

Allelopathic effects of *Eucalyptus salubris* F. Muell. and *E. brockwayii* C.A. Gardner on germination and seedlings growth of prairie ground cherry (*Physalis hederifolia* A. Gray)

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ABSTRACT

We evaluated the allelopathic potential of two selected Eucalyptus species (*Eucalyptus salubris* F. Muell. and *E. brockwayii* C.A. Gardner) on noxious perennial weed prairie ground cherry (*Physalis hederifolia* A. Gray). Steam distillation of eucalyptus leaves produced two bioactive fractions: essential oil fraction and the aqueous volatile fractions (AVFs). Both the essential oils and the AVFs inhibited the germination and growth of prairie ground cherry, however, the essential oils of *E. salubris* and *E. brockwayii* was more inhibitory than commercial eucalyptus essential oil. In addition, the inhibitory effects of *E. salubris* on the germination and growth of weed were higher than *E. brockwayii*. At 0.15 ml/L oil concentration, *E. salubris* inhibited the germination and seedling growth of prairie ground cherry by 94-95% and completely suppressed these at concentrations > 0.45 ml/L. The AVFs of both eucalyptus species also completely inhibited the germination and seedling growth at 75% AVF concentration. In glass house, the foliar application of essential oils severely damaged the seedlings of prairie ground cherry. At 21 days after foliar application, the *E. salubris* essential oil at 20% concentrations complete killed 5-leaf old seedlings. Thus eucalyptus essential oils could be further exploited to control weeds in integrated weed management.

Key Words: Allelopathy, aqueous volatile fractions, essential oil fraction, eucalyptus, *Eucalyptus salubris*, *Eucalyptus brockwayii*, *Physalis hederifolia*, prairie ground cherry, seed germination, seedlings growth.

INTRODUCTION

Eucalyptus (family Myrtaceae) has > 800 species (21). Most of these species are native to Australia and few species from neighbouring countries of Papua New Guinea and Indonesia (6). Eucalyptus has been introduced to more than 100 countries and its plantations around the world are > 20 million ha (18). Its large scale plantations are used as shelterbelts, windbreaks, erosion control, land reclamation and carbon sink (5,8,22). It is major source of biomass for paper pulp, fibreboard, industrial charcoal and biofuel feedstock (32). Its essential oils are used in perfumery and honey industries (8,29). The eucalyptus species often cause biodiversity loss and soil degradation, with very limited understorey vegetation (19,37) due to allelopathy and resource competition. Further

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research found that eucalyptus allelopathy is more important than its resource competition (26).

The eucalyptus allelopathy affects the growth of other plant species in vicinity by release of bioactive compounds into the environment through volatilization, leaching, foliage litter decomposition and root exudation (20,40). The allelopathic effects of eucalyptus have been intensely explored for use in weed management. Dadkhah (7) found that the aqueous extract of eucalyptus (*E. camaldulensis* L.) inhibited the seed germination, growth and photosynthesis of *Amaranthus retroflexus* L. Puig *et al.* (24) found that the aqueous extract from *E. globulus* Labill leaves exhibited both pre- and post-emergent activities on *Lactuca sativa* L. and *Agrostis stolonifera* L. The decomposing leaf litter of *E. grandis* W. Hill. inhibits the growth of *Setaria viridis* P. Beauv. (19). Kaur *et al.* (15) reported that *Eucalyptus tereticornis* Sm. volatile oil and its two major chemical constituents (α -pinene and 1,8-cineole) significantly reduced the early seedling growth and seedling vigour of *Amaranthus viridis* L. The essential oils of *Eucalyptus citriodora* Hook inhibited the seed germination and seedling length, chlorophyll content and respiratory activity of *Parthenium hysterophorus* L. in laboratory bioassays and were also effective in controlling seedlings when sprayed with the oils (29). 1,8-cineole, one of the major compounds in eucalyptus essential oil is herbicidal to many weed species (2,29). 1,8-cineole at 0.30 mmol/L concentration inhibited the germination and seedling growth of *L. sativa*, with the root elongation being the most sensitive parameter (25). However, Zhang *et al.* (37) reported that 1,8-cineole was less effective in suppressing the germination and seedling growth of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) than essential oils from *E. salubris*, *E. dundasii* Maiden, *E. brockwayii* and *E. spathulata* Hook.

Prairie ground cherry (*Physalis hederifolia* A. Gray), is one of eight *Physalis* species found in Australia (13). It is native to North and South America (23,31). Globally, prairie ground cherry is a weed in South Africa, Chile, Argentina, Brazil, Uruguay and western United States. Within Australia, it is a declared noxious weed in New South Wales, Victoria and Western Australia. Grice (12) reported that 96 000 ha were infested with prairie ground cherry in Australia. Its infestations are now reported from Queensland, New South Wales, Victoria, South Australia and Western Australia, with the potential to infest up to 409 million hectares (17). It is deep-rooted summer perennial weed that competes with summer pastures and crops, reducing the yield, forage value and carrying capacity. Grain quality is affected due to contamination of prairie ground cherry. The weed is spread by seeds and by roots. Root fragments > 1.5 cms are capable of producing new plants (10). Therefore, established populations cannot be readily eradicated using current control techniques. Alternative control options are therefore needed to reduce the impact of existing infestations and prevent its further spread.

It is observed that understory vegetation is limited within the dripline of certain eucalyptus species such as *E. salubris* and *E. brockwayii* in the field (37). This study aimed to determine the herbicidal potential of the selected two eucalyptus species against prairie ground cherry under laboratory and glasshouse conditions.

MATERIALS AND METHODS

Plant materials and chemicals

Two eucalyptus species *E. salubris* and *E. brockwayii* were selected based on our previous studies (37,38). Two Kg fresh leaves of each eucalyptus species were randomly collected from 6-years old trees growing in fields in Ungarie (33°35'53.06"S, 146°55'41.33"E), New South Wales, Australia. The leaves were then stored in cool room (10°C) before steam distillation. A commercial eucalyptus essential oil purchased from local supermarket was used for comparison. Seeds of prairie ground cherry were collected from a field site near Tocumwal (35°47'49.08"S, 145°41'25.38"E), NSW in April 2007. Fresh seeds were washed thoroughly to remove the sticky coating and air-dried prior to use.

Steam distillation of essential oils and aqueous volatile fractions

Leaves of each eucalyptus species were subjected to steam-distillation as per Wu *et al.* (36). Three hundred g of fresh leaves of each eucalyptus species were cut into 5 mm strips and subjected to steam-distillation for 2.5 h using a Pyrex oil distillation apparatus with a flat bottom flask (2L) containing 1,200 ml distilled water to generate steam. The volatile components from the leaves were condensed through a cooling tube. Two volatile fractions, which included condensed water and the fractions (defined as "essential oil") afloat on it, were obtained. The former was collected through a separation funnel and designated as the aqueous volatile fraction (AVF) (full strength, 100%). Both essential oil and the AVF were stored in a sealed bottle at 4 °C before use.

Bioassays

(i). Essential oils: Seeds of prairie ground cherry were dipped in distilled water for 5 h before germination bioassays. Fifty seeds of prairie ground cherry were sown in 9-cm Petri dishes lined with one layer of Whatman No.1 filter paper. Distilled water (5 mL) was initially added to each Petri dish. An aliquot of 0, 10, 30, 90 and 270 µL of each essential oil was loaded using Eppendorf micro pipette onto a piece of filter paper (2 x 2 cms) attached to the inner side cover of Petri dish. The concentration series was equivalent to 0, 0.15, 0.45, 1.35 and 4.05 ml/L. Immediately after the treatment, each Petri dish with its cover was sealed with parafilm to reduce evaporation. All Petri dishes were kept in growth incubator [diurnal temperature cycle of 25°C in light and 15°C in dark and a photoperiod of 12 h]. A randomized complete block design with three replicates was used. Germinated seeds with > 1 mm radicle were recorded and shoot lengths were measured 20 d after incubation.

(ii). Aqueous volatile fractions: The full strength (100%) aqueous volatile fraction (AVF) of each eucalyptus species was used to make concentration series of 0 (water control), 25, 50, 75 and 100%. Fifty seeds of prairie ground cherry were sown into the Petri dishes lined with one layer of Whatman No.1 filter paper. Five mL of each concentration of aqueous volatile fraction was added to Petri dishes as per treatments. The management of Petri dishes and measurements were the same as aforementioned.

Foliar application of essential oils

In glass house, pre-germinated seeds of prairie ground cherry were sown in pots filled with commercial potting mix. After two weeks, seedlings were thinned to two plants each pot. Essential oils were sprayed onto seedlings at 5-leaf stage. Three eucalyptus essential oils (*E. salubris*, *E. brockwayii* and commercial eucalyptus essential oil) were evaluated at 5-concentrations (0, 5 %, 10 %, 20 % and 40 %) with four replications. Tween 80 (0.05 %, v/v) was used as a non-ionic surfactant and emulsifier to dilute the essential oils for the required concentration series. Each seedling was sprayed with 40 μ l volume by hand-held atomizer. Visual injury rating was assessed at 1 day and 21 days after treatment.

Statistical Data analysis

All experiments were arranged in a completely randomized block design and repeated twice. Combined data were used due to the non-significant differences between bioassays at two different times. The germination count data were square-root transformed to meet the ANOVA assumptions. Data analysis was performed with ANOVA using Genstat with means separated by Fisher's Protected LSD.

RESULTS AND DISCUSSION

I. Weed germination and seedling growth

(i) **Seed germination:** Eucalyptus essential oils differed in their herbicidal activities on the germination of prairie ground cherry. The essential oil of *E. salubris* was most inhibitory and the commercial eucalyptus essential oil the least (Fig. 1). At the 0.15 ml/L oil concentration (10 μ l/Petri dish), *E. salubris* inhibited the prairie ground cherry germination by 95%, while there was only 65% inhibition by *E. brockwayii* and the commercial essential oils. The *E. salubris* essential oil at 0.45 ml/L of almost completely suppressed the germination (99%).

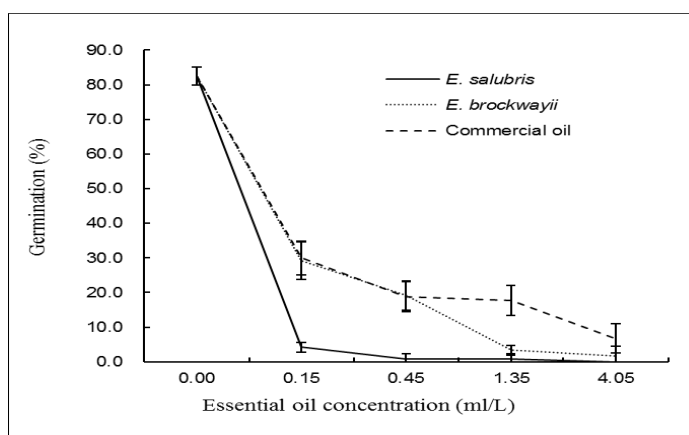


Figure 1. Germination inhibition of prairie ground cherry by eucalyptus essential oils. Bars represent standard error of the mean.

(ii). **Seedling growth:** The seedling growth of prairie ground cherry was significantly inhibited by eucalyptus essential oils, depending on the oil and its concentration. The essential oil of *E. salubris* caused the strongest suppression of root and shoot growth of prairie ground cherry. The 0.15 ml/L *E. salubris* oil concentration drastically inhibited the seedling growth (94-95%), while, the commercial oil at the same concentration, inhibited the root growth only by 39% (Fig. 2A) and shoot growth by 70% (Fig. 2B). Among the three essential oils, *E. brockwayii* had the intermediate activity on the seedling growth of the weed.

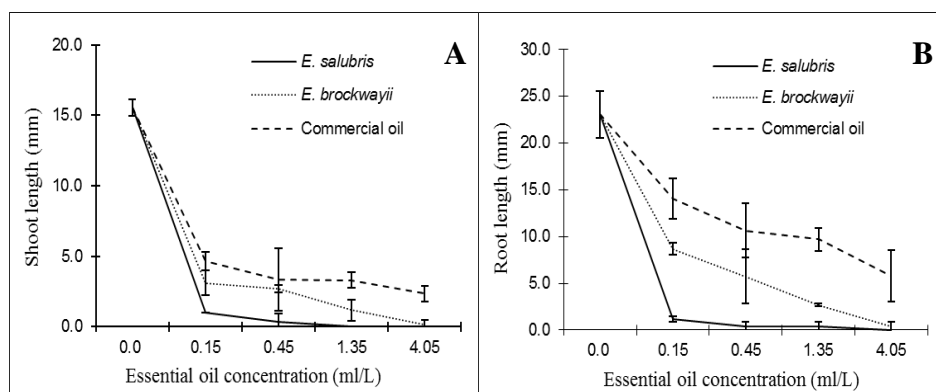


Figure 2. Growth inhibition of prairie ground cherry by eucalyptus essential oils. Bars represent standard error of the mean.

II. Aqueous volatile fractions of eucalyptus

(i). **Germination:** The aqueous volatile fractions (AVFs) from both *E. salubris* and *E. brockwayii* inhibited the germination of prairie ground cherry (Fig. 3). At 50% AVF concentrations, both eucalyptus species inhibited the germination by > 97% than control, while at low AVF concentration of 25%, *E. salubris* was more inhibitory to germination than *E. brockwayii*.

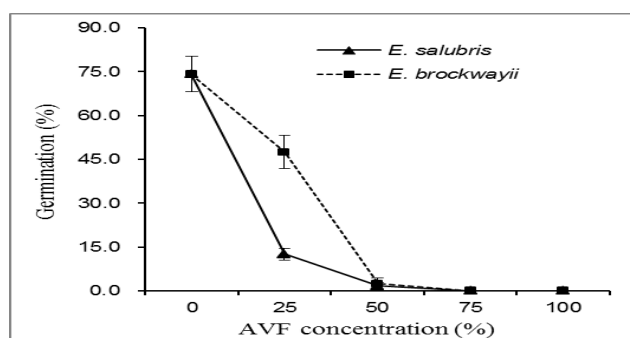


Figure 3. Germination inhibition of prairie ground cherry by the aqueous volatile fractions (AVF) from eucalyptus. Bars represent standard error of the mean.

(ii). **Seedling growth:** The seedling growth of prairie ground cherry was also inhibited by both eucalyptus AVFs (Fig. 4). At 25 % and 50 % concentrations, the AVFs of *E. salubris* and *E. brockwayii* inhibited the root growth by 60 % and 97 %, respectively, while they suppressed the shoot growth only by 41% and 87%, indicating that root growth is more sensitive to essential oils than shoot growth. The AVF concentrations > 75 %, completely suppressed the seedling growth.

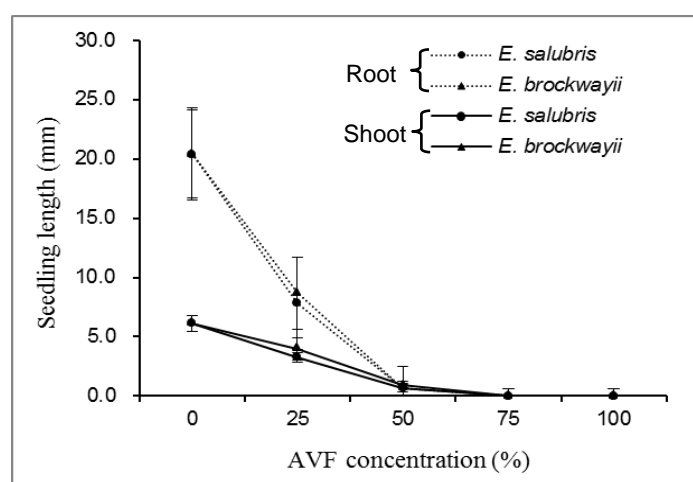


Figure 4. Growth inhibition of prairie ground cherry by the aqueous volatile fractions (AVF) from eucalyptus. Bars represent standard error of the mean.

III. Foliar application of essential oils

(i). **Seedling growth:** In glasshouse, the foliar application of essential oils significantly damaged the seedlings of prairie ground cherry (Fig. 5). The injury increased with the increase in oil concentration used. *E. salubris* and *E. brockwayii* showed higher herbicidal activities than commercial eucalyptus oil. The essential oil of *E. salubris* at 40% concentration completely killed the plant, one day after foliar treatment. The damage to seedlings also increased as the time after treatment progressed. For example, application of 10% essential oil of *E. brockwayii* caused 15% injury to prairie ground cherry seedlings at 1 day after treatment (DAT) than 50% injury at 21 DAT. *E. salubris* essential oils at higher concentrations > 20% caused the complete seedling death at 21 DAT.

Eucalyptus essential oil contains complex mixture of terpenoids (39), dominated by monoterpenes (1,8-cineole, *p*-cymene, citronellal, citronellol, limonene, α -phellandrene, β -phellandrene, α -pinene, β -pinene, *trans*-pinocarveol, terpinolene, α -terpineol and α -thujene) and sesquiterpenes (β -caryophyllene, β -eudesmol, globulol, spathulenol and viridiflorol). Some of these chemicals such as 1,8-cineole and citronellal are herbicidal to many weed species (15,27,28). Each eucalyptus specie has its unique chemical profile of

essential oil, regulating the differential bioactivities between eucalyptus species (37). In the present study, the eucalyptus oils of *E. salubris* and *E. brockwayii* inhibited the germination and seedling growth of prairie ground cherry in laboratory bioassays, which was consistent with the allelopathic effects reported for these two eucalyptus species on silverleaf nightshade (*Solanum elaeagnifolium* Cav.) (37). Further foliar application of essential oils also suppressed the seedling growth of prairie ground cherry under glasshouse conditions. The inhibition was concentration dependent and varied between eucalyptus oils, with *E. salubris* oil showing the strongest bioactivity in both laboratory and glasshouse bioassays than *E. brockwayii* and the commercial essential oil. The commercial eucalyptus oil was a mixture of essential oils from various eucalyptus species, thereby diminishing its inhibitory activities. Each eucalyptus species has its unique chemical constituents in the essential oil.

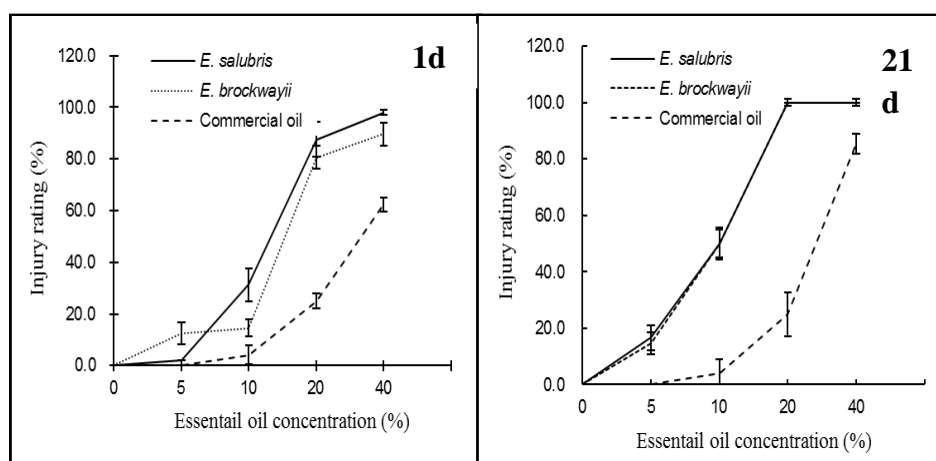


Figure 5. Growth injury of prairie ground cherry seedlings by eucalyptus essential oils. Bars represent standard error of the mean. Assessment was conducted at 1 day and 21 days after the treatment of essential oils. Tween 80 (0.05%) was used as an inert medium to dilute the essential oils. A volume of 40 μ L was sprayed to each seedling.

Our previous studies had reported the differences in chemical profiles in essential oils between *E. salubris* and *E. brockwayii* (37). There were 56 identifiable compounds in *E. salubris* essential oil, with the 1,8-cineole (57.6%), α -pinene (10.9%) and *p*-cymene (8.3%) being the main component. In the essential oil of *E. brockwayii*, 56 compounds were identified with α -pinene (31.1%), isopentyl isovalerate (20.2%) and 1,8-cineole (16.9%) as the most abundant components. The two eucalyptus species therefore differed in their major constituents and their relative abundance, regulating the different herbicidal activities between essential oils. The constituent monoterpenes might also be acting synergistically like other allelochemicals (9). The herbicidal activities therefore are not only determined by each individual compound but also by the joint actions of mixtures in unknown ratios. Further studies of herbicidal activities of individual constituents and their

joint actions are needed to identify not only the responsible compounds, but also their mixtures for the observed herbicidal activities. The identified compounds could then be used as lead molecules to develop herbicides through structure modification to improve the potency and reduce the volatility. For example, 1,8-cineole has been successfully used to develop grass herbicide cinmethylin, for use in broadleaf crops such as soybeans (*Glycine max* L. Merr.) (4).

Although essential oils are highly volatile, these compounds are partly soluble in water (35), with the herbicidal activities on annual ryegrass and barley grass (36). The herbicidal activities of aqueous volatile fractions were also confirmed on the germination and growth of prairie ground cherry in the present study, with the AVF from *E. salubris* being more effective than *E. brockwayii*. We have previously reported that the two eucalyptus species also differed in their chemical compositions of AVFs (38). The AVF of *E. salubris* contained a total of 29 compounds, with the main components being 1,8-cineole (47.5%), isomenthol (15.9%), pinocarvone (1.5%), *trans*-pinocarveol (4.7%) and α -terpineol (1.9%), while there were thirty-five compounds identified in the AVF of *E. brockwayii*, with 1,8-cineole (37.1%), isopentyl isovalerate (5.9%), pinocarvone (4.2%), *trans*-pinocarveol (7.0%), α -terpineol (5.8%) and globulol (6.9%) being the predominant compounds. The essential oil and AVF of *E. salubris* consistently contained higher levels of 1,8-cineole, carvacrol, carvotanacetone, cuminaldehyde, dihydro-linalool acetate, isomenthol, piperitone, terpinen-4-ol, thymol and verbenone than the counterparts of *E. brockwayii*. Some of these individual compounds have been bioassayed in previous studies, such as 1,8-cineole, isomenthol, terpinen-4-ol and thymol (16,34). The terpinen-4-ol was most potent, causing complete suppression of germination and growth of *L. sativa*, *Amaranthus retroflexus* L., *Chenopodium album* L. and *Rumex crispus* L., while 1,8-cineole, isomenthol and thymol were less effective and their activities also depended on bioassayed species (16,34). Further research is needed not only to screen and identify the most potent individual compounds in eucalyptus, but also to study the joint actions of mixtures of various compounds in different ratios, as compounds in mixtures can act independently, synergistically or antagonistically on germination and seedling growth (34). Despite the high volatility of eucalyptus essential oil, the identification of some essential oil constituents in the aqueous volatile fractions suggests that essential oils can be partly soluble in water and could be leached into the ground by rainfall to perform their biological activities on understorey vegetation. This natural ecological phenomenon can be used for weed management. Well-designed row-planting of selected eucalyptus species along the boundaries of farms or paddocks could provide an understorey “weed-free” corridor, proactively preventing weed incursion from roadsides into the cropping areas.

The herbicidal activities of essential oil and other plant parts from eucalyptus are known on many weed species (7,15,29). The eucalyptus leaf residues, aqueous extracts and essential oil reduced the chlorophylls a and b and carotenoids, and cellular respiration in treated seedlings (3,15), accumulated the reactive oxygen species (11,14,20), damaged the membrane integrity and electrolyte leakage (11,19,29). A recent study showed that leaf residues of *E. globulus* caused damages to *G. max* L. genome (1), indicating that

eucalyptus treatment might induce the internucleosomal fragmentation of genomic DNA, an active process of cell death, which is referred to an autolytic kind of programmed cell death process (33). The results obtained in this study suggest that the herbicidal activities of eucalyptus are due to diverse modes of action, making eucalyptus a suitable biological source for identifying natural compounds with herbicidal activities. The herbicidal activity of essential oils of certain eucalyptus species could be further explored as an alternative option for weed control.

CONCLUSIONS

Laboratory and glasshouse studies confirmed the allelopathic effects of *E. salubris* and *E. brockwayii* on germination and seedlings growth of prairie ground cherry. Both eucalyptus species in their essential oils, contained mixture of phytotoxic compounds, which leached into the ground to suppress the understorey vegetation within the drip line. This natural phenomenon could be used to control the weeds in integrated weed management.

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