INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-1, Jan-Mar-2016 Coden IJABFP-CAS-USA Copyrights@2016

Received: 7th Dec-2015 *Revised: 24th Dec -2015* *Accepted: 29th Dec -2015*

Research article

HERBICIDAL ACTIVITY OF *EUCALYPTUS ASTRINGENS* AND ITS PHYTOTOXIC COMPONENTS

Aida GRICHI[1,2], Zouhair NASR[2], Mohamed Larbi KHOUJA[2]

[1]Faculty of Sciences of Bizerta, 7021 Jarzouna, Tunisia

[2]National Institute for Research in Rural Engineering, Water and Forests Street Hédi Elkarray, Elmanzeh IV, Ariana 2080, Tunisia

ABSTRACT: A study was undertaken to assess the phytotoxic effect of *E.astringens* essential oil on seed germination, root and shoot length, chlorophyll content, membrane integrity, malondialdehyde (MDA) and proline content of *Triticum durum, Vicia faba, Phaseolus vulgaris, Sinapis arvensis, Erica vesicaria* and *Scorpiurus muricatus* with a view to exploit them for the future weed management . Dose-response studies were conducted under laboratory and greenhouse conditions. Germination, emergence and seedling growth of test species were significantly reduced 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.astringens* 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 eucalypt oil exhibiting a differential species-specificity. Indeed, the weeds were affected more strongly than the crops. *E.astringens* essential oil exhibit strong phytotoxicity activity against weeds especially and possesses weed-suppressing abilities. Hence, these could be a base for developing bioherbicides.

Key words: Allelopathy, *Eucalyptus astringens*, Essential oil, Bioherbicide.

*Corresponding author: Aida GRICHI, [1]Faculty of Sciences of Bizerta, 7021 Jarzouna, Tunisia, aida.grichi@gmail.com

INTRODUCTION

Traditional agrochemicals, such as herbicides, fungicides, insecticides and nematocides, have been used intensively in current agriculture and several useful chemicals products have been discovered during the last decade (Dayan et al., 2009). The overuse of synthetic agrochemicals causes different environmental hazards, nutrients deficiency and a global degradation of soil physiochemical properties (Chou et al., 2010). Thereby, developing naturally occurring agrochemicals to replace the synthetic agrochemicals becomes an interesting subject in sustainable agriculture (Cantrell et al., 2012, Dayan et al., 2009). Allelopathy was discovered by Molish and meant that a "plant releases compounds to either stimulate or inhibit the growth of other plant growing in the same habitat in natural and agricultural ecosystem" (Molish, 1937), can play an eco-friendly role in weed management. These studies of allelopathic interactions between plants are one of the most important strategies for herbicide discovery. Indeed, the use of allelochemicals involved in allelopathic interactions could satisfy the requirements for weeds management and crops protection (Singh et al., 2003). 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 increased 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 proposed 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. astringens* on generation, in situ detection, and metabolism of ROS aren't mentioned before. Therefore, we conducted this study to explore the effect of *E.astringens* essential oils on: seed germination, radical length, seedling growth, chlorophyll content, membrane integrity, lipid peroxidation (MDA) and proline content of three crops and three weeds species with a view to exploit them for the future weed management.

MATERIALS AND METHODS

Extraction of essential oil from *E.astringens*

Leaves were collected in April 2011 from *E.astringens* trees acclimated in Korbous arboreta (located in Nabeul, northeast of Tunisia, with a sub-humid bioclimatic stage). The *E.astringens* 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 (Wiley, 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 essential oil from *E.astringens* 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 chlorophyll content levels. Each concentration was replicated five times.

Estimation of chlorophyll content

Chlorophyll content was measured using a chlorophyll content meter CCM-200 (SPAD).The CCM-200 is a handheld, battery operated instrument used for the non-destructive and rapid determination of chlorophyll content in intact leaf samples. This innovative instrument can be applied to a multitude of crop production and research projects.

The chlorophyll content meter provides instantaneous measurements which can be done in the field under normal conditions. All obtained data can be downloaded to a computer for additional analyses using the software and data cable of the CCM-200.

Lipid peroxidation

In order to explore the phytotoxic effect of *E.astringens* 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 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 (Singh et al., 2006).

In order 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.astringens* leaves, which gave an oil of 1.2% yield. The GC-MS analyses indicated that *E.astringens* essential oil shared a high proportion of the 1, 8-cineole (44.7%) and a relatively high mean percentage of α-pinene (20.2%). The aromadendrene (Sesquiterpene hydrocarbons) and the β-cymene (monoterpene hydrocarbons) represent respectively (9.1%) and (8.3%) of the total *E.astringens* essential oil (Table.1). The presence of 1,8-cineole as the major compound in *E.astringens*, α-pinene and β-cymene representing high proportion is in agreement with earlier studies (Elaissi et al., 2010).

Growth studies under laboratory conditions

The germination of all the test species was significantly reduced. In general, a dose-response relationship was observed and the emergence decreased with the increase in concentration of *E.astringens* essential oil. At 0.12µl/ml *E.astringens* essential oil, there was no significant effect on emergence of test species, except in *S.arvensis* and *S.murucatus*, where 82 and 87% respectively decrease was observed (Fig.1). However, at 0.75µl/ml *E.astringens* essential oil, 3% emergence was observed in *S.murucatus*, *7% in S.arvensis* and 15% in *E.vesicaria* (Fig.1a).

Not only germination, even the seedling growth measured as root and shoot length was significantly reduced even at 0.12µl/ml *E.astringens* essential oil. At 0.25µl/ml *E.astringens* essential oil 23 to 94 % reduction was observed in root length of tested species. The reduction was greater with increasing amount of *E.astringens* essential oil (Fig.1b and c). Indeed, at highest concentration (0.75 µl/ml), the maximum inhibition in root length was observed in *S.murucatus* and *E.vesicaria* (98 and 88% respectively) (Fig.1b). Likewise, the shoot length of test weeds was significantly reduced in response to *E.astringens* 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 (Fig.1a, b and c).

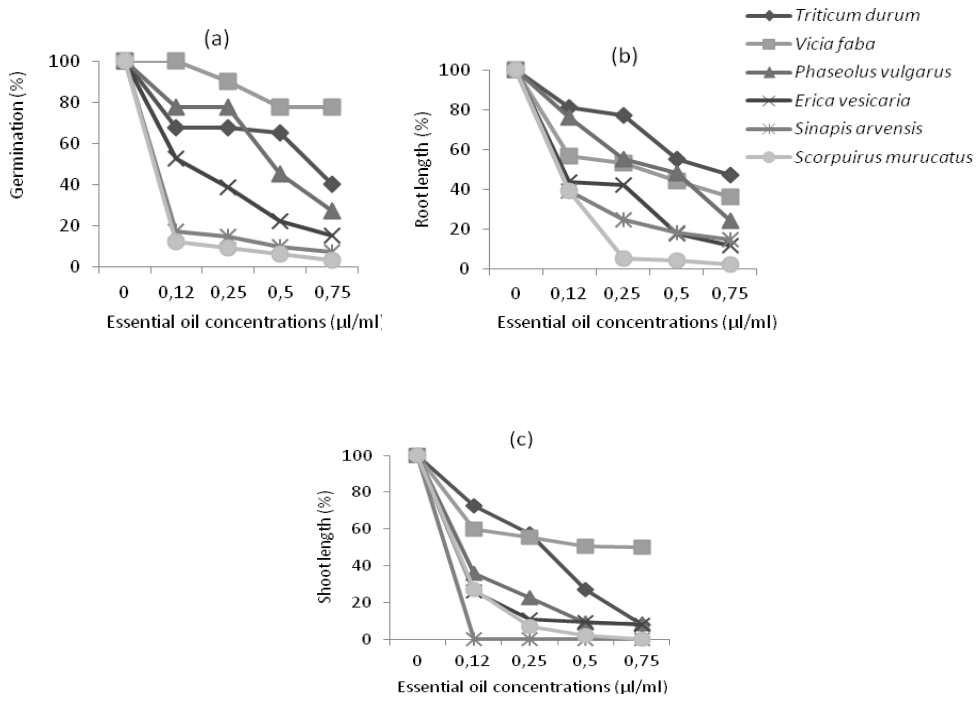


Fig.1. The effect of *E.astringens* essential oil on germination rate (a), root (b) and shoot length (c) of test species.

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

Compound class and name	RI[a]	Composition (%)
Monoterpenehydrocarbons		**30.9**
α-Thujene	922	0.1
α-Pinene	930	20.2
β-Pinene	970	0.1
Verbenene	975	0.2
β-Myrcene	980	0.1
Limonene	1006	1.2
ρ-Cymene	1015	8.3
γ-terpinene	1067	0.3
α-Terpinolene	1089	0.4
Oxygenatedmonoterpenes		**52.8**
Camphor	1125	Tr
Myrtenal	1137	0.2
Borneol	1150	1.2
Terpinene-4-ol	1163	1.1
α-Terpineol	1176	3.3
Fenchylacetate	1203	1.1
Geranial	1224	0.7
Carvacrolmethylether	1226	0.2
Linalylacetate	1240	0.2
Carvacrol	1279	0.1
1,8-Cineole	1282	44.7
Sesquiterpenehydrocarbons		**10.2**
Aromadendrene	1434	9.1
Alloaromadendrene	1477	0.7
β-Gurjunene	1506	0.1
δ-Cadinene	1517	0.1
α-Humulene	1519	0.2
Oxygenatedsesquiterpenes		**0.7**
β-Eudesmol	1362	0.2
α-Eudesmol	1466	0.1
Palustrol	1562	0.2
Caryophylleneoxide	1575	0.1
Ledol	1585	0.1
Ketones		**0.1**
Torquatone	2102	0.1
Aliphatics compound		**2.9**
(z)-2-heptenal	926	1.1
1-Octen-3-ol	959	1
Decane	1000	0.2
Nonanal	1081	Tr
2-phenylethanol	1119	0.3
Decanal	1182	Tr
Octylacetate	1191	0.1
Decanol	1253	0.1
Viridiflorol	1579	Tr
Tricosene	2300	0.1
Total identified (%)		**97.6**

R.I[a]: Retention Index

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, 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, 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* (Lopez, 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, 2009). The inhibition of seedling growth may either be due to synergistic or additive effect of compounds in *E.astringens* oil. Allelopathy is the result of the accumulative action of various compounds and often includes compounds with divergent chemistry (Einhellig, 2002). 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 affects the chlorophyll content

Leaves of weed plants sprayed with *E.astringens* oil showed a significant reduction in chlorophyll content. Indeed, after treatment with 25µl/ml, 1-DAS, the chlorophyll content was reduced for all weed species. Further, the chlorophyll content decreased in response to treatment with higher concentrations of eucalypt oil (Fig.2). The greatest reduction in chlorophyll content was observed in *E. vesicaria,* 1-DAS, with 100µl/ml eucalypt oil. In addition, in the crops, the reduction in chlorophyll content was greatest in *P.vulgarus* 1-DAS. In addition, in response to 100µl/ml eucalypt oil, 3-DAS, the inhibition in chlorophyll content was greatest in *S.arvensis* (96%) followed by *E.vesicaria* (91%), *S.murucatus* (90%), *P.vulgarus* (84%), *V. faba* (63%) and *T.durum* (17%) (Fig.2). The observed loss in chlorophyll content is in agreement to earlier studies reporting that volatile oils reduce chlorophyll content and thus interferes with photosynthetic activity of the plants (Singh, 2002; (Batish et al., 2004). The yellowing of weed leaves upon eucalypt oil spray may be the secondary effect due to decline in chlorophyll content. Kaur (2010) demonstrated that volatile oil from *Artemisia scoparia* reduced chlorophyll content and cellular respiration in *Cassia occidentalis, Parthenium hysterophorus, Ageratum conyzoides, Echinochloa crus-galli and Achyranthes aspera*.

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.astringens* 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 ~37%, 42.5%, 38%, 82.5%, 85.5% and 80.5% for *T.durum, V.faba, P.vulgarus, Erica vesicaria, S.arvensis* and *S.murucatus*, respectively.

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 and Savour, 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, 1989).

Eucalypt oil causes ion leakage

Eucalyptus astringens 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 specie.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 ~9%, 10.5%, 12%, 26.5%, 41% and 36.5% for *T.durum, V.faba, P.vulgarus, Erica vesicaria, S.arvensis* and *S.murucatus*, respectively.

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; Kaur et al., 2010). Singh et al. (2006) mentioned that terpenes of volatile oils disrupt fluxes across plasma membrane and damage membrane permeability resulting in oxidative burst. Essential oils penetrate cell membranes, particularly mitochondrial, disturb their permeability and induce alteration (Bakkali et al., 2008). Singh et al. (2009a) reported that *Artemisia* oil disrupts membrane integrity and increase electrolyte leakage from the roots of *Cyperus rotundus*.

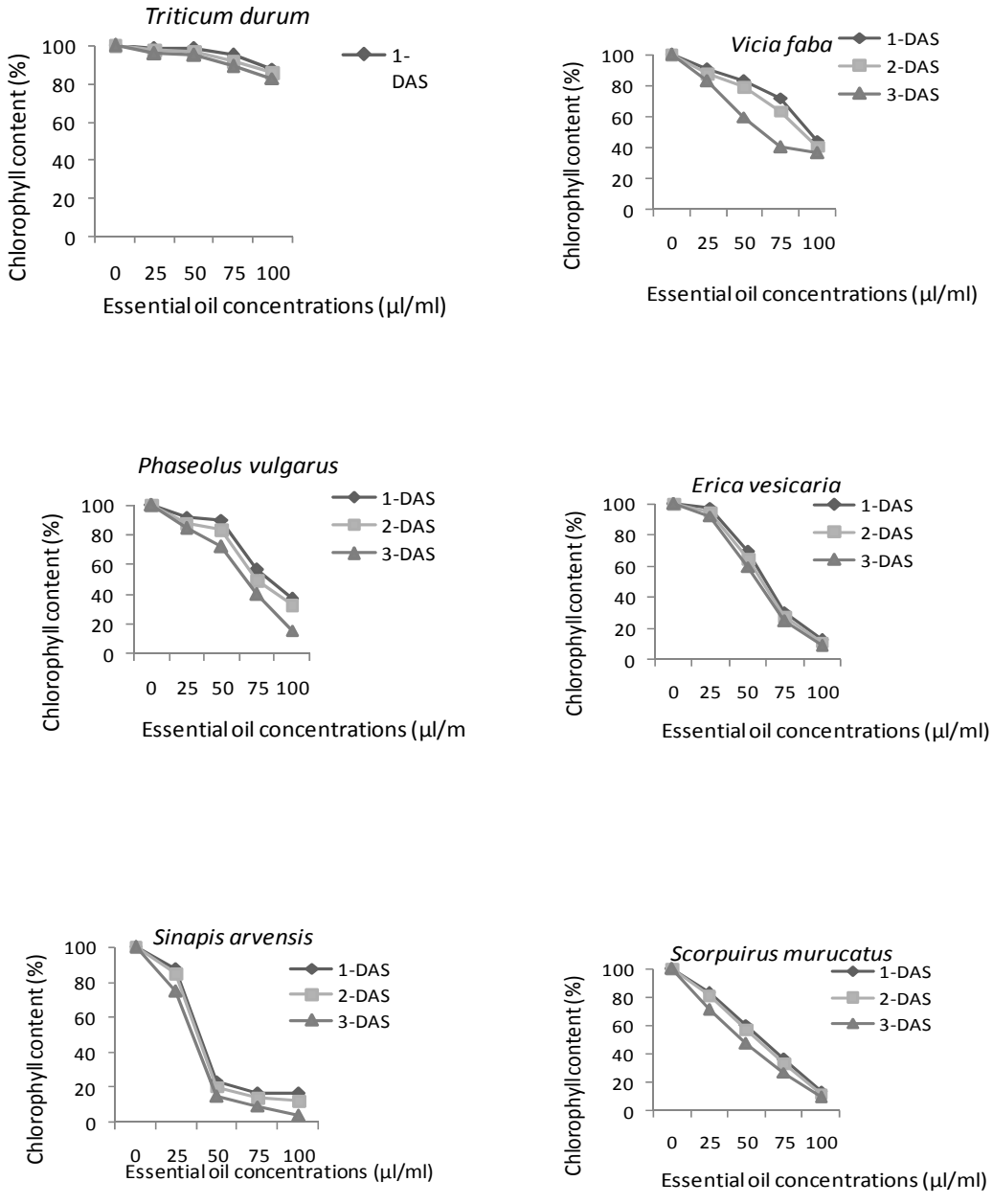


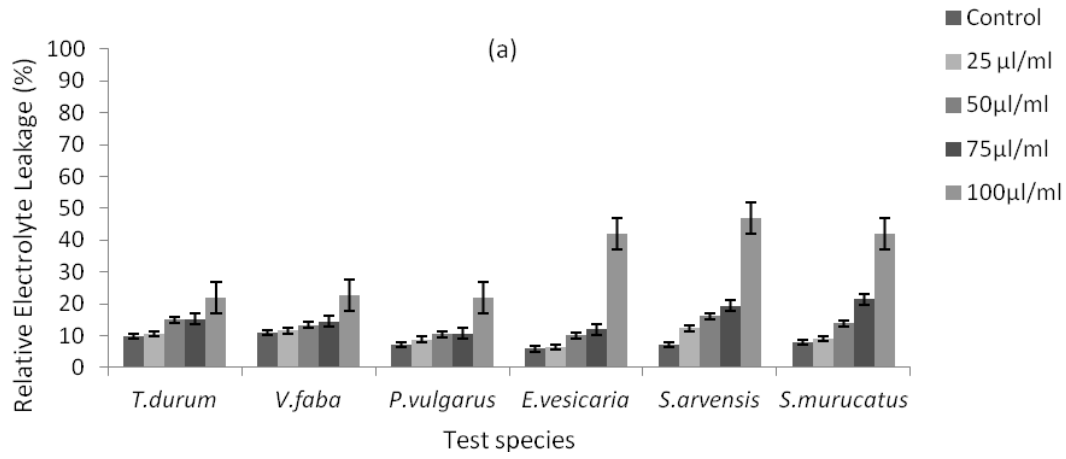
Fig.2. Effect of *E.astringens* essential oil on chlorophyll content in 4 –week-old plants of test weeds measured 1-2and 3 days after spray (DAS).

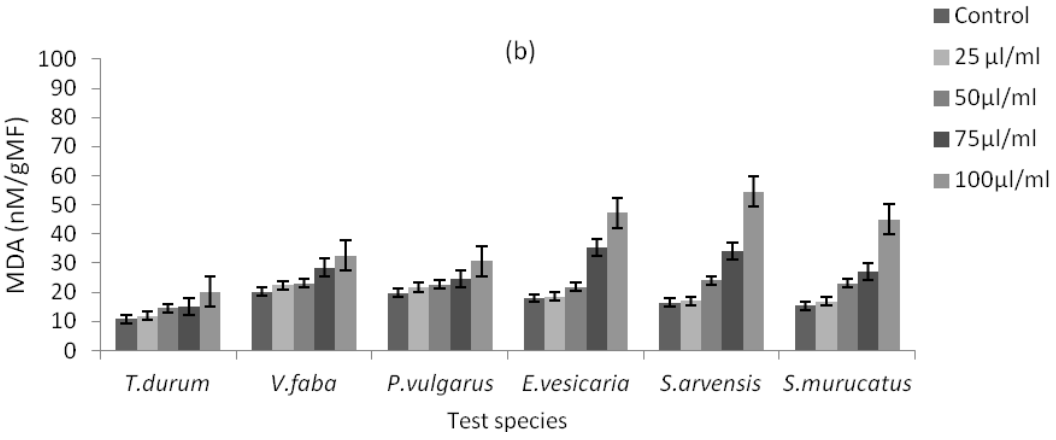
Allelopathic compounds are known to depolarize and disrupt cell membranes thereby enhancing their permeability, inducing lipid peroxidation and finally leading to cell death (Yu et al., 2003; Singh et al., 2006, 2009a).The change of membrane permeability in turn affects other physiological and biochemical activity linked to membrane function as lipid peroxidation.

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 ~9.5%, 12.5%, 11%, 29%, 38%, 30% 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 (Fig.3a).

Enhanced MDA content is an indicator of lipids peroxidation (Heath and Packer, 1968). The oxidative degradation of lipids induce 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, 2009; Mutlu et al., 2011). 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).





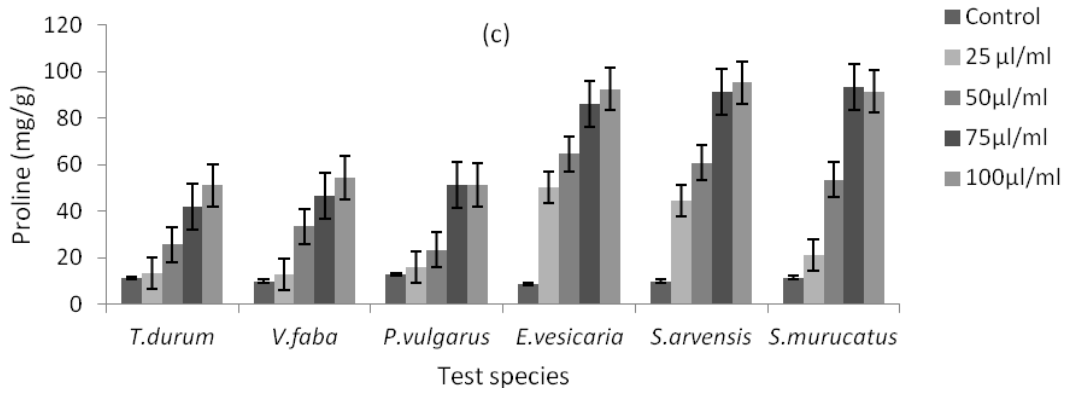


Fig.3. Effect of *E.astringens* essential oil on electrolyte leakage, MDA and proline contents of six test species.

CONCLUSION

From the present study, it could be concluded that *E.astringens* essential oil possesses a strong phytotoxicity against weeds and hence could be useful for developing as a bioherbicide. Indeed, use of *Eucalyptus* oil as allelopathic agent will be eco-friendly, cheaper and effective mode of weed control. Nevertheless, further studies are required to explore the exact sequence of events that are responsible for growth inhibitory potential of *E.astringens* essential oil.

ACKNOWLEDGMENTS

This research was financially supported by National Institute for Research in Rural Engineering, Water and Forests. We are also thankful to Faculty of Sciences of Bizerta.

REFERENCES

Angelini, L.G., Carpanese, G., Cioni, P.L., Morelli, I., Macchia, M., Flamini, G., (2003). Essential oils from Mediterranean lamiaceae as weed germination inhibitors. J. Agric. Food Chem., Vol. 51: 6158-6164.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., (2008). Biological effects of essential oils-A review. Food Chem. Toxicol., Vol. 46: 446-475.

Barney, J.N., Hay, A.G., Weston, L.A., (2005). Isolation and characterization of allelopathic volatiles from mugwort (*Artemisia vulgaris*).J. Chem.Ecol., Vol. 31: 247-265.

Bates, L.S., Walderen, R.D., Taere, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil.,Vol. 39: 205-207.

Batish, D.R., Singh, H.P., Kohli, R.K., Kaur, S., (2008). *Eucalyptus* essential oil as natural pesticide. For. Ecol. Manage., Vol. 256: 166-2174.

Batish, R.D., Setia , N., Singh, H.P., Kohli, R.K., (2004). Phytotoxicity of lemon-scented eucalypt oil and its potential use a bioherbicide. Crop Protection., Vol. 23:129-1214.

Cantrell, C.L., Dayan, F.E. and Duke, S.O., (2012). Natural products as sources for new pesticides. Journal of Natural Products.,Vol. 75: 1231-1242.

Charoenying, P., Teerarak, M., Laosinwattana, Ch., (2010). An allelopathic substance isolated from *Zanthoxyllum limonella* Alston fruit. Scientia Horticulturae., Vol. 125:411-416.

Chou, C.H. (2010). Role of allelopathy in sustainable agriculture: Use of allelochemicals as naturally occurring bio-agrochemicals. Allelopathy Journal., Vol. 25: 3-16.

Cruz-Ortega, R., Lara-Nunez, A., Anaya, A.L., (2007). Allelochemical stress can trigger oxidative damage in receptor plants: mode of action of phytotoxicity. Plant Signal. Behav., Vol. 4:269-270.

Dayan, F.E. and Duke, S.O.,2009. Biological activity of allechemicals. In: Plant-derived Natural Products: Synthesis, Function and Application (Eds., A.E. Osbourn and V. Lanzotti), pp 361-384, Springer, New York, NY.

Dayan, F.E., Cantrell, C.L. and Duke, S.O., (2009). Natural products in crop protection. Bioorganic and Medicinal Chemistry., Vol. 17: 4022-4034.

Einhellig, F.A., (2002). The physiology of allelochemical action: clues and views. In: Reigosa, M.J., Pedrol, N. (Eds.), Allelopathy, from Molecules to Ecosystems. Science Publishers, Enfield, New Hampshire, pp. 1-24.

Elaissi, A., Medini, H., Khouja, M.L., Simmonds, M., Lynene, F., Farhat, F., Chemli, R., Harzallah, F., (2010). Variation in Volatile Leaf Oils of Eleven *Eucalyptus* Species Harvested from Korbous Arboreta (Tunisia). Chemistry and Biodiversity., Vol. 7: 1841-1854.

Ens, E.J., Bremner, J.B., French, K., Korth, J., (2009). Identification of volatile compounds released by roots of an invasive plant, bitou bush (*Chrysanthemoides monilifera spp. Rotundata*), and their inhibition of native seedling growth. Biol. Inv., Vol. 11: 275-287.

Heath, R.L., Packer, L., (1968). Photoperoxidation in isolated chloroplasts.I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., Vol. 125:189-198.

Kaur, S., Singh, H.P., Mittal, S., Batish, D.R., Kohli, R.K., (2010). Phytotoxic effects of volatile oil from *Artemisia Scoparia* against weeds and its possible use as a bioherbicide. Ind. Crops Prod., Vol. 32: 54-61.

Kaur, S., Singh, H.P., Batish, D.R., Kohli, R.K., (2012). *Artemisia scoparia* essential oil inhibited root growth involves reactive oxygen species (ROS)-mediated disruption of oxidative metabolism: In vivo ROS detection and alterations in antioxidant enzymes. Biochemical Systematics and Ecology., Vol. 44:390-399.

Kohli, R.K., Batish, D.R., Singh, H.P., (1998). Eucalypt oil for the control of *parthenium (Parthenium hysterophorus L.*). Crop Prot., Vol. 17: 119-122.

Kohli, R.K., Singh, D., (1991). Allelopathic impact of volatile components from *Eucalyptus* on crop plants. Biol. Plant., Vol. 33: 475-483.

Koitabashi, R., Suzuki, T., Kawazu, T., Sakai, A., Kuroiwa, H., Kuroiwa, T. (1997).1,8-cineole inhibits root growth and DNA synthesis in the root apical meristem of *Brassica campestris* L.J. Plant Res., Vol. 110: 1-6.

Liza López, M., Bonzani, N.E., Zygadlo, J.A., (2008). Allelopathic potential of *Tagetes minuta* terpenes by a chemical, anatomical and phytotoxic approach. Biochem. Syst. Ecol., Vol. 36: 882-890.

Molish, H., (1937). Der Einfluss einer Pflanze auf die andere Allelopathie., Fisher, Jena.

Mutlu, S., Atici, □., Esim, N., Mete, E. (2011). Essential oils of catmint (*Nepeta meyeri Benth.*) induce oxidative stress in early seedlings of various weed species. Acta Physiol. Plant., Vol. 33:943-951.

Scrivanti, L.R., (2010). Allelopathic potential of *Bothriochloa laguroides var. laguroides* (DC.) Herter (Poaceae: Andropogonae). Flora., Vol. 205: 302-305.

Scrivanti, L.R., Zunino, M.P., Zygadlo, J.A., (2003). *Tagetes minuta* and *Schinus areira* essential oils as allelopathic agents. Biochemical Systematics and Ecology., Vol. 31: 563-572.

Singh, H.P., Batish, D.R., Kaur, S., Arora, K., Kohli, R.K., (2006). α-Pinene inhibits growth and induces oxidative stress in roots. Ann. Bot., Vol. 98: 1261-1269.

Singh, H.P., Batish, D.R., Kohli, R.K., (2002). Allelopathic effects of two monoterpenes against bill goat weed (*Ageratum conyzoides L.*). Crop Protection., Vol. 21: 347-350.

Singh, H.P., Batish, D.R., Kohli, R.K., (2003). Allelopathic interactions and allelochemicals: new possibilities for sustainable weed management. Crit. Rev. Plant Sci., Vol. 22:239-311.

Singh, H.P., Batish, D.R., Setia, N., Kohli, R.K., (2005). Herbicidal activity of volatile essential oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. Annals of Applied Biology., Vol. 146: 89-94.

Singh, H.P., Kaur, S., Mittal, S., Batish, D.R., Kohli, R.K., (2009). Essential oil of *Artemisia scoparia* inhibit plant growth by generating reactive oxygen species and causing oxidative damage.J.Chem.Ecol., Vol. 35: 154-162.

Szabados, L., Savour, A., (2010). Proline: a multifunctional amino acid. Trends Plant Sci., Vol. 15: 89-97.

Venekemp, J.H., (1989). Regulation of cytosolic acidity in plants under condition of drought. Plant Physiol., Vol. 76:112-117.

Weir, T.L., Park, S.W., Vivanco, J.M., (2004). Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin.Plant Biol., Vol. 7: 472-479.

Willey & Sons., (1998). Willey Registry of Mass Spectral Data, 7th ed., NIST Spectral Data CD Rom, J., New York.

Yang, G.Q., Wan, F.H., Liu, W.X., Guo, J., (2008). Influence of two allelochemicals from *Ageratina adenophora Sprengel* on ABA, IAA and ZR contents in roots of upland rice seedlings. Allelopathy Journal., Vol. 21:253-262.

Yu, J.Q., Ye, S.F., Zhang, M.F., Hu, W.H., (2003). Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals on photosynthesis and antioxidant enzumes in cucumber. Biochem. Syst. Ecol., Vol. 31:129-139.

ISSN : 0976-4550

INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Email : ijabpt@gmail.com

Website: www.ijabpt.com