, allelochemicals causes several effects on the cellular processes implicated in plant growth and in the inhibition of seed germination (Koitabashi *et al*., 1997; Yang *et al*., 2008). On the other hand, plants have several strategies to avoid, detoxify and repair the damage caused by reactive oxygen species such as an increase of proline content and the antioxidative enzyme activities: catalase, superoxide dismutase and many peroxidases (Weir *et al*., 2004; Yang *et al*., 2008). Among the allelochemicals released by plants,

volatile essential oils were considered the most important. Many studies have proved that volatile oils or their components inhibit and delay seed germination and inhibit seedling growth of many weeds and crops (Angelini *et al*., 2003). The phytotoxic effects of essential oils have attracted the interest in exploring volatile oil extracted from aromatic plants for potential weed management (Singh *et al*., 2003; Dayan *et al*., 2009; Batish *et al*., 2008). These studies are fundamental in view of the environmental and human health preoccupation of chemical weed control and increasing resistance in weeds caused by chemicals products. Consequently, there is necessary to search for environmentally safer compounds. It was reported that the essential oil from *Eucalyptus tereticornis* inhibits the growth of *Lens culinaris* seedlings and that from Tasmanian blue gum (*Eucalyptus globulus* Labill.) inhibits the growth of *Phaseolus aureus*, *Hordeum vulgare* and *Avena sativa* (Kohli *et al*., 1991). Also, it was demonstrated that the volatile oil from *Eucalyptus citriodora* and Tasmanian blue gum inhibits the germination and seedling growth of ragweed *Parthenium hysterophorus*. Thus, these could be used for weed management (Kohli *et al*., 1998). Of late, the essential oils induce ROS generation and cause oxidative damage (Singh *et al*., 2006; Mutlu *et al*., 2011) and reactive oxygen species (ROS) generation resulting in oxidative stress has been suggested as one of the modes of action of allelochemicals caused plant growth inhibition (Weir *et al*., 2004; Cruz-Ortega *et al.,* 2007). Nevertheless, the details concerning the effect of *E. maidenii* on generation, in situ detection, and metabolism of ROS aren't mentioned before. This research aimed to identify and quantify the phenolic compounds by HPLC and determine phytotoxic effects of *E.maidenii* essential oil. This research will promote a better understanding of allelopathy mechanisms in the natural and agricultural ecosystems by investigating the allelopathic effect and quantification of causative allelochemicals.

## MATERIALS AND METHODS

### Extraction of volatile oil from *E.maidenii*

Leaves were collected in April 2011 from *E.maidenii* trees acclimated in Souiniet arboreta (located in Ain Draham, north of Tunisia). The *E.maidenii* essential oils were extracted by hydrodistillation of 100g of dried leaves for 4h according to the standard method described in the European Pharmacopoeia. Hydrodistillations were performed in triplicate. The yield in essential oil was expressed in % (v/w) of the dry material.

### *Chemical characterization of the oil*

The chemical composition of the extracted essential oil was determined by gas chromatography-mass spectroscopy (GC-MS).

***GC Analysis*:** GC Analysis was carried out with a Hewlett-Packard 6890 apparatus equipped with FID and an intermediately polar Supelco SPB-20 cap. Column (30m×0.32 mm i.d., film thikness 0.25 µm). The oven temp. was programmed isothermal at 35°C for 1 min, rising from 35 to 250°C at 5°/min, and then held isothermal at 250°C for 3 min; injector temp., 250°C; detector temp., 280°C; carrier gas, N2 (1.2 ml/min). The injected volume was 1 µl (10% essential oil in purified hexane). The relative concentration was determined using the software HP Chemstation, which allowed assimilating the percentages of the different compounds. Retention indices (RI) were determined according to the retention times ($t_R$) of a series of n-alkanes (C9-C28) (Elaissi *et al*., 2010).

***GC/MS Analysis*:** The essential oils were analyzed with a Hewlett-Packard 5890 series II apparatus equipped with a 5972 mass-selective detector and an intermediately polar Supelco SPB-20 cap. Column (30m*0.32mm i.d., film thickness 0.25 µm). He was used as the carrier gas. The operating conditions of the mass spectrometer were: ionization voltage, 70 eV; ion source, 230°C. The GC anal. Conditions were as described in GC Analysis.

***Compound Identification*:** The identification of the compounds was based on the comparison of their RI and mass spectra with those of principal constituents by means of the NBS75K.L. and Wiley 275 databases and with literature data (Willey *et al.,* 1998).

### Dose-response studies

**Seeds of all test species:** *Sinapis arvensis, Erica vesicaria, Scorpiurus muricatus*, *Triticum durum, Vicia faba* and *Phaseolus vulgaris* were collected locally from agricultural fields on Ousseltia (located in Kairouan, centreast of Tunisia, with arid bioclimatic stage). These were surface-sterilized with sodium hypochlorite (0.1%, w/v) for 3 min, washed under running tap water (for 3min) followed by distilled water and stored for further use. Dose-response studies were conducted under laboratory conditions to determine the effect of eucalypt oil on growth of test species. Briefly, 10 seeds of each test plants were placed in Petri dishes (15cm diameter) on two layers of Whatman filter paper wetted with 7ml of distilled water (control) or with the different assayed doses of eucalypt oil (0.12, 0.25, 0.5 and 0.75µl/ml) after spacing the seeds on the base. Each concentration was replicated five times. Then, Petri dishes were closed immediately with an adhesive tape to avoid escaping of volatile compounds and were kept in a growth chamber maintained at 16/18h light/dark period at 25±2°C temperature. Seven days after treatment, the germination rate and root and shoot lengths of test plants were measured. The percent of germination inhibition, root and shoot lengths were calculated according to the following equation: Inhibition (% of control) = (100-(sample extracts/control) ×100) (Charoenying *et al*., 2010).

### Greenhouse study

Experiments were conducted in the greenhouse in order to test the herbicidal activity of the volatile oil from *E.maideni* under field conditions. Seeds of all test species were sown manually in 15cm pots. For this, 1200g of garden soil was taken in each pot and seeds of *Sinapis arvensis, Erica vesicaria, Scorpiurus muricatus*, *Triticum durum, Vicia faba* and *Phaseolus vulgaris* were sown. Pots were placed in experimental house with natural light conditions (Temperature 21°C, Humidity 32%, Sunshine7hj-1) and irrigated daily. When the plants were 4-

week-old, they were spray treated with 25, 50, 75 and 100µl/ml solution of eucalypt oil (or distilled water to serve as control) in such a manner that each plant received 6ml of treatment. One- two and 3-days after spray (DAS), the treated test plants were examined for lipid peroxidation, proline content and electrolyte leakage. Each concentration was replicated five times.

### Lipid peroxidation

In order to explore the phytotoxic effect of *E.maidenii* allelochemicals on lipid peroxidation, malondialdehyde (MDA) content was measured following Heath and Packer method (1968). Indeed, 100mg of test species leaf were homogenized in TCA (5 ml, 0.1%, w/v) and centrifuged at 10 000*g for 10 min. To 4 ml of thiobarbaturic acid (0.5%, w/v, in 20%, w/v, TCA) was added 1ml of the supernatant. After, the mixture was, for 30min, heated at 95°C, cooled over ice, and centrifuged at 95°C, cooled over ice, and centrifuged at 10 000 g for 10 min. At 532 nm, the supernatant absorbance was recorded and corrected for non-specific absorbance at 600 nm. Finally, MDA content was calculated using =155 $mM^{-1}$ $cm^{-1}$ and expressed as nmol $g^{-1}$ (Kaur *et al.*, 2012).

### Proline content

The proline content measurements were made according the method described by Bates *et al*. (1973) was used. Indeed, 0.1g of leaf tissues was digested, for 30 min at 100°C, in 3 ml of 3% sulfosalicylic acid followed by centrifugation at 2000 g for 5 min at 25°C. Two ml of the reagent mixture (30 ml glacial acetic acid, 20 ml distilled water and 0.5 g Ninhydrin) and 0.4 ml of distilled water was added 0.2 ml of the extract. After boiling for 1h, the samples were cooled and extracted with 4 ml of toluene. The toluene phase absorbance was determined at 520 nm and proline content was calculated using a standard curve and expressed as µM $g^{-1}$ f.wt (Bates *et al.,* 1973).

### Relative electrolyte leakage

Relative electrolyte leakage was determined in leaf of test species treated with eucalypt oil, to study the phytotoxic effect on solute leakage and consequently their effects on loss of membrane integrity. To measure the medium conductivity ($C_1$), leaf tissues were immersed in distilled water for 60 min. After boiling for 30min, the conductivity ($C_2$) was again measured in test tubes containing leaf tissues [17]. To calculate the relative electrolyte leakage (REL), following formula was used: $\%REL= (C_1/C_2)*100$. The REL was expressed in percent.

### Statistical analyses

All data obtained from seed germination, seedling growth, chlorophyll content, membrane integrity, malondialdehyde (MDA) and proline content assays of test species were expressed as mean values and were, on the condition of significant ANOVA, analyzed by means of multiple comparison SNK tests in order to investigate if significant differences existed between eucalypt oil concentrations and test species. Values of $p \leq 0.05$ were considered significantly different.

## RESULTS AND DISCUSSION

### Chemical characterization of the eucalypt oil

The essential oil was obtained by hydrodistillation of *E.maidenii* leaves, which gave an oil of 1.54% yield. The chemical composition of *E.maidenii* oils, the percentage content of the individual components and the retention indices are summarized in table1. 40 compounds were identified accounting for 95.2% of the total oil. The monoterpene hydrocarbons fraction represents 20.2% and the main compounds were α-Pinene (8.7%) and ρ-Cymene (7.3%). Whereas, the Oxygenated monoterpenes fraction amounted 65.1% and the major compound was 1,8-Cineole (58.2%). The sesquiterpene hydrocarbons accounted (3.1%) and Oxygenated sesquiterpenes (3.9%). Finally, the lowest fraction was accorded to the aliphatics compound (2.9%) (Table 1). The presence of 1,8-cineole as the major compound in *E.maidenii essential oil*, α-pinene and ρ-Cymene representing high proportion is in agreement with earlier studies (Elaissi *et al.,* 2010).

**Table 1. Chemical composition of the essential oils extracted from freshly collected mature leaves of *E.maidenii***

| Compound class and name | RI | Composition (%) |
|---|---|---|
| *Monoterpenehydrocarbons* | | 20.2 |
| α-Thujene | 922 | 0.1 |
| α-Pinene | 930 | 8.7 |
| β-Pinene | 970 | 0.1 |
| Verbenene | 975 | 0.2 |
| β-Myrcene | 980 | 1 |
| Limonene | 1006 | 2.4 |
| ρ-Cymene | 1015 | 7.3 |
| α -terpinene | 1067 | 0.1 |
| Terpinolene | 1089 | 0.3 |
| *Oxygenated monoterpenes* | | 65.1 |
| Camphor | 1125 | 0.1 |
| Myrtenal | 1137 | 0.2 |
| Borneol | 1150 | 1 |
| Terpinene-4-ol | 1163 | 0.9 |
| α-Terpineol | 1176 | 3 |
| Fenchol | 1203 | 1.1 |
| Geranial | 1224 | 0.1 |
| Carvaol | 1226 | 0.2 |
| Linalool | 1240 | 0.1 |
| Carvacrol | 1279 | 0.2 |
| 1,8-Cineole | 1282 | 58.2 |
| *Sesquiterpene hydrocarbons* | | 3.1 |
| Aromadendrene | 1434 | 2.3 |
| Alloaromadendrene | 1477 | 0.5 |
| β-Gurjunene | 1506 | 0.1 |
| δ-Cadinene | 1517 | 0.1 |
| α-Humulene | 1519 | 0.1 |
| *Oxygenated sesquiterpenes* | | 3.9 |
| Viridiflorol | 1579 | 0.3 |
| β-Eudesmol | 1362 | 2.5 |
| α-Eudesmol | 1466 | 0.8 |
| Palustrol | 1562 | 0.1 |
| Caryophyllene oxide | 1575 | 0.1 |
| Ledol | 1585 | 0.1 |
| *Aliphatics compound* | | 2.9 |
| (z)-2-heptenal | 926 | 1.1 |
| 1-Octen-3-ol | 959 | 1 |
| Decane | 1000 | 0.2 |
| Nonanal | 1081 | 0.1 |
| 2-phenylethanol | 1119 | 0.1 |
| Decanal | 1182 | Tr |
| Octylacetate | 1191 | 0.1 |
| Decanol | 1253 | 0.1 |
| Tricosene | 2300 | 0.2 |
| *Total identified (%)* | | 95.2 |

*R.I: Retention Index; Tr: Trace (<0.1%)*

**Growth studies under laboratory conditions**

Phytotoxic effects of *E.maidenii* essential oil were tested on germination and seedlings growth of the six tested species. Providing statistical analysis, the germination of all tested species was significantly reduced. In general, a dose-response relationship was observed and the emergence decreased with the increase in concentration of *E.maidenii* essential oil. At 0.25µl/ml *E.maidenii* essential oil, there was no significant effect on emergence of test species, except in *S.murucatus*, where 62.5% decrease was observed. However, at 100µl/ml *E.maidenii* essential oil, 85% and 62.5% emergence was observed respectively in *Vicia faba* and *Triticum durum*. But, any germination was observed in the case of *P.vulgarus* and *S.arvensis* (Table 2). Not only germination, even the seedling growth measured as root and shoot length was significantly reduced even at 0.25µl/ml *E.maidenii* essential oil. At 0.5µl/ml *E.maidenii* essential oil 73.3 to 97.69 % reduction was observed in root length of tested species. The reduction was greater with increasing amount of *E.maidenii* essential oil (Table 2). Likewise, the shoot length of tested species was significantly reduced in response to *E.maidenii* essential oil, but with varying degrees of susceptibility. Also, the shoot growth was further reduced when eucalypt oil concentration increased. In general, the phytotoxic effect was greater on weeds than on crops except for *P.vulgarus* (Table 2).

The results obtained in the present study are parallel to earlier reports documenting the growth inhibitory activity of aromatic plants, including *Eucalyptus* species and their volatile oils. For instance, volatile oil (0.12-0.30 mg/ml) from *Eucalyptus citriodora* inhibit seedling growth and reduced dry weight accumulation in *Cassia occidentalis, Amaranthus viridis* and *Echinochloa crus-galli* by ≥ 50% (Batish *et al.,* 2004). It was demonstrated that essential oils from *Thymus vulgaris, Rosmarinus officinalis* and *Satureja montana* (at 500ppm) severely reduced germination potential and seedling growth of weeds such as *Portulaca oleracea, Chenopodium album* and *E.crus-galli* (Angelini *et al.,* 2003). Later, it was reported that *E.citriodora* oil (at 0.2-5.0 nl/ml) reduced seed germination and seedling growth of *P.hysterophorus* by 56-100% (Singh *et al*., 2005). The volatile oil from *Tagetes minuta* (at 100-1000ppm) was demonstrated that inhibited the germination of weed species such as *Mikania cordifolia, Taraxacum officinale* and *Cynodon dactylon* (López *et al*., 2008). Recently, it was reported that volatile oil from *Artemisia scoparia* (at 0.14-0.35mg/ml) inhibited radical emergence and seedling growth in *Cyperus rotundus* and *Phalaris minor* (Singh *et al.,* 2009). The inhibition of seedling growth may either be due to synergistic or additive effect of compounds in *E.maidenii* essential oil. Allelopathy is the result of the accumulative action of various compounds and often includes compounds with divergent chemistry (Einhellig *et al.,* 2002).

**Table 2. The effect of E.maidenii essential oil on germination rate, root and shoot length of tested species**

| *Triticum durum* | Germination (% of Control) ±SD | Root length (% of Control)±SD | Shoot length (% of Control) ±SD |
|---|---|---|---|
| Doses | | | |
| 0.25 µl/ml | 95.00±5.77 | 31.00±3.67 | 21.04±2.20 |
| 0.5 µl/ml | 75.00±5.77 | 26.67±5.86 | 13.61±1.25 |
| 0.75 µl/ml | 65.00±5.77 | 24.67±3.17 | 11.39±0.57 |
| 1 µl/ml | 62.50±5.00 | 12.67±1.33 | 10.64±0.95 |
| *Vicia faba* | Germination (% of Control) ±SD | Root length (% of Control)±SD | Shoot length (% of Control) ±SD |
| Doses | | | |
| 0.25 µl/ml | 95.00±5.77 | 27.50±5.69 | 26.11±2.80 |
| 0.5 µl/ml | 92.50±5.00 | 20.00±1.36 | 23.89±3.33 |
| 0.75 µl/ml | 87.50±5.00 | 19.17±1.67 | 23.33±1.28 |
| 1 µl/ml | 85.00±5.77 | 17.08±0.83 | 10.00±1.28 |
| *Phaseolus vulgarus* | Germination (% of Control) ±SD | Root length (% of Control)±SD | Shoot length (% of Control)±SD |
| Doses | | | |
| 0.25 µl/ml | 47.50±5.00 | 2.31±0.89 | 7.73±1.74 |
| 0.5 µl/ml | 20.00±0.00 | 2.31±0.89 | 0.45±0.91 |
| 0.75 µl/ml | 17.50±2.89 | 1.62±0.15 | 0.00±0.00 |
| 1 µl/ml | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| *Erica vesicaria* | Germination (% of Control) ±SD | Root length (% of Control)±SD | Shoot length (% of Control)±SD |
| Doses | | | |
| 0.25 µl/ml | 52.78±5.56 | 18.14±1.88 | 13.46±1.47 |
| 0.5 µl/ml | 25.00±5.56 | 8.82±1.96 | 8.46±1.99 |
| 0.75 µl/ml | 22.22±0.00 | 2.45±0.98 | 6.92±1.99 |
| 1 µl/ml | 19.44±5.56 | 1.96±0.00 | 2.69±0.77 |
| *Sinapis arvensis* | Germination (% of Control) ±SD | Root length (% of Control)±SD | Shoot length (% of Control)±SD |
| Doses | | | |
| 0.25 µl/ml | 75.00±5.77 | 19.32±1.31 | 0.00±0.00 |
| 0.5 µl/ml | 42.50±5.00 | 18.18±3.21 | 0.00±0.00 |
| 0.75 µl/ml | 37.50±5.00 | 2.84±1.14 | 0.00±0.00 |
| 1 µl/ml | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| *Scorpuirus murucatus* | Germination (% of Control) ±SD | Root length (% of Control)±SD | Shoot length (% of Control)±SD |
| Doses | | | |
| 0.25 µl/ml | 37.50±0.00 | 18.48±4.16 | 13.00±2.00 |
| 0.5 µl/ml | 28.13±6.25 | 10.87±2.51 | 9.00±2.00 |
| 0.75 µl/ml | 18.75±7.22 | 4.35±0.00 | 5.00±2.00 |
| 1 µl/ml | 15.63±6.25 | 4.02±0.65 | 3.00±2.00 |

This ecological phenomenon is considered the main cause of dominance and successful colonization of a particular exotic species in invaded community of plant (Barney *et al.,* 2005; Ens *et al.,* 2009).

**Eucalypt oil induce Proline accumulation**

Plants have developed several defense strategies such as proline accumulation to prevent the cellular damage due to reactive oxygen species generation. Thus, the increased proline content in leafs may be evaluated as an important response against the increasingly oxidative stress resulting from essential oils. Our results indicated that essential oil of *E.maidenii* induce a significant accumulation of proline in the leaf tissues of all the test species especially for the weeds species. The increasing of proline content was concentration-dependant. At 100 µl/ml, the increase, over the control, was ~45, 56, 49, 83, 84 and 73 mg/g for *T.durum, V.faba, P.vulgarus, Erica vesicaria, S.arvensis* and *S.murucatus*, respectively (table3). An increased level of proline is a common response of plants to abiotic stress. Although the mechanism of proline accumulation in stressed plants is largely unknown; nevertheless, it gets accumulated as osmolyte and regulates cytosolic acidity, avoids oxidation of membranes, acts as singlet oxygen quencher and protect against free radicals (Szabados *et al.,* 2010). Stress reduced activity of electron transport system, thereby resulting in NADH and $H^+$ accumulation. Since these two molecules are used for the synthesis of proline from glutamic acid, there occurs an accumulation of proline (Venekemp *et al*., 1989).

**Eucalypt oil causes ion leakage**

*Eucalyptus maidenii* volatile oil caused significant loss of membrane integrity and cell death in test species leaf, as measured by the increasing of relative electrolyte leakage (REL). The electrolyte leakage obviously showed a difference depending on the concentration of essential oil and the test species. There was no significant difference in treatment of 25 and 50 µl/ml compared with the control. However, at 100 µl/ml eucalypt oil concentration, the electrolyte leakage was clearly increased by ~17%, 21%, 20%, 35%, 37% and 32% for *T.durum, V.faba, P.vulgarus, E.vesicaria, S.arvensis* and *S.murucatus*, respectively (Table 3). The observations made in present study are in agreement with earlier reports on the essential oils and their constituents that reduce plant growth through electrolyte leakage (Singh *et al*., 2005). Terpenes of volatile oils disrupt fluxes across plasma membrane and damage membrane permeability resulting in oxidative burst (Singh *et al*., 2006). Essential oils penetrate cell membranes, particularly mitochondrial, disturb their permeability and induce alteration (Bakkali *et al*., 2008). *Artemisia* oil disrupts membrane integrity and increase electrolyte leakage from the roots of *Cyperus rotundus* (Singh *et al*., 2009). Allelopathic compounds are known to depolarize and disrupt cell membranes thereby enhancing their permeability, inducing lipid peroxidation and finally leading to cell death (Singh *et al*., 2009; Yu *et al*., 2003).The change of membrane permeability in turn affects other physiological and biochemical activity linked to membrane function as lipid peroxidation.

**Table 3. Effect of *E.maidenii* essential oil on electrolyte leakage, MDA and proline contents of six test species**

| *Triticum durum* | MDA content (nM/gMF)±SD | Electrolyte leakage (%)±SD | Proline content (mg/g) ±SD |
|---|---|---|---|
| Control | 10.90±0.17 | 9.67±0.58 | 11.33±1.15 |
| 25 µl/ml | 12.73±0.55 | 12.61±0.42 | 13.80±0.50 |
| 5 µl/ml | 14.30±0.66 | 20.60±1.13 | 33.23±0.59 |
| 75 µl/ml | 17.07±1.19 | 23.10±0.46 | 41.20±1.21 |
| 100 µl/ml | 20.67±1.12 | 26.83±0.96 | 56.20±1.51 |
| *Vicia faba* | MDA content (nM/gMF) ±SD | Electrolyte leakage (%) ±SD | Proline content (mg/g) ±SD |
| Control | 20.23±0.68 | 11.00±1.00 | 9.83±0.29 |
| 0.25 µl/ml | 20.67±0.42 | 11.97±0.25 | 13.77±0.25 |
| 0.5 µl/ml | 22.43±0.38 | 14.03±0.55 | 32.10±0.85 |
| 0.75 µl/ml | 24.97±0.31 | 18.90±0.17 | 39.37±0.91 |
| 1 µl/ml | 34.33±0.85 | 31.73±1.27 | 66.07±1.07 |
| *Phaseolus vulgarus* | MDA content (nM/gMF) ±SD | Electrolyte leakage (%)±SD | Proline content (mg/g) ±SD |
| Control | 19.83±0.29 | 7.33±0.58 | 12.67±0.58 |
| 25 µl/ml | 20.6±0.66 | 9.07±0.40 | 16.13±0.57 |
| 5 µl/ml | 22.87±1.03 | 17.07±0.57 | 26.13±3.23 |
| 75 µl/ml | 25.43±0.75 | 23.27±0.95 | 44.60±2.35 |
| 100 µl/ml | 28.73±0.81 | 27.77±0.96 | 61.60±1.49 |
| *Erica vesicaria* | MDA content (nM/gMF) ±SD | Electrolyte leakage (%) ±SD | Proline content (mg/g) ±SD |
| Control | 18.00±1.00 | 5.83±0.29 | 8.53±0.50 |
| 25 µl/ml | 19.10±0.36 | 10.70±0.62 | 32.90±2.26 |
| 5 µl/ml | 26.63±1.44 | 18.27±0.93 | 65.17±1.93 |
| 75 µl/ml | 34.67±1.56 | 24.23±0.68 | 85.00±2.93 |
| 100 µl/ml | 47.73±1.07 | 41.07±1.33 | 91.73±1.5 |
| *Sinapis arvensis* | MDA content (nM/gMF) ±SD | Electrolyte leakage (%)±SD | Proline content (mg/g) ±SD |
| Control | 16.50±0.50 | 7.17±0.76 | 9.83±0.76 |
| 25 µl/ml | 18.37±1.06 | 14.27±2.00 | 25.53±2.80 |
| 5 µl/ml | 24.5±0.95 | 16.50±1.00 | 75.93±1.70 |
| 75 µl/ml | 34.43±0.93 | 26.57±0.95 | 85.27±1.72 |
| 100 µl/ml | 52.13±1.46 | 44.67±1.88 | 93.90±3.29 |
| *Scorpuirus murucatus* | MDA content (nM/gMF) ±SD | Electrolyte leakage (%) ±SD | Proline content (mg/g) ±SD |
| Control | 15.33±0.58 | 7.83±0.29 | 11.43±0.51 |
| 25 µl/ml | 17.30±1.61 | 11.93±1.68 | 20.57±1.05 |
| 5 µl/ml | 26.20±0.80 | 17.77±1.79 | 54.37±1.83 |
| 75 µl/ml | 28.70±0.95 | 28.07±0.60 | 75.00±2.36 |
| 100 µl/ml | 40.20±1.15 | 40.07±0.40 | 84.70±1.23 |

**Eucalypt oil affects the lipid peroxidation**

Our results revealed that MDA production didn't have a significant change at lower concentration (25µl/ml) for all the test species. The amount of MDA increased significantly in response to higher concentrations (75 and 100µl/ml). It increased by ~10, 14, 9, 30, 36, 25 nM/gMF for *T.durum, V.faba, P.vulgarus, Erica vesicaria, S.arvensis* and *S.murucatus*, respectively, over the control in response to 100µl/ml. In general, the increase of lipid peroxidation was greater on weeds than on crops (Table 3). Enhanced MDA content is an indicator of lipids peroxidation (Heath *et al*., 1968). The oxidative degradation of lipids induces lipid peroxidation. As fatty acids and other lipids are known as membranes structural constituents, it is correct to suppose that membrane disruption could result free lipids in the cytoplasm of targeted cells. The free lipids of the cytoplasm could be the target of an oxidative action (Scrivanti *et al*., 2003). Some studies have reported that volatile oil from various allelopathic plants and their constituents caused accumulation of $H_2O_2$ in some plant species (Singh *et al*., 2006; Singh *et al*., 2009). In addition, it has been reported that the effect of essential oils can disturb the permeability of weeds cell membrane structure. This is due to the penetration of allelochemicals through the cell wall and cell membrane, or induces a leakage of cellular potassium that inhibits respiration (Mutlu *et al.,* 2011). Root exudates and root extracts of *Cucumis sativus* and phenolic acids increased membrane peroxidation in cucumber (Yu *et al*., 2003).

**Conclusion**

Essential oil of *E.maidenii* showed a strong phytotoxicity against weeds more than crops. According to our knowledge, this is the first data related to the herbicidal activity of this *Eucalyptus* specie. These results may serve as benchmark information for further studies on the elucidation of chemicals involved in phytotoxicity against weeds.

**Acknowledgements**

This study was funded by grants from the National Institute for Research in Rural Engineering, Water and Forests and the Faculty of Sciences of Bizerta.

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