

Effects of *Ocimum gratissimum* L. extract on the germination, respiration and growth of *Euphorbia heterophylla* L.

C.O. Martendal*, G.C. Mantovanelli, B. Reis¹, C. Cavaleiro²,
E.L.I. Iwamoto³ and C.M. Bonato⁴

Postgraduate Program in Biological Sciences of the State University of Maringá ,
5790, Colombo Avenue, Maringá, PR 87020-900, Brazil,
E-mail: cintiamartendal@hotmail.com

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ABSTRACT

We investigated the effects of *O. gratissimum* extract on the germination, growth, respiratory activity and survival of the weed *E. heterophylla*. The bioherbicidal effects of extract on adult plants were also evaluated. The gas chromatography-mass spectrometry (GC/MS) analysis detected the phenylpropanoid eugenol was the primary constituent in the extract and was 43% of the total. The increase of extract concentration inhibited the germination (%) and germination speed index. Total respiration and KCN-insensible respiration at the prominent apices treated with extract increased with increasing extract concentrations, whereas KCN-sensitive respiration was inhibited. When the extract was applied to the incubation medium the total oxygen consumption was inhibited at all concentrations tested. In adult plants, the application of extract (> 2.5%) resulted in 100% weed plants death. These results indicate that *O. gratissimum* extract constituents, most likely eugenol, may have modified the cell membrane permeability. In the mitochondria, the damage caused by the extract can be associated with the mitochondrial oxidative phosphorylation decoupling that reduces the energy production of the germination process and initial growth of the seedlings.

Key words: Allelochemicals, allelopathy, bioherbicide, extract, eugenol, *Euphorbia heterophylla* L., germination, *Ocimum gratissimum* L., physiological processes, seeds, seedling growth, weed.

INTRODUCTION

Weeds cause serious damage to crops through competition for growth resources (water, nutrients and physical space). In addition, the weeds may be hosts of insects and diseases that reduces the growth and development of crops (18). Currently, weeds are controlled with synthetic herbicides and many of these have caused environmental problems (26). Xenobiotics that remain on the ground or reach groundwater can intoxicate animals and accumulate in the food chain, depending on the compound (7). In addition, indiscriminate herbicidal use has resulted in the development of highly resistant weed biotypes, leading to demand for new chemical compounds with mechanisms and actions that are distinct from those currently used (25,29). There are currently 437 herbicide-resistant

*Correspondence author, ¹Postgraduate Program in Agronomy of the State University of Maringá, 5790, Colombo Avenue, Maringá, PR 87020-900, Brazil. ²Pharmaceutical Studies Center - Faculty of Pharmacy of the Coimbra University, 3000-548 Coimbra, Portugal. ³Department of Biochemistry of the State University of Maringá, 5790, Colombo Avenue, Maringá, PR 87020-900, Brazil. ⁴Department of Biology, State University of Maringá, 5790, Colombo Avenue, Maringá, PR 87020-900, Brazil.

weed biotypes distributed among 238 species [138 dicotyledons and 100 monocotyledons (13)]. In many cases, this resistance is associated with successive applications of herbicides with the same mechanism of action. Thus, in the present time the replacement of synthetic chemicals by allelopathic substances less harmful to the agroecosystem is of fundamental importance. Studies with allelochemicals may lead to the production of other types of herbicides for direct use or as molecular models for the synthesis of new agrochemicals (23). Natural compounds are safer for the environment, as most are more water-soluble and non-halogenated than most synthetic compounds, which are lipophilic and halogenated (35). The study of allelopathy and its allelochemicals is considered an attractive method for weed control, as these are ecofriendly (34) and sustainable. We investigated the allelopathic spp. *Ocimum gratissimum* L. its essential oil is of great interest, as it contains allelochemicals [eugenol, methyl eugenol, linalool, 1,8-cineol and alfa-terpineol (32)] in superficial glandular trichomes (10). In particular, eugenol is phenylpropanoid derivative, chemically referred to as 4-allyl-2-methoxy-phenol or 2-methoxy-4- (2-propenyl-phenol) (23). It is synthesized biologically from phenylalanine amino acid, through metabolic route of the phenylpropanoides (chiquinate, phenylalanine and unamic acid) (30). This phenylpropanoid is highly lipophilic compound in membranes (17). Some agronomic studies have been done with the applications of eugenol, such as (i). protection of agricultural products (2,5), (ii). control of stored grain pests (24), (iii). management of mites (11) and (iv). its allelopathic effect on plants (4,21). This study aimed to investigate the effect of *O. gratissimum* extract on germination, initial growth and energetic metabolism of *Euphorbia heterophylla* L. This weed competes for water and nutrients and decreases summer crops yield and also liberates large quantities of latex, which can facilitate disease propagation and hinders crop (3).

MATERIALS AND METHODS

The experiments were conducted in the Plant Physiology and Biological Oxidation Laboratories and Experimental Didactic Garden (23°24'13.4"S 51°56'19.9"W, altitude 550 m), Biology Department, State University of Maringá, Maringá – Paraná State, Brazil.

I. Obtention of *O. gratissimum* extract

Fresh leaves were hydro distilled for 4 h in a modified Clevenger-type apparatus followed by exhaustive distillate extraction with hexane. Thereafter, the extract was filtered over anhydrous Na₂SO₄ to remove the solvent.

II. Analysis of *O. gratissimum* extract

Analysis was conducted by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Analytical GC was carried out in a Hewlett-Packard 6890 (Agilent Technologies, Palo Alto, CA, USA) gas chromatograph with an HP GC ChemStation Rev. A.05.04 data handling system, equipped with a single injector and two flame ionization detection (FID) systems. A graphpak divider (Agilent Technologies, part no. 5021-7148) was used for simultaneous sampling of two Supelco (Supelco, Bellefonte, PA, USA) fused

silica capillary columns with different stationary phases, SPB-1 (polydimethylsiloxane 30 m × 0.20 mm i.d., film thickness 0.20 μm) and SupelcoWax-10 (polyethyleneglycol 30 m × 0.20 mm i.d., film thickness 0.20 μm). The oven temperature program was 70-220 °C (3 °C min⁻¹), 220 °C (15 min). Injector temperature was 250 °C. The carrier gas was helium, adjusted to a linear velocity of 30 cm.s⁻¹. The splitting ratio was 1:40. The detector temperature was 250 °C. GC/MS was conducted in a Hewlett-Packard 6890 gas chromatograph fitted with an HP1 fused silica column (polydimethylsiloxane 30 m × 0.25 mm i.d., film thickness 0.25 μm), interfaced with a Hewlett-Packard mass selective detector 5973 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. GC parameters were as described previously with an interface temperature of 250 °C; MS source temperature of 230 °C; MS quadrupole temperature of 150 °C; ionization energy of 70 eV; ionization current of 60 μA; scan range of 35 to 350 units; and scans/sec of 4.51.

Extract components were identified by their retention indices on both SPB-1 and SupelcoWax-10 columns and from their mass spectra. Retention indices, calculated by linear interpolation relative to retention times of C₈-C₂₃ of *n*-alkanes, were compared with those of reference samples included in the Pharmaceutical Studies Center (P.S.C.) / Faculty of Pharmacy, University of Coimbra laboratory database. Acquired mass spectra were compared with reference spectra from our laboratory database, Wiley/NIST libraries and literature data (1,16). Relative amounts of individual components were calculated based on GC raw data areas without FID response factor correction.

III. Effects of *O. gratissimum* extract on germination of *E. heterophylla*

Seeds of *E. heterophylla* (Cosmos Agrícola Produtos e Serviços Rurais Ltda®) were washed in deionized water. Fifty seeds were sown evenly in each petri dish (12 cms dia) two germitest paper sheets moistened with 10 mL solution. The extract was prepared with *O. gratissimum*, dissolved in dimethylsulfoxide (1.5% [v/v] DMSO) and deionized water. The experimental treatments consisted of 9 concentrations (0 a 0.14% v/v) of *O. gratissimum* with 4 replications. The experimental design was completely randomized. The control consisted of DMSO (1.5%, [v/v]) prepared in deionized water. Petri dishes were sealed with clear plastic wrap to prevent water evaporation. Experimental were randomly arranged in a BOD- germination chamber [12 h photoperiod (230 μmol m⁻² s⁻¹ photon flux density), 25 °C]. Seeds were considered germinated when the root protrusion reached ≥ 2 mm. The germinated seeds were counted every 12 h. After 7 days, germination percentage (%G), germination average time (GAT), germination average speed (GAS), germination speed index (GSI), root system (RS), total length (TL), shoot (SM), root system (RSM) and total mass (TM) were measured. Dry mass was recorded after fresh material was dried in an oven at 80 °C till obtain constant weight.

The study was done to determine the direct effects of *O. gratissimum* extract on radicle growth of *E. heterophylla* were submitted to the same treatment as the previously described assay but were first placed in plastic trays and maintained in a Biological Oxygen Demand (BOD) germination chamber for 24 h. After root protrusion (≥ 2 mm), 10 selected seeds were evenly distributed in each Petri dish (12 cms dia) line with two sheets of

germitest paper moistened with 10 mL of solution. The solution consisted of different concentrations of *O. gratissimum* extract (0 to 0.07% [v/v]) dissolved in dimethylsulfoxide (1.5% [v/v] DMSO) and deionized water. After the seventh day, the biometric variables were evaluated as described in the previous section.

IV. Respiratory activity in root apices pretreated treated with *O. gratissimum* extract

Weed seeds were germinated with *O. gratissimum* extract (0 to 0.08% [v/v]) and DMSO (1.5% [v/v]) and respiratory activity of the root apices was determined 72 h after sowing. Root apices of 20 mg samples were removed using scissors and surgical tweezers. Then, they were chopped and inserted immediately into an acrylic camera containing 2 mL (pH 5.8) nutrient solution, [Ca (NO₃) 2 mM, KNO₃ 2.2 mM, FeCl₃ 27 μM, NH₄Cl 0.43 mM, MgSO₄ 0.75 mM and NaH₂PO₄ 20 μM (20)]. The acrylic camera was connected to a Clark-type oxygen electrode inserted into a polarograph. Cytochrome oxidase (COX, KCN-sensitive respiration), alternative oxidase (AOX) and extra-mitochondrial oxidase (KCN-insensitive respiration) contribution were evaluated by addition of potassium cyanide, 200 μM (KCN). For oxygen measurement, 240 μM dissolved oxygen in the reaction medium at 25 °C was assumed (6). In the evaluation, we considered the total respiration-oxygen consumption rate without KCN, KCN-insensitive-respiration after KCN addition and KCN-sensitive differences between oxygen consumption measured in the absence and presence of KCN.

The study was done to determine the direct effects of *O. gratissimum* extract on respiration of *E. heterophylla* seeds were submitted to the same methodology used in the previously described experiment, but in this case, only water was used for germination. The extract of *O. gratissimum* (0 to 0.2% [v/v]) and DMSO (1% [v/v]) was added directly onto the acrylic camera.

V. Bioherbicidal effects of *O. gratissimum* extract on *E. heterophylla* in greenhouse

Fifteen *E. heterophylla* seeds were sown per pot (0.5 kg, 12x12cm) containing soil (horizon C) and organic substrate (Fertimax®) in : 1:1 (v/v), containing the mixture of following characteristics: pH 7.1, C (30.1%), MO (54.8%), N-total (0.54%), CaO (12.2%), MgO (6.11%), K₂O (0.94%), P₂O₅ (0.72%); Fe (3.062 mg kg⁻¹), Cu (512 mg kg⁻¹), Mn (977 mg kg⁻¹) and Zn (208 mg kg⁻¹). The experimental treatments consisted of 5 concentrations (0.1 to 2.5% v/v) with 4 replicates. The experimental design was completely randomized. The thinning was done 10-days after sowing, leaving 8-plants per pot. The remaining plants were sprayed with 10 mL of *O. gratissimum* extract solution 0.1 to 2.5% concentration (v/v), diluted in DMSO (1.5% [v/v]) and deionized water. The control consisted of DMSO (1.5% [v/v]) and deionized water. Extract was sprayed with hand sprayer. Plants were irrigated when necessary. On 30th day after sowing, plant survival percentage, length and dry mass of shoot and root system were evaluated. We used DMSO concentration of 1.5% (v/v), because in preliminary tests this concentration was not inhibitory.

In previous experiments it was observed that *Euphorbia* seeds were more sensitive to extract than seedlings grown in pots. In this way, smaller doses were used for seed germination assays and larger for the control of seedlings grown in pots.

VI. Statistical analysis

Data were statistically analyzed with ANOVA and polynomial regression ($p \leq 0.05$) using SISVAR software (9). The graphs are displayed in GRAPHPAD PRISM (version 5.0).

RESULTS AND DISCUSSION

Analysis of *O. gratissimum* extract

GC/MS analyses of *O. gratissimum* extract revealed that the major constituents are: phenylpropanoid eugenol (42.7%), germacrene D (9.6%), Z- β -ocimene (8.2%), E-cariofilene (6.4%), 1,8-cineole (6.2%), δ -cadinene (3.1%) and α -copaene (2.7%) (Table 1). The results resemble those found by other authors (6,19,32) describing eugenol as a major component of *O. gratissimum* extract. Eugenol ($C_{10}H_{12}O_2$) is slightly water soluble but soluble in organic solvents (17). Studies have shown that eugenol has anti-inflammatory, antioxidant, antimicrobial and anticonvulsant action (12). An anti-mutagenic (22) and anti-cytogenic function (23) in microorganisms, animals and humans have also been demonstrated. In addition, it is important to note that the use of this compound in the protection of fruits against post-harvest decay has been reported (5,2) Phenylpropanoid interactions on growth and development in plants are scarce (21). Some studies have shown positive effects of eugenol in control of stored grain pests (14,22,24) and control of mites, such as *Tetranychus urticae* (11). There are no studies on eugenol effects on metabolism or action mechanisms of plants. The results presented from our study highlight some considerations of the eugenol effect on microorganisms and animals, since many of the effects in these organisms can be closely related to plants, mainly in regards to their action on the membrane systems.

Germination and seedlings growth of *E. heterophylla*

Seed germination of *E. heterophylla* was completely inhibited at 0.14% concentration of the extract (Figure 1A) and GSI was markedly inhibited with increasing extract concentrations (Figure 1B).

It was observed that initial weed growth was notably different when the seeds received *O. gratissimum* extract before germination as compared with the seeds that received the treatment post-germination. In addition, seedlings showed radicle necrosis with increasing oil concentration, both before and after germination, preventing its growth.

Table 1. Composition of the extract of *O. gratissimum* L. analyzed by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/MS).

RI ^a	RI ^b	Compounds	%
1339	2166	Eugenol	42.7
1465	1702	Germacrene D	9.6
1025	1234	Z- β -Ocimene	8.2
1407	1596	E-Cariofilene	6.4
1020	1216	1,8-Cineole	6.2
1505	1752	δ -Cadinene	3.1
1369	1490	α -Copaene	2.7
1480	1727	Biciclogermacrene	1.7
1211	1762	Citronellol	1.7
1130	1564	Isopulegol	1.5
1377	1515	β -Bourbunene	1.4
931	1031	α -Pinene	1.3
1118	1372	allo-Ocimene	1.2
1130	1478	Citronellal	1.1
1241	1557	Linalil acetato	1.5
981	1163	Mircene	0.7
1138	1557	neo-isopulegol	0.7
1557	1972	Cariofilene oxyde	0.5
1628	2223	α -Cadinol	0.5
1438	1663	α -Humulene	0.5
1237	1844	Geraniol	0.5
1117	1690	α -Terpineol	0.5
1085	1542	Linalol	0.5
1050	1458	E-Sabinene hidrate	0.5
1047	1248	γ -Terpinene	0.5
1011	1275	p-Cimene	0.5
969	1118	β -Pinene	0.4
1617	2191	α -Murolol	0.3
1158	1595	Terpinene-4-ol	0.3
1419	2141	γ -Elemene	0.3
1543	1818	Germacrene B	0.1
1000	1171	α -Felandrene	0.1
1020	1206	Limonene	t
1515	n.d.	Cadine-1,4-diene	t
1521	n.d.	α -Cadinene	t
		Phenilpropanoids	42.7
		Sesquiterpene hidrocarbons	25.8
		Monoterpenes	12.9
		Oxygen-containing monoterpenes	8.2
		Oxygen-containing sesquiterpenes	1.4
		Total of compounds identified	91.0

t: traces.

n.d.: not detected.

a Retention indices in SPB-1 column relative to C₈-C₂₃ n-alkanes.b Retention indices in the Supelcowax-10 column relative to the C₈ to C₂₃ n-alkanes.

** Quantification based on SPB-wax peak areas.

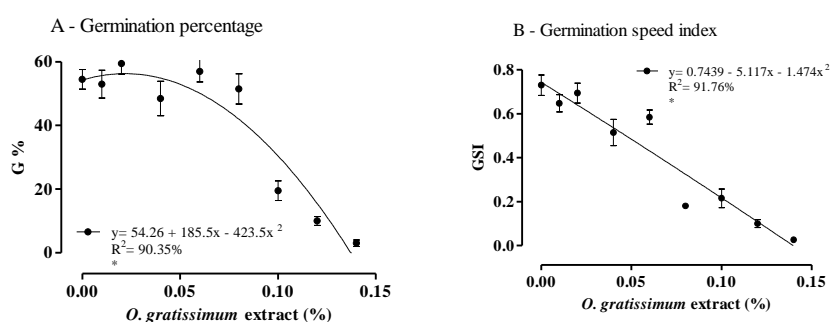


Figure 1. Effects of *O. gratissimum* extract on germination variables [(A) germination percentage (G%), (B) germination speed index (GSI)] of *E. heterophylla* evaluated at day 7. The means are the results of 4 replicates. *Significant results were analyzed by second degree polynomial regression with significance level of $p \leq 0.05$.

All variables, shoot length (SL), root system length (RSL), total length (TL), shoot dry mass (SDM), root system dry mass (RSDM) and total dry mass (TDM) in both treatments were inhibited with increasing *O. gratissimum* extract concentration (Figures 2A to I). It was observed that germination and biometric variables were more affected when extract was added to seeds already germinated. A low concentration of extract (0.07%) inhibited 100% of the variables studied when the oil was applied to pre-germinated seeds (Figure 2). Seeds treated with extract at the beginning of germination showed less inhibition (approximately 60%). These results of the germination variables show that *O. gratissimum* extract strongly affects both the germination percentage and the rate of the germination speed index of *E. heterophylla* (Figures 1A and B). The effects of *O. gratissimum* extract on germination and the germination speed index may be due to different causes. The extract's effect on germination may be linked to the influence of their components on the mechanisms of germination and/or vital embryonic processes, such as the energy production process.

Respiratory activity in root apices of *E. heterophylla* treated with *O. gratissimum* extract

Total and KCN-insensitive respiration of root apices pretreated with extract for 168 h increased with increasing extract in the growth medium, reaching 1.9 and 8 times, respectively, at the highest concentration (Figures 3A and B). KCN-sensitive respiration was inhibited (approximately 25%) by the extract at the concentration of 0.08% (Figure 3C). It was observed that the increase in total respiration with the increase in the extract concentration occurred mainly from greater KCN-insensitive pathway activation; in other words, by alternative pathway oxygen consumption along the routes added by extra-mitochondrial peroxidases consumption (Figures 3A, B and C).

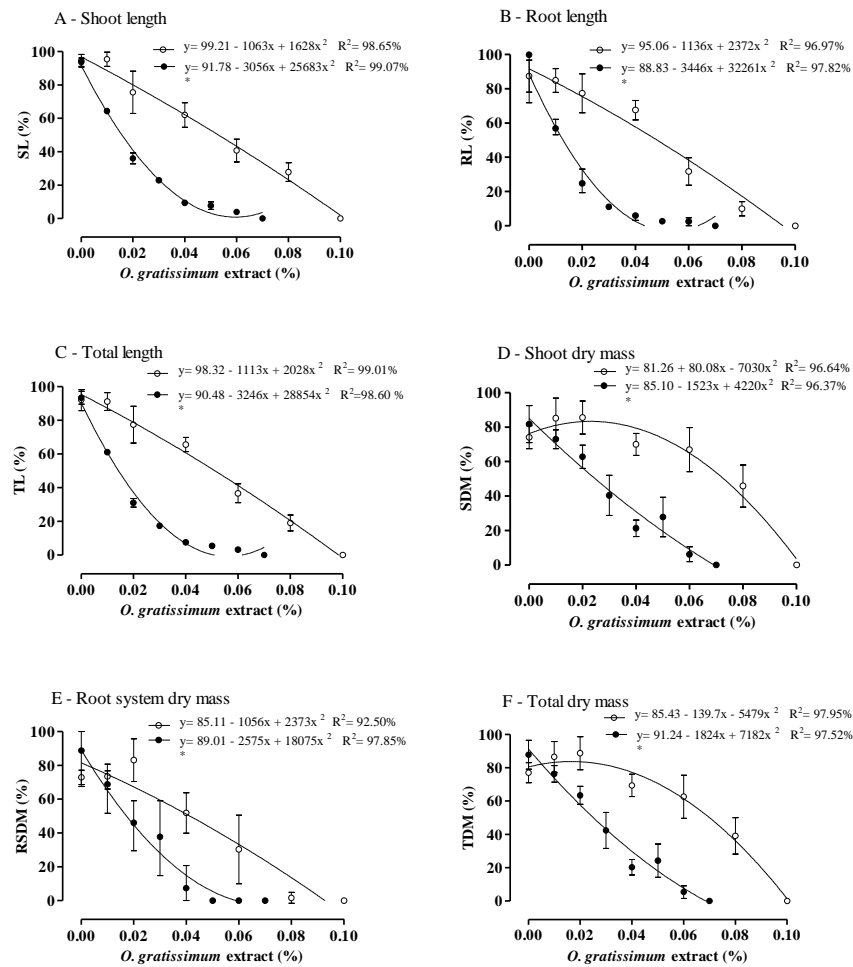


Figure 2. Effects of *O. gratissimum* extract applied before germination (o) and after germination (•) on growth variables of *E. heterophylla* on day 7: (A) shoot length (SL), (B) root length (RL), (C) total length (TL), (D) shoot dry mass (SDM), (E) root system dry mass (RSDM), (F) total dry mass (TDM). Means are the four replicates. *Significant results were analyzed by second degree polynomial regression with significance level of $p \leq 0.05$.

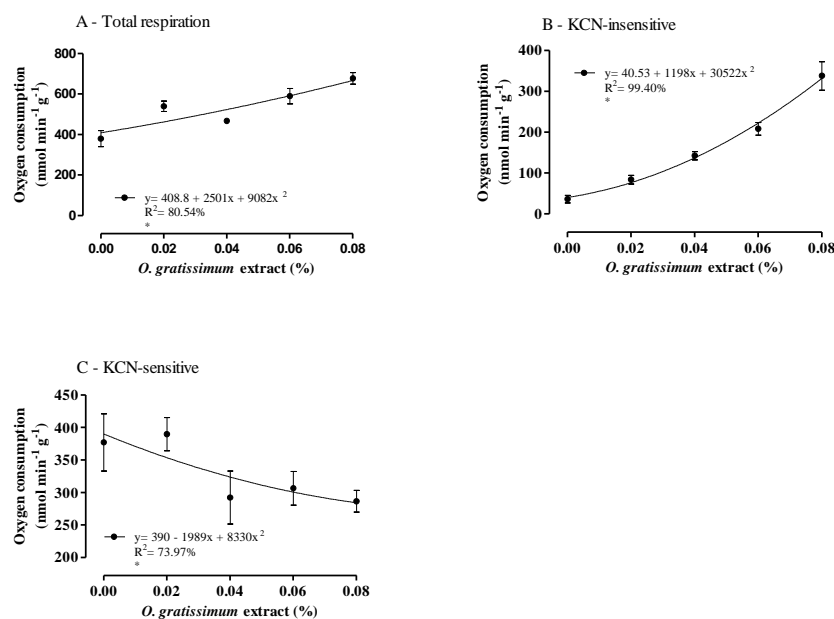


Figure 3. Effects of extract of *O. gratissimum* on respiratory activity on *E. heterophylla* root apices (72 h after sowing) pretreated with (A) Total respiration rate of oxygen consumption in the absence of KCN, (B) KCN-insensitive - respiration after addition of KCN and (C) KCN-sensitive - difference between oxygen consumption measured in absence and presence of KCN. Results are means of 5 replicates. *Significant results were analyzed by second degree polynomial regression with significance level of $p \leq 0.05$.

Respiratory activity in root apices treated with *O. gratissimum* extract added to incubation medium

Total, KCN-sensitive and KCN-insensitive respiration were inhibited with increasing extract concentration in the incubation medium (Figures 4A, B and C). Consumption by the insensitive KCN pathway was the most affected by increased extract concentration, being completely inhibited by the 0.20% concentration (Figure 4B). KCN-sensitive respiration (oxidase pathway) was less inhibited (Figure 4C).

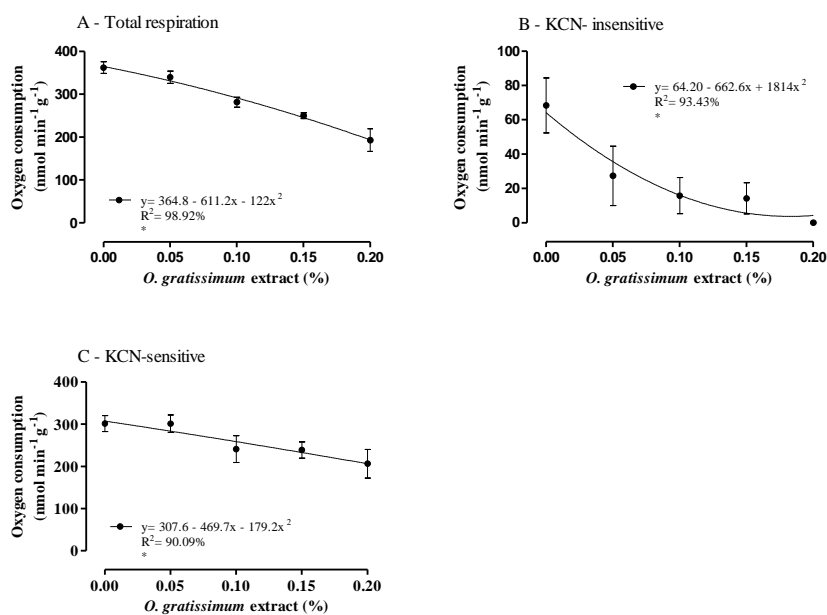


Figure 4. Effects of *O. gratissimum* extract on applied in the incubation medium respiratory activity of *E. heterophylla* root apices (72 h after sowing) : (A) Total respiration - oxygen consumption in the KCN absence , (B) KCN-insensitive - respiration after KCN addition and (C) KCN-sensitive - difference between oxygen consumption measured in absence and presence of KCN. The means are results of 5 replicates. *Significant results were analyzed by second degree polynomial regression with significance level of $p \leq 0.05$.

Although total respiration in the presence of the extract increased, normal-cytochrome-oxidase respiration (KCN-sensitive) was drastically inhibited (Figure 3A and C). The reduction of oxygen consumption by the normal pathway and the increase in the alternative pathway oxidase consumption suggest that the extract may be decoupling the mitochondrial oxidative phosphorylation, that is, reducing ATP production and its availability for the germination process. This increase in oxygen consumption by the alternative route oxidase may also be associated with external lipoxygenases. In this case, the extract may cause oxidative stress in the plant, increasing the reactive oxygen species (ROS) activity. It is known that seed germination is highly metabolic energy dependent, especially ATP (33). Thus, low energy supply for the embryonic metabolic processes may explain, at least in part, the lethal effect of the extract on the germination percentage. Effects of the extract on energy metabolism may also delay seed germination, as shown by the low rate of germination. It is known that germination speed depends on many factors linked to the embryo and degradation and mobilization of seed reserves. Part of the germination delay can also be explained by the lower energy supply. Reducing the energy supply can delay the degradation of reserves and, consequently, reduce germination.

Bioherbicidal potential of *O. gratissimum* extract on *E. heterophylla* growth in greenhouse conditions

There was a drastic reduction in the variable values measured with the extract application (Figures 5A, B and C). At the highest concentration of extract tested (2.5%), there was 100% inhibition. At the concentration 1%, no plant was able to complete its cycle. Control plants and plants treated with 0.1% extract concentration were in excellent condition, as they were intensely green even after 30 days of growth (Figure 5A).

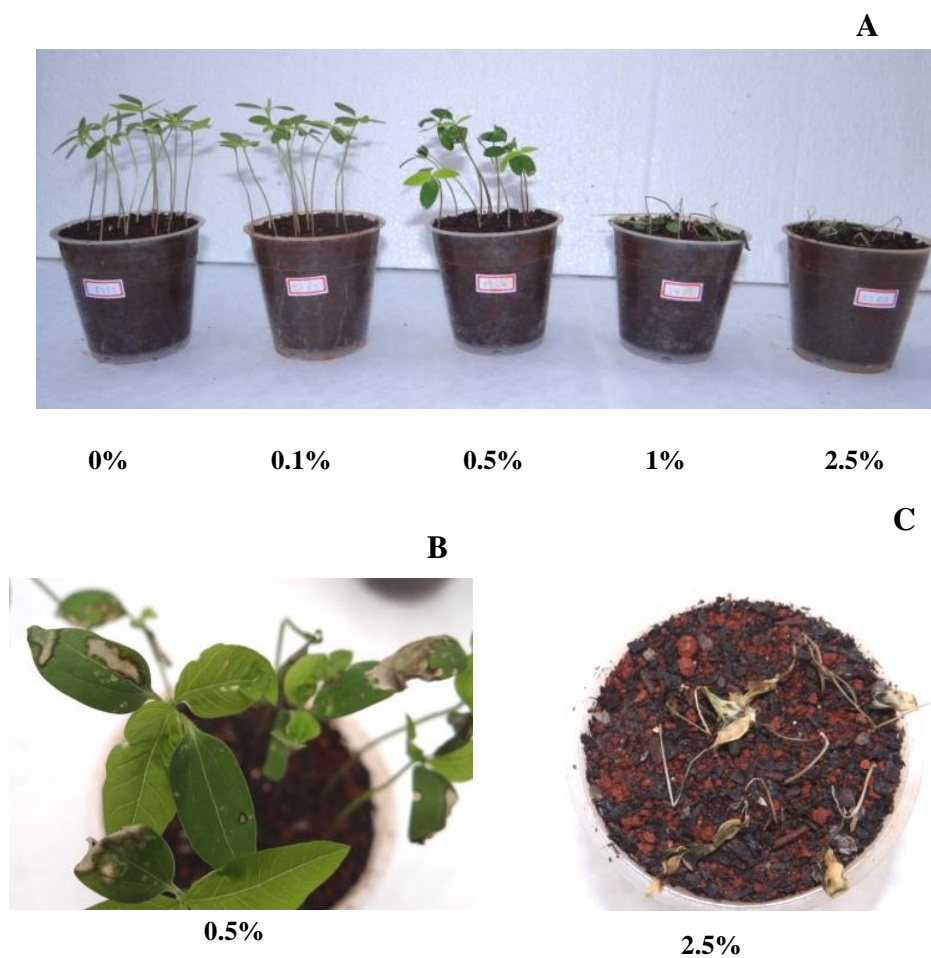


Figure 5. Effects of applied *O. gratissimum* extract on *E. heterophylla* treated with increasing concentration (A), plants damaged by the extract (B, C).

Extract application at concentrations 0.5% and higher resulted in leaf damage and decreased biometric variable values. At concentrations 1 and 2.5% the damage was greater and the plants wilted and dried almost completely. At the highest concentration (2.5%), there was 100% inhibition for all variables (Figures 5B and C).

The percentage of surviving plants corresponds to plants that still had at least one green part (live) 30 days after application of the extract. Extract application reduced the survival plant percentage, reaching 100% of death (Figure 6A). The plants treated with the extract lowest concentration (0.1%) and the control presented similar results. For the other concentrations tested, inhibition increased with increasing extract concentration (Figures 6B to J).

The effect of *O. gratissimum* extract on adult plants was as effective as observed in germination and post-germination events (Figures 5A to C). All the biometric variables studied were significantly inhibited by the extract, especially the percentage of survival plants (SP) (Figures 6A to J). The extract caused large lesions, especially in the leaf blade and doses close to 2.0% were lethal. The explanation for the effect of the extract on adult plant biometric variables may be the same as those described for the biometric variables of the seedlings (Figures 2A to J).

In general, we can consider that the results obtained from the extract applied at germination and after germination (Figures 2) show that in both cases, the extract significantly affected all studied variables (length, dry of both root and shoot), leading to total inhibition at relatively low doses (Figures 2A to J). However, it is clear that the effect of the extract was greater when it was added to seeds already germinated than when it was applied at the beginning of germination. These results suggest that the presence of the extract at the beginning of germination acclimatizes the seeds to withstand adverse conditions (hardening) and/or could be also because of the mode of action of the extract. It is not possible to determine the probable causes of this behavior from the data obtained. However, certain relationships may be conjectured in regards to respiration. It is observed that the application of extract in the incubation medium, or in the intact tissues, causes a significant decrease in total respiration, KCN-insensitive and KCN-sensitive respiration. This impairment of *O. gratissimum* extract may explain, at least in part, the results of biomass production inhibition and effects on seedling length (root, shoot and total). Mitochondrial damage or the global respiratory process can also affect energy availability (27) and the carbon skeleton supply.

Apparently, the extract caused more damage to respiration when was applied directly to the root apices (Figure 3) than when applied to the seeds at the beginning of germination. It is possible that the extract compounds, when applied at the beginning of germination, do not easily penetrate into the seeds' internal structures due to the seed integument impediment. Conversely, when there is radicle protrusion, the extract constituents may not be in the same initial concentrations because the constituents may be complexed, undergoing natural oxidation or volatilizing. In germinated seeds, this may not occur; therefore, extract constituents may act directly on the tissue without integument obstacles.

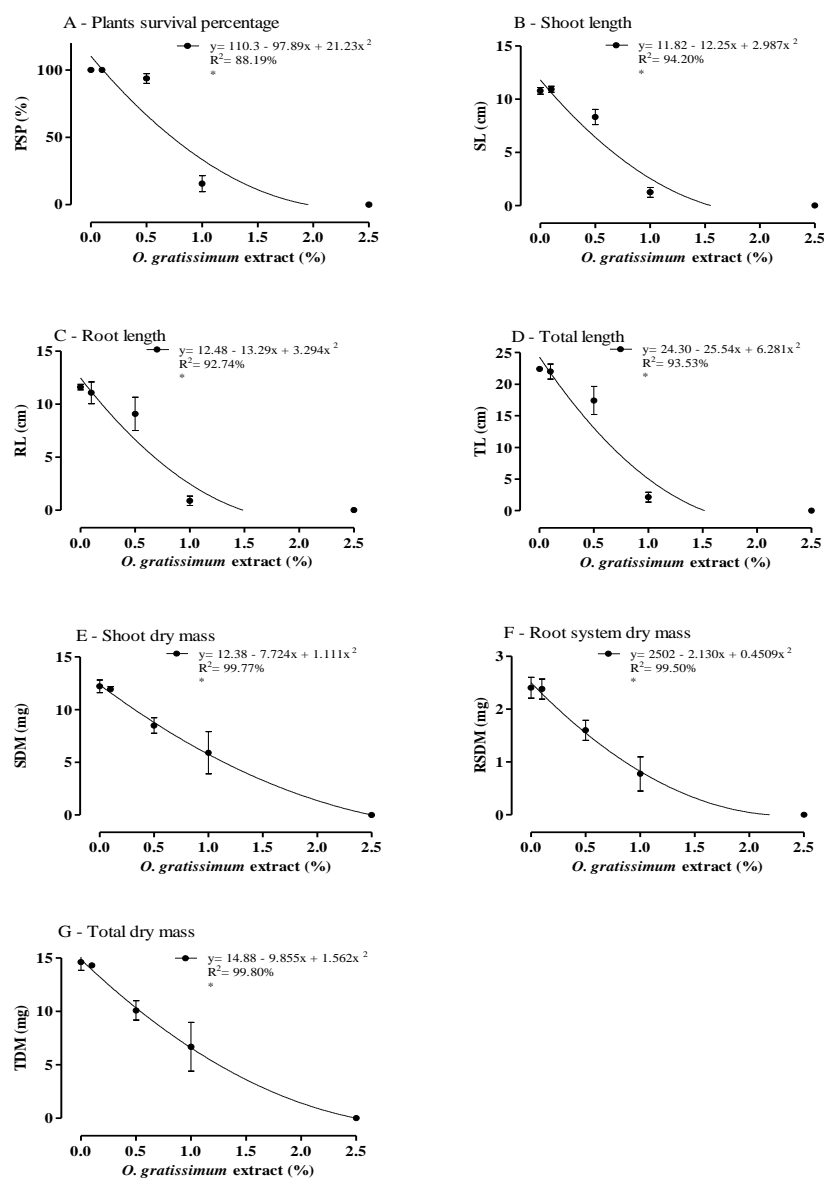


Figure 6. Effect of *O. gratissimum* extract on some *E. heterophylla* growth variables evaluated at 30 days after sowing: (A) plants survival percentage (PSP), (B) shoot length (SL), (C) root length (RL), (D) total length (TL), (E) shoot dry mass (SDM), (F) root system dry mass (RSDM), (G) total dry mass (TDM). Means are results of 5 replicates. *Significant results were analyzed by second degree polynomial regression with significance level of $p \leq 0.05$.

Studies have shown that eugenol can alter the membrane composition (lysis) and the structure cell wall of bacteria (*Listeria monocytogenes*, *Streptococcus pyogenes*, *Proteus vulgaris* and *Escherichia coli*), causing leaching of lipids and proteins after 120 min of exposure of bacteria to phenylpropanoid (27). This experiment proved that fungal lethality was due to damage to membrane integrity and cell cycle paralysis. In addition, ultra-structural changes such as pronounced swelling, internal membrane disorganization and increased cristal numbers were observed in the parasite mitochondria, causing leishmaniasis (35). Colon cancer cells treatments with eugenol caused an early increase in lipid bilayer breakage. Additionally, membrane potential dissipation and the reactive oxygen species generation accompanied eugenol-induced apoptosis (13).

Eugenol, which is lipophilic, can easily disperse between the fatty acid chains that compose the cell membrane bilayers and modify the fluidity and permeability of its membranes, disturbing cell growth (29,31). Considering that eugenol is the main constituent of the *O. gratissimum* extract, this evidence explains well the data obtained in our experiment. Membrane damage may compromise not only the plasma membrane but also the structure and/or functioning of the mitochondrial membranes. These changes in energy metabolism of seeds and seedlings may be one of the causes of the inhibitory effects on seed germination and initial growth of seedlings.

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