Potent Physiological Allelopathic Effect of Eucalyptus Leaf Extract on *Malva parviflora* L (mallow) Weed

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Received: 19 January 2016      Revised: 29 January 2016      Accepted: 05 February 2016

**ABSTRACT**

The present study is an investigation of the physiological allelopathic effect of the aqueous extract of eucalyptus leaves on *Malva parviflora* weed and its establishment as a tool of herbicidal potential. Seed germination and length of *Malva parviflora* seedlings exhibited different degrees of inhibition by application of different concentrations of aqueous extract of eucalyptus leaves (2.5, 5, 10, 15, 20, 25 and 40%) as compared with respective controls. Maximum inhibitions were recorded by 40% leaf extract. The reduction of growth of *Malva parviflora* weed (60 days old) was accompanied by reduction in photosynthetic pigments, total carbohydrates, nucleic acids and nitrogenous compounds content. The activities of hydrolytic enzymes amylase, invertase and protease decreased as concentration gradient increased from 15 to 40%. The inhibitory effects were correlated with accumulation of the internal contents of total phenols, compared to their corresponding controls with all treatments.

**Keyword:** Allelopathy; *Malva parviflora*; Aqueous extract; Eucalyptus; Herbicide; Phenols

**INTRODUCTION**

*Malva parviflora* (little mallow) is a winter annual broadleaf weed in the family Malvaceae. It associates growth of many plants and represents a constraint to their production. It reproduces by seeds which have a rounded kidney shape, reddish brown, and are 2 mm long. It grows to 0.5 m. Because of the environmental and toxicological effects of herbicides, besides increasing herbicidal resistance among weeds, more alternative strategies against weeds must be developed. Using allelopathy for weed management leads to improved water quality and reduced environmental contamination. Allelopathy is defined as the effect(s) of one plant on other plants through the release of chemical...
compounds in the environment [1]. Chemical identification procedures have recently become more advanced, and biologically active substances with phytotoxic potential, that can explain allelopathic behaviour, have been found [2]. The chemicals causing the allelopathic effects are called allelochemicals. Natural plant products known for their structural and chemical diversity offer a challenging new area for the discovery of new herbicides. Essential oils from a number of higher plants are known to possess greater toxicity and are responsible for allelopathic activity. Previous studies have shown that various Eucalyptus species can yield allelopathic chemicals which may be effective in suppressing vegetation. Allelopathy is associated with Eucalyptus spp. due to the presence of allelochemicals in these plants; several studies have demonstrated the release of phenolic and volatile compounds in its foliage [3]. Eucalyptus reduces the growth of neighboring crops through the release of allelochemicals [4]. Aqueous extract from bark and leaf, and volatiles from leaves of Eucalyptus citriodora showed allelopathic effect on the growth of nine species, including the weeds Bidens pilosa, Digitaria pertenuis, Eragrostics ciliaris, Setaria geniculata, and crops such as corn, rice, cucumber and bean [5]. In laboratory bioassay germination, seedling length, chlorophyll content and respiratory ability of weed plants was drastically affected [6]. The inhibitory effects of E. citriodora aqueous extract against wild oat seeds were correlated with accumulation of the internal contents of total phenols, compared to their respective controls [7]. The objective of this study is evaluation of the physiological allelopathic effect of eucalyptus aqueous extract against Malva parviflora weed. Generally, allelochemicals are secondary metabolites exhibit a wide range of action mechanisms. They affect DNA, photosynthetic and mitochondrial functions, phytohormone activity, ion uptake and water balance.

**MATERIALS AND METHODS**

**Preliminary laboratory experiment**

Seeds of Malva parviflora were obtained from the Agricultural Research Center, Ministry of Agricultural, Giza, Egypt. Samples of eucalyptus fresh leaves grown in agroforestry plantation were collected and washed with tap water to remove the dirt. Samples were first air dried in hot air 2-3 days and completely dried in oven at 40°C for 48hrs. The dried leaves samples were ground to get fine powder. To prepare aqueous extract at different concentrations, 25, 50, 100, 150, 200, 250 or 400 g powder from leaves was shaken well by hand and allowed to soak for 5 days in 1000 ml sterile, deionized, distilled water at room temperature and filtered to get solutions of 2.5, 5, 10, 15, 20, 25 and 40% concentration. The solutions were preserved in refrigerator until need. A preliminary Petri dish assay was carried out for screening the physiological effect of the seven concentrations of aqueous extracts of eucalyptus leaves (2.5, 5, 10, 15, 20, 25 and 40 %) on germination and growth of Malva parviflora seedlings (15 days old). Seed germination, shoot and root lengths of Malva parviflora exhibited different degrees of inhibition according to the concentration of the aqueous extract. Maximum inhibitions of germination percentage and shoot and root lengths were recorded when using 40% leaf extract. Based on this preliminary experiment (Petri dish assay), studies were conducted under greenhouse conditions to evaluate the effects of soil treatments of aqueous extracts of eucalyptus leaves on Malva parviflora weed using the higher concentrations which are the most effected concentrations (15, 20, 25 and 40%). In the preliminary experiment, seeds of Malva parviflora were surface sterilized with 0.03% formalin for one hour and thoroughly washed with tap water following with distilled water. The sterilized seeds were germinated in Petri dishes containing 1-layer Whatman no.3
filter paper with 6 mL of different concentrations of eucalyptus leaves extracts, as follows: a- Fresh leaves extracts at 2.5, 5, 10, 15, 20, 25 and 40%. b- Untreated control (distilled water). Germination was carried out in the laboratory in December at average maximum and minimum temperatures 25.5 ± 1 and 18.5 ± 1 ºC. The experiment was repeated twice with one week interval. Each treatment was represented by five replicates, with each Petri dish representing one replicate. After three days, 2 mL of the previous treatments were added. Germination percentage, root and shoot length of *Malva parviflora* seedlings were recorded 15 days after germination.

Pot experiments were conducted under greenhouse conditions. The pots were infested with *Malva parviflora* seeds at 2 cm depth from the soil (10 seeds/pot). Routine fertilizers were added as calcium super phosphate (15.5% P₂O₅) before planting at the rate of 3 g per pot. Based on the preliminary work (Petri dish assay), the leaf extracts were used at concentrations of 15, 20, 25, and 40% as follows:

**Preparation of the extract**

Finely ground dry eucalyptus leaves (oven dried at 40°C) were used. To get solution of 15, 20, 25 and 40%, 300, 400, 500 or 800 g was shaken well by hand and allowed to soak for 5 days in 2000 ml sterile, deionized, distilled water at room temperature and filtered through very fine mesh. The solutions were preserved in refrigerator until need and prepared according to the quantity of extract needed. The extracts were applied early in the morning 3 days after sowing. The treatments were carried out weekly thrice, as follows: The previous aqueous extracts were applied in the soil at the rate of 250 mL kg⁻¹ soil. The pots were arranged in a complete block design. The infested weed was collected from each pot at 60 days after treatments for estimation of plant height (cm), fresh weight (g) and chemical analyses. The effect of the aqueous extract of eucalyptus leaves on photosynthetic pigments, Hill reaction, some enzyme activities, total carbohydrates, DNA, RNA, nitrogenous compounds was estimated. The studies involved estimation of the endogenous contents of total phenols in *Malva parviflora* weed.

Photosynthetic pigments were estimated in 85% acetone extracted leaves according to Metzner et al. [8]. For isolation of chloroplasts, according to the method of Aronoff [9] and Osman et al. [10], fresh leaves were blended in cold buffer with 0.4 M sucrose, (pH 7.8), 3 mM MgCl₂, 4 mM sodium ascorbate and 0.1% bovine serum albumin. The suspension was centrifuged at 4°C (1 min at 800 g). The pellet was resuspended in the isolation buffer and centrifuged for 5 min at 300 g and the supernatant was then centrifuged for 10 min at 1000 g. Chloroplasts (residue) were resuspended in the buffer solution. Hill reaction of the isolated chloroplasts was measured by using potassium ferricyanide as electron acceptor. For enzyme assay, plant material was prepared by macerating the tissues with a chilled pestle and mortar at 0-4°C. The tissue homogenate was centrifuged at 10 000 g for 20 min and the supernatant obtained was used directly for determining enzyme activity. Assaying of α and β-amylases, invertase, and protease activities was performed according to the method described by Bergmeyer [11]. Total carbohydrates were estimated using anthrone reagent according to Umbriet et al. [12] method. Borate buffer (pH 8.0) extract of dry leaves was used according to Naguib [13] for determination of peptide-N and total soluble-N. Total nitrogen was measured by digesting the dry leaves in 50% sulphuric acid and 35% perchloric acid and its ammonia content was estimated using Borthelot reaction which carried out according to Chaney and Marbach [14]. DNA was measured according to Dische and Schwartz [15] by using diphenylamine reagent. RNA was determined using the
method adopted by Ashwell [16] using orcinol reagent. Total phenol compounds in the weed were extracted from drying finely ground tissues (powdered). Drying was carried out in an electric oven at 60 ºC until constant weight was achieved. Total phenols content was determined colorimetrically according to the method defined by Snell and Snell [17], using Folin and Ciocalteu phenol reagent. Statistical analysis was carried out according to Snedecor and Cochran [18].

RESULTS AND DISCUSSIONS

Effect of different concentrations of aqueous extract of eucalyptus leaves on seed germination, shoot and root length of Malva parviflora seedlings (15days old)

In the preliminary experiment, the application of eucalyptus leaf extract at all used concentrations led to pronounced inhibition of germination percentage of Malva parviflora seeds to become 8% at concentration 40% compared to respective control seeds (96.5%) (Fig.1a). Also, Malva parviflora shoot and root lengths are negatively affected by aqueous leaf extract of eucalyptus at different concentrations, compared to untreated control. Remarkable inhibitions in seedling shoot and root lengths were observed by the same treatment (40%) that affected seed germination (Fig.1b). These results are in line with those of El-Rokiek and Eid [7] on wild oat weed and Allolli and Narayanareddy on cucumber [19].

Effect of different concentrations of aqueous extract of eucalyptus leaves on chemical constituents of Malva parviflora weed (60 days old)

Results in Fig. 2a & b show that the weed length and fresh weight were significantly reduced by applying different extracts of eucalyptus leaves. These results were true with different applications. The highest reductions in length and fresh weight caused by the extracts were observed by 40% of eucalyptus leaf extract. This agrees with Florentine and Fox [20], Batish et al. [6], and El-Rokiek and Eid [7]. In general, reduced growth of many weed species in response to different plant extracts is well reported [21], [22]. High weed growth suppression may be attributed to the presence of toxin compounds in the aqueous extracts, as reported by Shiming [23]. These compounds are volatile oils and phenolic acids [24], [7].

Effect of different concentrations of aqueous extract of eucalyptus leaves on chemical constituents of Malva parviflora weed (60 days old)

Different treatments of eucalyptus leaf extracts revealed significant decrease in chlorophyll a & b, and carotenoids in fresh leaves of Malva parviflora weed over the control (Fig.3 a ). Also, the results show a significant decrease in the photosynthetic activity (Hill reaction) of Malva parviflora weed (Fig.3b). The inhibitory effect of eucalyptus leaf extract application on photosynthetic pigments content could be attributed to retardation of the rate of biosynthesis of chlorophyll a and b. The decreased content of chlorophyll was in parallel with the retarding of plant growth i.e., there is an intimate relationship between growth and chlorophyll content. El-Maghraby and Gomaa [25] reported that eucalyptus leaf extract application decreased number of green leaves and leaf area per plants. Fig.4 reveals that different of eucalyptus leaf extracts led to significant decreases in the hydrolytic enzymes α and β-amylases, invertase and protease of Malva parviflora weed. Also, it is evident that eucalyptus leaf extract treatments caused significant decreases in total carbohydrates, total soluble nitrogen, total nitrogen and peptide nitrogen contents of the weed compared with respective controls which may be attributed to the decrease in the activities of the previously mentioned
hydrolytic enzymes (Figs. 5 & 6). The effect of higher concentrations was more obvious.

Figs.7 & 8 show that great differences were found between the total nucleic acids and total phenols contents in *Malva parviflora* weed treated with different extracts of eucalyptus and those in untreated weeds. Eucalyptus leaf extract might have effects on cell division of the weed and possibly be involved in blocking the DNA or protein synthesis required for normal cell division process. The results also indicated that the reduced nucleic acids content and the increased accumulation of total phenols were correlated with extract concentration. Accumulation of phenols is often a characteristic of stress condition [26], [7]. Allelopathic compounds in the leaves of eucalyptus, coumaric, gallic, hydroxybenzoic, syringic, vanillic acids, ferulic, gallic, syringic acids, and catechol revealed significant reduction in germination, growth and metabolic activities of *Cajanus cajan* over controls [27].

Fig. 1a: Effect of different concentrations of aqueous extract of eucalyptus leaves on germination percentage of *Malva parviflora*. L.S.D. at 5%: 1.52

Fig. 1b: Effect of different concentrations of aqueous extract of eucalyptus leaves on root and shoot lengths of *Malva parviflora* seedlings. L.S.D. at 5%: 1.16

Fig. 2a: Effect of different concentrations of aqueous extract of eucalyptus leaves on weed height (cm) (60 days old). L.S.D. at 5%: 3.21

Fig. 2b: Effect of different concentrations of aqueous extract of eucalyptus leaves on fresh weight (g) of *Malva parviflora* weed (60 days old). L.S.D. at 5%: 2.04
Fig. 3a: Effect of different concentrations of aqueous extract of eucalyptus leaves on photosynthetic pigments content (mg/g.f.wt) of *Malva parviflora* weed. L.S.D. at 5%: 1.52

Fig. 3b: Effect of different concentrations of aqueous extract of eucalyptus leaves on Hill reaction (µM [ferricyanide] g⁻¹ chlorophyll s⁻¹) of *Malva parviflora* weed. L.S.D. at 5%: 1.52

Fig. 4: Effect of different concentrations of aqueous extract of eucalyptus leaves on hydrolytic enzyme activities (µg g⁻¹ fm h⁻¹) of *Malva parviflora* weed. L.S.D. at 5%: 1.52

Fig. 5: Effect of different concentrations of aqueous extract of eucalyptus leaves on total carbohydrates content (mg g⁻¹ wt) of *Malva parviflora* weed. L.S.D. at 5%: 1.52

Fig. 6: Effect of different concentrations of aqueous extract of eucalyptus leaves on nitrogenous content (mg g⁻¹ dm) of *Malva parviflora* weed. L.S.D. at 5%: 1.52

Fig. 7: Effect of different concentrations of aqueous extract of eucalyptus leaves on nucleic acids contents (mg g⁻¹ fm) of *Malva parviflora* weed. L.S.D. at 5%: 1.52
Fig. 8: Effect of different concentrations of aqueous extract of eucalyptus leaves on total phenol content of *Malva parviflora* weed. L.S.D.at 5%: 1.52

Plate 1. Effect of different concentrations of aqueous extract of eucalyptus leaves on *Malva parviflora* weed (60 days after sowing). A: control, B: 15%, C: 20%, D: 25% and E: 40%.

**CONCLUSION**

From the results of this study it may be concluded that the leaf extracts of eucalyptus have the potential of acting as a natural herbicide against *Malva parviflora* weed which is associated with many plants. Their effectiveness in controlling weed may favor their use in agricultural systems, with a concomitant decrease in the need for synthetic herbicides.

**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no competing interests.

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Cite this article as: