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Allelopathic Effects of *Eucalyptus camaldulensis* on Seed Germination and Growth Seedlings of *Acroptilon repens*, *Plantago lanceolata* and *Portulaca oleracea*

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Abstract: A petri dish assay was carried out to study allelopathic potential of foliar aqueous extract of *Eucalyptus camaldulensis* on germination and seedling growth of some weeds including *Acroptilon repens*, *Plantago lanceolata* and *Portulaca oleracea*. Seed germination, rate of germination, root and shoot length of weeds exhibited different degree of inhibition according to the concentration of the aqueous extract. Maximum inhibitions on germination percentage, rate of germination and seedling growth were recorded when using the highest concentration of the aqueous extract (20 g L^{-1}) of Eucalyptus. Seed and rate germination of Portulaca weed were not affected by aqueous extract of *Eucalyptus camaldulensis*. However, seed germination and germination rate of *Acroptilon repens* and *Plantago lanceolata* were severely affected by aqueous extract of *Eucalyptus camaldulensis.* Aqueous extract of *Eucalyptus camaldulensis* affected seedling growth of all weeds even *Portulaca oleracea* so that root and shoot length of *Portulaca oleracea* decreased by 83.3 and 68% at high aqueous extract concentration (20 g L^{-1}), respectively compared to control. Root length was more affected than other parameters by aqueous extract of *Eucalyptus camaldulensis*.

Key words: Allelopathy, germination, Eucalyptus, weeds, seed germination, Iran

INTRODUCTION

Weeds are one of major constraints to plant production worldwide. Weeds affect crop growth and production that may be significantly reduced when weeds compete with them for light, water and minerals (Hussein, 2001). Existing weed control methods are either expensive or hazardous. Heavy use of chemical herbicides in most integrated weed management systems is a major concern since, it causes serious threats to the environment, public health and increase cost of crop production. Therefore, alternative strategies against weed must be developed. Rice (1983) defined allelopathy as the effect (s) of one plant on other plants through the release of chemical compounds in the environment.

Allelopathy interactions are primarily based on the ability of certain species to produce secondary chemical compounds that exert some sort of biological effects on other organisms, many of which unknown. The chemical causing the allelophatic effects are called allelochemicals. Allelopathy is characterized by a reduction in plant emergence or growth, reducing their performance in the association (Florentine *et al*., 2006). Allelopathy provides a relatively cheaper and environmental friendly weed control alternative. This can be considered as a possible alternative weed management strategy (Cheema *et al*., 2000). Purslane (*Portulaca oleracea*) as an important widespread weed is a fleshy annual herb, reproducing by seeds or stem-fragments rooting when lying on moist soil. The stems are succulent, often reddish and 20-50 cm in length. The leaves are alternate frequently clustered at the end of branches. Flowers are yellow, sessile and self pollinated. Flowers open on sunny mornings and produce numerous (up to 243000 per plant), tiny (0.5 mm diameter) and black seeds (Waterhouse, 1994).

Ribwort (*Plantago lanceolata*) is a rosette-forming perennial herb with leafless, silky, hairy flower stems (10-40 cm). The basal leaves are lanceolate spreading or erect, scarcely-toothed with 3-5 strong parallel veins narrowed to short petiole. Grouping leaf stalk deeply furrowed, ending in an oblong inflorescence of many small flowers each with a pointed bract. Each flower can produce up to two seeds. It is present and widespread in Iran as an introduced species (Hjelle *et al*., 2006).

Acroptilon repens is a herbaceous perennial that propagates by seeds and vegetative means. It has erect stems and ranges in height from 30-80 cm. Its natural range extends from Turkey throughout Central Asia to China. *A. repens* is an invasive weed causes problems in disturbed habitats in its native range in Asia including

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Iran where the soil is regularly tilled in order to reduce competition for water between the fruit trees and herbaceous vegetation (Uygur *et al*., 2004). Previous studies have shown that various Eucalyptus species possess the allelopathic potential to suppress some weeds (May and Ash, 1990). Allelopathy associated with Eucalyptus due to the presence of allelochemicals such as phenolic and volatile compounds in its foliage (El-Rokiek and Eid, 2009). Coa and Luo (2005) reported that aqueous extract from bark and leaf and volatiles from leaves of *Eucalyptus citriodora* showed allelopathic effect on growth of 9 species including the weeds *Bidens pilosa*, *Digitarie pertenuis*, *Eragrostics cilianesi* and *Setaria geniculata*.

It was reported that seed germination, seedling growth, chlorophyll content and respiratory ability of some weed plants was drastically affected by allelochemical extracted from *Eucalyptus citriodora* (Batish *et al*., 2005). Therefore, this study aimed to evaluate the effect of *Eucalyptus camaldulensis* aqueous extract on germination and seedling growth of some widespread weed species such as *Acroptilon repens*, *Plantago lanceolata* and *Portulaca oleracea* which cause major constraints to crop production in Iran.

MATERIALS AND METHODS

Aqueous extract preparation: Foliar section of *Eucalyptus camaldulensis* that were gathered from north area of Iran (Golestan province) were washed with distilled water and dried at shade room temperature. Dried tissues were ground into a fine powder (using an electric mill until homogeneity was achieved). About 20 g of ground tissue were placed in a 1 L Erlenmeyer flask and 1000 mL deionized water was added to it. The flask were covered with aluminum foil to protect them from photodecomposition, then placed on a rotary shaker (~250 revolutions per min) for 24 h. The mixtures were filtered through Whatman paper no.1 using vacuum pump. The pH and electrical conductivity of extract were determined using a digital pH meter and conductivity meter. This filtrate was considered as stock solution.

A series of solutions including the stock solution (extract of 20 g dry weight L^{-1} of water (S_1) and concentration dilution of 10 g L^{-1} (S_2) (was developed from the stock solution) and deionized distilled water (control) were used for germination tests. The extra solutions were kept in -18°C for later use.

Petri dish bioassays: A petri dish assay based on a randomized complete block design with 6 replications was carried out for screening the effect of different concentrations of aqueous extracts of *Eucalyptus camaldulensis* on germination and seedling growth of *Acroptilon repens*, *Plantago lanceolata* and *Portulaca oleracea*. Weed seeds were pre-sterilized with 2% sodium hypochlorite for 5 min and washed with distilled water. About 30 seeds of each weed (pre-tested seeds with >95% germinabilities) were evenly distributed on two layers of Whatman no. 1 filter papers in each 9 cm disposable petri dish. About 5 mL of a dilute series was added to each petri dish covered with a lid.

Germination was carried out in germinator at average maximum and minimum temperatures 25±1 and 16±1°C for 14 days. Germinated percentage, root and shoot length of seedlings were recorded 10 days after germination.

This experiment was carried out base on randomized complete block design. The data for all characters were analysed using the analysis of variance procedure of Statistical Analysis System (SAS) software, version 6.12. Means were compared by Duncan's multiple range tests at the 0.05 probability level for all comparisons.

RESULTS AND DISCUSSION

Germination percentage and seedling growth of target weed seeds (*Acroptilon repens*, *Plantago lanceolata* and *Portulaca oleracea*) were negatively affected by different concentrations of aqueous extracts from foliage tissues of *Eucalyptus camaldulensis*. The maximum germination percentage was reached in distilled water (control) for all tested weeds (Fig. 1). Germination was progressively inhibited with increasing concentration of foliar dry powder aqueous extract of *Eucalyptus camaldulensis*. However, the sensitivity of the different weeds and characters to aqueous extract of *Eucalyptus camaldulensis* varied. Germination percentage of Portulaca was not affected by different concentration of foliar aqueous extract of *Eucalyptus camaldulensis*

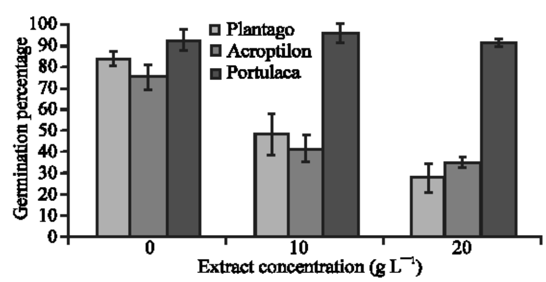

Fig. 1: Germination percentage of studied weed seeds in the presence of various concentrations of aqueous extracts of *Eucalyptus camaldulensis*. Each reported value represents the means±S.D of 6 replications

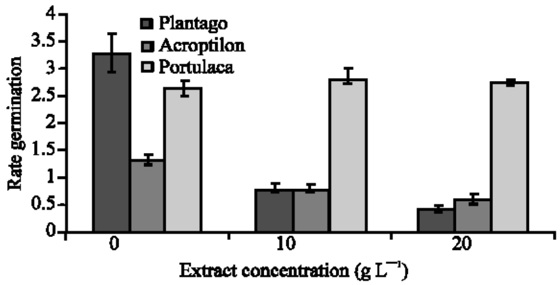

Fig. 2: Germination rate of studied weed seeds in the presence of various concentrations of aqueous extracts of *Eucalyptus camaldulensis*. Each reported value represents the means±S.D of 6 replications

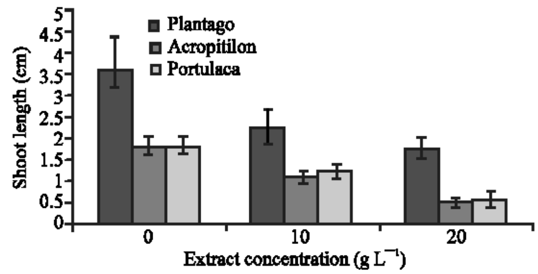

Fig. 3: Seedling shoot length of studied weed species in the presence of various concentrations of aqueous extracts of *Eucalyptus camaldulensis*. Each reported value represents the means±S.D of 6 replications

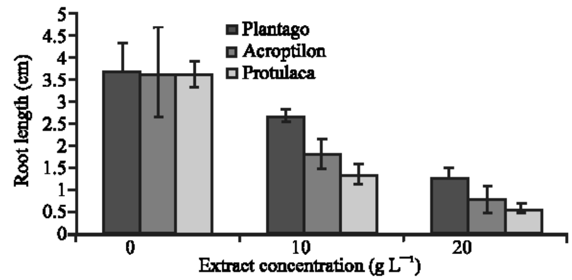

Fig. 4: Seedling root length of studied weed species in the presence of various concentrations of aqueous extracts of *Eucalyptus camaldulensis*. Each reported value represents the means±S.D of 6 replications

(Fig. 1) while the germination percentage of Plantago and Acroptilon decreased by 66.9 and 53.6% at the highest concentration of aqueous extract (20 g L^{-1}), respectively compare to control. Germination rate of Portulaca also was not affected by aqueous extract of Eucalyptus. However, germination rate of Plantago and Acroptilon decreased by 86.9 and 54.6%, respectively at the highest concentration of aqueous extract of Eucalyptus compare to control (Fig. 2). This indicated that Eucaliptus allelochemicals were not inhibitor on Portulaca germination.

Although, seed germination and germination rate of portulaca were not affected by aqueous extract concentrations, growth of young seedlings of Portulaca was severely reduced especially at the higher aqueous extract concentration. Root length of Portulaca decreased by 61.9 and 83.3% at low (10 g L^{-1}) and high (20 g L^{-1}) concentrations of aqueous extract of Eucalyptus, respectively (Fig. 3). Shoot length of Portulaca also decreased by 32 and 68% at low (10 g L^{-1}) and high (20 g L^{-1}) concentrations of aqueous extract of Eucalyptus compare to control (Fig. 4). Shoot length of Plantago and Acroptilon decreased by 51 and 72% while root length decreased by 64.5 and 79%, respectively at highest extract concentration. It can be said that changes in shoot length were on average smaller than those in root length. This is in agreement with Ahn and Chung (2000) who found that the length and dry weight of roots of *Echinochloa crusgalli* were more affected by hull extract than the shoots.

An *et al*. (2001) on evaluation of Vulpia (*Vulpia myuros*) allelochemicals also found all phenolic compounds caused greater inhibition on root elongation than on shoot length.

The present finding corroborate the earlier report by El-Rokiek and Eid (2009) who found that the inhibitory effect of foliar extract of Eucalyptus on germination of some weeds was proportional to the concentration of the extract. In the present study, responses indices revealed that the inhibition of growth parameters of seedlings was more pronounced than that of seed germination. In other words, the seedling growth of the target weeds was more suppressed than the germination. Smith (1991) and Ben-Hammounda *et al*. (1995) found aqueous leaf extracts of several species have suppressed seedling growth in target plants more than seed germination.

The inhibitory effect of Eucalyptus on seed germination and seedling of weeds may be related to the presence of allelochemicals including phenolic contents and volatile compounds in its foliage. Furthermore, the toxicity might be due to synergistic effect rather than single one. Phenolic acids have been shown to be toxic to germination and plant growth processes (May and Ash, 1990; Enhilelling, 1995; Asghari and Tewari, 2007). El-Rokiek and Eid (2009) reported that the inhibitory effects of Eucalyptus on weeds correlated with accumulation of internal contents of total phenols compared to their respective controls. Accumulation of phenols is often a characteristics stress condition (Hopkins, 1999; El-Rokiek, 2002, 2007). Some researchers reported that allelochemicals exhibited inhibitory effects on physiological processes that translate to growth (Jefferson and Pennacchio, 2003).

The effects of allelopathy on germination and growth of plants may occur through a variety of mechanisms including reduced mitotic activity in roots and hypocotyls, suppressed hormone activity, reduced rate of on uptake, inhibited photosynthesis and respiration, inhibited protein formation, decreased permeability of cell membranes and/or inhibition of enzyme action (Rice, 1983). The nature of the inhibitory effect of allelochemical to seed germination could be attributed to inhibit water absorption which is a procusor to physiological processes that should occur in seed before germination is triggered (Oyun, 2006).

Similarly, the nature of the effect of the allelochemicals on seedling growth was likely to be that of inhibition to nutrient uptake by weeds thereby reducing growth parameters.

CONCLUSION

Results show that the effect of extract concentration strongly decreased germination percentage, rate of germination and seedlings growth. However, the sensitivity of weeds target differed. The seedling growth of the target weeds was more suppressed than the germination.

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