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# Effects of Aqueous Eucalyptus Extracts on Seed Germination, Seedling Growth and Activities of Peroxidase and Polyphenoloxidase in Three Wheat Cultivar Seedlings (*Triticum aestivum* L.)

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**Abstract:** Evaluation of allelopathic effects of this plant on other near cultivations especially wheat is the aim of this study. Effects of water extracts of eucalyptus leaves examined on germination and growth of three wheat cultivar seeds and seedlings. Results showed that: germination percentage strongly decreased, leaf and root lengths also affected and dry and wet weights of both roots and shoots showed similar change patterns. Activities of peroxidase and polyphenoloxidase as antioxidant enzymes in roots and shoots measured. Activity of peroxidases increased in stress conditions and roots showed more increased enzyme activity than leaves. Activity of polyphenoloxidases increased only in one of three cultivars and again roots showed more activity of this enzyme in response to eucalyptus extract. Suggest that detoxification process were conducted mainly in roots of seedlings.

**Key words:** Allelopathy, eucalyptus, peroxidase, polyphenoloxidase, wheat seedling

## INTRODUCTION

Plants compete with each other for light, water and nutrients. Production of secondary metabolites, accumulation and release of these compounds is one of several complex defense strategies that have evolved by plants (Rice, 1984; Swain, 1977). The intractions of plants through release of chemicals is called allelopathy. There are hundreds of secondary metabolites in the plant kingdom and many are known to be phytotoxic (Einhellig, 2002). Allelopathic effects of these compounds are often observed to occur early in the life cycle, causing inhibition of seed germination and/or seedling growth. These compounds exhibit a wide range of mechanisms of action and interpretations of mechanisms of action are complicated by the fact that individual compounds can have multiple phytotoxic effects (Einhellig, 2002).

The chemical interaction of plants through chemical signals or allelopathy has many possible agricultural applications and decline in crop yields in cropping and agro-forestry systems in recent years has been attributed to allelopathic effects. Eucalyptus is a native species in Australian rain forests and recently cultivated on dry and saline lands in south of Iran and in Mazandaran in soils with high level of underground water especially near the Caspian sea located on north of Iran. These trees also used as wind shelter for agricultural lands in many parts of Iran (Djazirehi, 2001). Eucalyptus species are considered one of the most notorious allelopathic trees and contain the most number of allelochemicals (El-Khawas and Shehata, 2005). It has been shown that eucalyptus affects on near plants by release of these allelochemicals to its surroundings (Willis, 1999). The allelopathic effects of eucalyptus have been examined in several crop plants including beans and cereals (Malik, 2004; Blaise, 1997; Singh *et al*., 1991; Kohli and Singh, 1991; May and Ash, 1990).

The evaluation of harmful effects of eucalyptus plant on three wheat cultivars (one of the most important crop plants in Iran) is the main aim of this study.

Germination percentage, fresh and dry weights and average length of both leaves and roots measured. Activities of two antioxidant enzymes; peroxidases and polyphenoloxidases in response to stress conditions also measured.

## MATERIALS AND METHODS

In this study seeds of three wheat cultivars prepared from Karaj seed and Seedling Research Institute.

Cultivar Roshan as a spring wheat and cultivars chamran and Dez as winter varieties.

All experiments conducted in laboratory of Islamic Azad University, Research and Science Campus located in east north of Tehran, from December 2004 to September 2005.

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**Eucalyptus extract:** Leaves of 10 years old Eucalyptus (*Eucalyptus camadulensis* Labill) gathered. After careful washing leaves dried for 72 h in 60°C. Leaves powdered by a grinder and 30 grams of powder added to 100 mL distilled water and stirred gently for 24 h by a shaker in room temperature. The suspension filtered two times by Wathmann filter paper No. 2 to remove the fiber. The resulting solution was passed through a sterilized 0.4 μm filter to avoid any contamination.

From this solution, 5, 10, 20, 30, 40 and 50% dilutions prepared and sterilized distilled water used as control. The germination and seedling growth conducted in autoclaved Petri dishes (15 cm diameter) covered by one layer of filter paper. Ten seeds of each cultivar planted in a petri dish and 10 mL of extract added to them as per treatment. The petri dishes were kept in germinator with 18 h light in 22°C and 6 h dark in 18°C.

Seed germination measured after 7 days and after 14 days fresh and dry weight of roots and leaves. Average length of 10 seedling leaves and roots measured. For measuring enzymatic activity, Fresh tissues of both roots and leaves were used. One gram fresh tissue were weighted and homogenized with 3 mL of Tris-glycine buffer at pH 8.3 in 4°C. The homogenate centrifuged at 12000 rpm in 4°C and supernatant used for enzyme activity measurement. POD activity was determined by spectrophotometry with $H_2O_2$ as a substrate and benzidine as chromogenic reagent at 530 nm (Mae-Adams, 1992) PPO activity was also followed by spectrophotometry using gayacol at 430 nm in 40°C (Raymonds, 1993). Results expressed in terms of dAbs $min^{-1}g^{-1}$Fw.

**Statistical analysis:** All experiments conducted with 3 replication per treatment and the data from each experiment were objected to an analysis of variance with significant amount means identified by Duncan` s multiple range test (p>0.05).

## RESULTS

The allelopathic potential of eucalyptus camadulensis on germination of three wheat cultivars is shown in Table 1 and it is obvious that aqueous extracts of eucalyptus leaves have inhibitory effects on germination of seeds especially on Dez and Chamran in high concentrations (40 and 50%).

Table 2 and 3 show the toxic effects of Eucalyptus leaf extracts on growth of first roots and leaves. Elongation of leaf and roots was reduced with increasing concentration of eucaluptus extracts and toxic effect of extract is much more pronounced on roots than leaves. Table 2 show that in Roshan leaf length is less affected than the two others and Table 3 show that root growth of Chamran is nearly inhibited totally in 50% concentration of extract.

Leaf extracts of eucalyptus reduces the total fresh weight of both leaves and roots with some exceptions: a small increase in 10% eucalyptus extract and then decrease in higher concentrations of extract for leaf and root fresh weight of all three cultivars and also in Dez fresh weight of leaves showed strong and meaningful increase in 5 and 10% eucalyptus extract (Table 4 and 5).

While in Roshan dry matter of leaves and roots decreased with increasing leaf extract concentrations, the dry matter of leaves in roots of Dez and Chamran not only did not show any decrease but also increased in 30 and 40% eucalyptus extracts (Table 4 and 5).

Root fresh weight of Chamran cultivar seedlings decreased in 40 and 50% aqueous extracts of eucalyptus leaves. Exceptional increase in root length of this cultivar in 5 and 10% aqueous extracts of eucalyptus leaves observed. Root fresh weights of cultivar Roshan seedlings decreased in 20, 30, 40 and 50% aqueous extracts of eucalyptus leaves comparing with control. In Dez cultivar seedlings fresh weight of roots increased in 5, 10 and 20% aqueous extracts of eucalyptus leaves (Table 6 and 7).

Table 1: Effects of aqueous leaf extracts of *E. camadulensis* on germination percentage of three wheat cultivar seeds (Mean±SE)

| Eucalyptus leaf extracts | Germination (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0% | 5% | 10% | 20% | 30% | 40% | 50% |
| Chamran | 98.33 | 74.33 | 52.33 | 27.33 | 25 | 11.66 | 5 |
| Roshan | 91.90 | 83.57 | 80.5 | 60.66 | 56.66 | 48.33 | 15 |
| Dez | 80.66 | 88.33 | 54 | 41.66 | 26.66 | 11.66 | 8.33 |

Table 2: Effects of aqueous leaf extracts of *E. camadulensis* on average leaf length (Mean±SE)

| Eucalyptus leaf extracts | Average leaf length of three wheat cultivars (cm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0% | 5% | 10% | 20% | 30% | 40% | 50% |
| Chamran | 6.166 | 5.000 | 5.000 | 4.500 | 3.633 | 2.333 | 1.666 |
| Roshan | 13.000 | 6.300 | 10.166 | 5.633 | 6.833 | 5.833 | 4.000 |
| Dez | 7.600 | 6.300 | 5.766 | 4.100 | 2.900 | 2.333 | 1.066 |

Table 3: Effects of aqueous leaf extracts of *E. camadulensis* on average root length (Mean±SE)

| Eucalyptus extract | Average root length of three wheat cultivar seedlings (cm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0% | 5% | 10% | 20% | 30% | 40% | 50% |
| Chamran | 5.360 | 6.713 | 4.100 | 2.533 | 2.180 | 2.165 | 0.283 |
| Roshan | 7.516 | 6.960 | 4.663 | 2.040 | 1.750 | 1.670 | 1.083 |
| Dez | 6.716 | 6.700 | 6.740 | 2.506 | 1.886 | 2.263 | 0.653 |

Table 4: Effects of aqueous leaf extracts of *E. camadulensis* on average fresh weight of leaves (Mean±SE)

| Eucalyptus extracts | Average fresh weights of leaves in three wheat cultivar seedlings (g $seed^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0% | 5% | 10% | 20% | 30% | 40% | 50% |
| Chamran | 0.055 | 0.0706 | 0.0753 | 0.0676 | 0.055 | 0.0421 | 0.044 |
| Roshan | 0.0945 | 0.0886 | 0.098 | 0.05 | 0.062 | 0.063 | 0.0736 |
| Dez | 0.0495 | 0.089 | 0.088 | 0.0735 | 0.069 | 0.060 | 0.0465 |

Aqueous extracts of eucalyptus leave also affected the dry weight of three wheat cultivar leaves. Significant dry matter increased in 30 and 40% aqueous extracts in Chamran cultivar seedlings were observed. Leaf dry matter decreased in Roshan cultivar according to increase of extract concentration. In wheat seedlings of Dez no significant change in leaf dry matter were observed.

Dry matter in roots of chamran cultivar seedlings increased in 10, 30 and 40% aqueous extracts of eucalyptus leaves. In roots of Roshan root dry matter decreased in 10, 30, 40 and 50% aqueous extracts of eucalyptus leaves. Root dry matter of Dez seedlings showed no difference with control (Table 6 and 7).

Leaf extracts of Eucalyptus strongly affects the activity of peroxidases in both roots and leaves. Activity of peroxidases is much higher in roots but it is obvious that increased activity of POD enzymes is much more pronounced in leaves than roots (Table 8 and 9).

Results on Table 10 and 11 show that Activity of PPO enzymes increased in roots and leaves of only one wheat cultivar (Roshan) seedlings by adding aqueous extracts of eucalyptus leaves.

Table 5: Effects of aqueous leaf extracts of *E. camadulensis* on average fresh weights of roots (Mean±SE)

| Eucalyptus extracts | Average fresh weight of roots in three wheat cultivar seedlings (g seed$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.0496 | 0.0853 | 0.081 | 0.0683 | 0.057 | 0.04 | 0.0253 |
| Roshan | 0.07 | 0.06 | 0.08 | 0.04 | 0.02 | 0.03 | 0.04 |
| Dez | 0.052 | 0.0803 | 0.0826 | 0.075 | 0.0403 | 0.033 | 0.0343 |

Table 6: Effects of aqueous leaf extracts of *E. camadulensis* on average dry weights of ten seedling leaves (Mean±SE)

| Eucalyptus extracts | Average leaf dry weight of three wheat cultivar seedlings (g 10 seed$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.0866 | 0.0853 | 0.096 | 0.091 | 0.116 | 0.107 | 0.089 |
| Roshan | 0.082 | 0.067 | 0.053 | 0.049 | 0.033 | 0.023 | 0.040 |
| Dez | 0.058 | 0.070 | 0.063 | 0.058 | 0.086 | 0.065 | 0.061 |

Table 7: Effects of aqueous leaf extracts of *E. camadulensis* on average dry weights of ten seedling roots (Mean±SE)

| Eucalyptus extract | Average root dry weights of three wheat cultivars (g 10 seed$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.0433 | 0.0513 | 0.079 | 0.053 | 0.081 | 0.072 | 0.053 |
| Roshan | 0.071 | 0.043 | 0.037 | 0.043 | 0.014 | 0.016 | 0.032 |
| Dez | 0.0583 | 0.059 | 0.058 | 0.055 | 0.066 | 0.060 | 0.055 |

Table 8: Effects of aqueous leaf extracts of *E. camadulensis* on POD activities of leaves (Mean±SE)

| Eucalyptus extract | Peroxidase activity of leaves in three wheat cultivar seedlings (dA dt$^{-1}$ g$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.41 | 0.56 | 0.72 | 0.38 | 0.70 | 1.30 | 0.93 |
| Roshan | 0.34 | 0.54 | 0.62 | 0.33 | 0.61 | 1.07 | 0.844 |
| Dez | 0.33 | 0.61 | 0.58 | 0.40 | 0.72 | 1.50 | 0.10 |

Table 9: Effects of aqueous leaf extracts of *E. camadulensis* on POD activities of roots (Mean±SE)

| Eucalyptus extracts | Peroxidase activity of leaves in three wheat cultivar seedlings (dA dt$^{-1}$ g$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.98 | 1.20 | 1.70 | 1.50 | 1.40 | 1.7 | 1.90 |
| Roshan | 1.02 | 1.53 | 2.03 | 1.29 | 1.22 | 1.6 | 1.73 |
| Dez | 1.11 | 1.42 | 1.90 | 1.33 | 1.35 | 1.6 | 1.70 |

Table 10: Effects of aqueous leaf extracts of *E. camadulensis* on PPO activities of leaves (Mean±SE)

| Eucalyptus leaf extracts | Polyphenoloxidase activity of leaves in three wheat cultivars (dA dt$^{-1}$ g$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.10 | 0.097 | 0.099 | 0.12 | 0.13 | 0.09 | 0.13 |
| Roshan | 0.105 | 0.446 | 0.772 | 0.28 | 0.462 | 0.634 | 0.466 |
| Dez | 0.11 | 0.12 | 0.10 | 0.098 | 0.097 | 0.079 | 0.12 |

Table 11: Effects of aqueous leaf extracts of *E. camadulensis* on PPO activities of roots (Mean±SE)

| Eucalyptus extracts | Polyphenoloxidase activity of roots in three wheat cultivars (dA dt$^{-1}$ g$^{-1}$) 0% | 5% | 10% | 20% | 30% | 40% | 50% |
|---|---|---|---|---|---|---|---|
| Chamran | 0.32 | 0.31 | 0.32 | 0.3 | 0.33 | 0.29 | 0.30 |
| Roshan | 0.30667 | 0.446 | 0.63 | 0.31 | 0.42 | 0.46 | 0.34 |
| Dez | 0.28 | 0.34 | 0.33 | 0.32 | 0.31 | 0.30 | 0.32 |

## DISCUSSION

The aqueous leaf extracts of *E. camadulensis* Labill strongly influence the germination ability of different wheat cultivar seedlings (Table 1). Active allelochemicals present in leaf extract of eucalyptus are commonly phenolics (Ballester *et al*., 1989) that show inhibitory effects on germination and growth of wheat cultivar seedlings. These results are in agreement with those obtained by El-Khawas and Shehata (2005) with very similar method of extraction on in seed germination and growth of bean and maize. Similar results have been obtained from different eucalyptus species on seed germination of plants: Effects of Eucalyptus globulous on germination and growth of maize, bean and potato (Malik, 2004); Eucalyptus globulous on green gram, black gram and coepea (Djanaguiraman *et al*., 2002) and also different Eucalyptus species on green gram, black gram and peanut (Singh *et al*., 2003) wheat, maize and cowpea (Blaise *et al*., 1997; Khan *et al*., 1999). Growth parameters changed in response to increasing leaf extracts concentrations of eucalyptus (Table 2-5). Leaf and root lengthening inhibited effectively. Similar results obtained by Khan *et al*. (1999) on wheat and maize and also in many similar studies with eucalyptus.

Fresh weights of both leaves and roots decreased meaningfully while the dry matter not. This reduction in fresh matter may be due to imbalances in water uptake or osmotic balances of the tissues because of allelohemical toxicity (Blum *et al*., 1999) and/or root growth inhibition (Chon *et al*., 2002). Seed germination of cultivar Roshan showed less sensibility to allelochemicals of extract and this maybe due to more resistance mechanisms that have been evolved in this plant. Chon *et al*. (2002) mentioned that some plants root tip growth nearly inhibited to escape from allelochemicals absorption. Nevertheless Sing *et al*. (2003) found that aqueous leaf leachates of *E. citriodora* inhibited seed germination and seedling growth of Vigna species and elongation of plumule more suppressed than radicles. These results are in contradiction with results observed in wheat radicle elongations (Table 3). High concentrations of allelochemicals caused more reduction of root elongation than leaves. Eucalyptus leaf extracts stimulate the peroxidase activity in both roots an first leaves in all three wheat cultivars (Table 8 and 9) and root enzymes are more stimulated than leaves because of direct contact with allelochemicals present in rooting medium. Allelochemicals absorbed by plant cells should be detoxified (Rice, 1984) and detoxification processes and also other responses of plant cells to these stressed conditions causes the increased activity of antioxidant enzymes: PPO and POD. These results are in agreement with previous studies in Eucalyptus extracts and also other allelochemicals on pland seedling (Szalay1 *et al*., 2005; Schulz and Friebe, 1999; Alscher and Cumming, 1990). Lar-Nunez *et al*. (2006) showed that allelochemicals stimulate the activity of many antioxidant enzyme activities in response to high level of free oxygen radicals in *lycopersicum esculentum*. Singh *et al*. (2006) also showed that $\alpha$-pinene; a common monotrepene present in many aromatic trees including eucalyptus stimulate antioxidant enzyme activities in some plant species including wheat. More activity of these enzymes observed in root parts suggest that detoxification and stress resistance mechanisms mainly occurred in root cells of these three cultivars. Increase in PPO activity only in one cultivar might be due to some special stress resistance abilities (Szalay1 *et al*., 2005). These experiments conducted in laboratory, seedling germination and seedling growth is conducted in soil free environment; but to exert the phytotoxic effects of one plant on other plant species, chemicals may have to move to the roots of the target plant through the soil. However, during movement, abiotic (physical and chemical) and biotic (microbial) soil barriers can limit the phytotoxicity of chemicals in terms of quality and quantity required to cause injury (Inderjit, 2001; Cheng, 1989). Therefore field experiments in natural soils can show more natural allopathic effects of plants including eucalyptus trees.

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