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Effects of Aqueous Eucalyptus (*E. camadulensis* Labill) Extracts on Seed Germination, Seedling Growth and Physiological Responses of *Phaseolus vulgaris* and *Sorghum bicolor*

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Abstract: Plants may be affected directly or indirectly by allelochemicals which may be released from plants or microorganisms. In this study, the allelopathic effect of leaf leachate of *Eucalyptus camaldulensis* Labill was examined on germination, growth, morphological and physiological criteria of sorghum (*Sorghum bicolor*) and kidney-bean (*Phaseolus vulgaris*). Leaf leachate was tried at 5, 10 and 20% concentrations and sterilized distilled water used as control. Seed germination, seedlings dry matter, shoot/root length were significantly reduced by all concentrations in both species (at 0/05 level). Decrease in chlorophyll content, soluble sugar content and consequently protein content is proportional to the increase in concentration of leaf leachate in both species.

Key words: Allelopathy, *Eucalyptus*, sorghum, seed germination, *Phaseolus vulgaris*, Iran

INTRODUCTION

Allelopathy, from the Latin words allelon of each other and pathos to suffer refers to the chemical inhibition of one species by another. Although, the term allelopathy is most commonly used to describe the chemical interaction between two plants, it has also been used to describe microbe-microbe, plant-microbe and plant-insect or plant-herbivore chemical communication. In plants, allelochemicals can be present in the leaves, bark, roots, root exudates, flowers and fruits. The delivery of allelochemicals into the rhizosphere is often thought to occur through leaching from leaves and other aerial plant parts, through volatile emissions by root exudation and by the breakdown of bark and leaf litter (Weir *et al*., 2004). Allelopathic effects of these compounds are often observed to occur early in the life cycle, causing inhibition of seed germination and or seedling growth (Einhellig, 2002). Therefore, the activity of allelochemicals can not be explained by just a single mode of action. The majority of effects such as reduction in seed germ inability and seedling growth, chlorosis, decreased ion up take and other physiological, morphological and an atomically abnormalities are caused by a variety of more specific interactions between allelochemicals and cellular or molecular systems. The degree of inhibition depends on their concentration. Some plants genotypes are likely to escape the allelopathic chemical (s) by being hypersensitive (Chon *et al*., 2002). Some new data indicating that allelochemicals may influence signal transduction pathway, since allelopathics from *Flourensia cernua* interacted with bovine brain CaM (calmoduline) (Mata *et al*., 2003). This example illustrates a complication and complexity of allelopathy phenomenon.

Eucalyptus camadulensis belongs to family mirtaceae. It is a large perennial woody tree. It is a native species in Australian rain forests and recently cultivated on dry and saline lands in south of Iran and in soils with high level of underground water especially near the Caspian sea on north of Iran (Ziaebrahimi and Khavari-Nejad, 2007). A large number of studies has confirmed that *Eucalyptus* leachates contained phenolic compounds. Bisal reported that *Eucalyptus* has harmful effects on germination and seedling growth of wheat, barley, lentil, chickpea, mustard and many weeds (khan *et al*., 2008). The large area of the ground surface beneath *Eucalyptus* remains completely bare or with very limited vegetation due the increase of cation exchange capacity and decrease in both pH and base saturation (Alexander, 1989). Phytotoxic substances may act in many biological processes such as to suppressing the mineral uptake by plants, inhibiting cell elongation and cell division as well as retarding the photosynthesis, respiration and enzymatic activities, resulting in the retardation of plant growth. They may also interfere with the action of plant growth regulators, e.g., gibberellins and auxins (Chou, 1980). It also included

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deterioration of membrane integrity and reduction of chlorophyll contents of leaves. The present research was carried out to study the allelopathic effects of leaf leachates of *E. camadulensis* on germination and some morphological and physiological criteria of *Phaseolous vulgaris* as a dicot plant and *Sorghum bicolor* as a monocot plant.

MATERIALS AND METHODS

Leaves of *Eucalyptus* gathered: After washing, leaves dried for 42 h in 50°C, leaves powdered by grinder. Then 30 g of powder added to 100 mL distilled water and stirred gently for 24 h by a shaker. The suspension filtered two times by wathmann filter paper No. 2 to remove the fiber. From this solution, 5, 10 and 20% dilutions prepared and sterilized distilled water used as control. Ten seeds of sorghum and kidney-bean were treated with 0.1% sodium hypochlorite, washed with distilled water and dried to eliminate fungal attack. The seeds were germinated on filter paper soaked in 10 mL of the aqueous leaf leachates of different concentrations, while distilled water was used for control treatment. The Petri dishes (15 cm diameter) were kept in germinator with 18 h light in 22°C and 6 h dark in 18°C. Seed germination measured after 7 days, shoot and root length, seedling dry matter, total chlorophyll content. Soluble protein, soluble sugar was recorded after 14 days. Chlorophyll contents were determined according to Arnon (1949). Soluble sugar was extracted by Homme *et al.* (1992). Soluble protein was determined according to the method described by Bradford (1976).

Statistical analysis: All experiments conducted with 4 replications per treatment and the data were objected to an analysis of variance with significant amount means identified by LSD ($p>0.05$).

RESULTS AND DISCUSSION

The leaf leachates of *E. camadulensis* inhibited the seed germination of sorghum and kidney-bean (Fig. 1a). In the present study, maximum inhibition was observed in 20%, followed by 10 and 5%. The inhibition effect in kidney-bean (84%) was more than in sorghum (15%). These results are in agreement to those obtained by El-Khawas and Shehata (2005) found that the use of aqueous extracts of *E. golobuse* leaves inhibited seed germination of maize and kidney-bean. Under field condition, the *Eucalyptus* trees reduced the wheat crop germination (Patil *et al.*, 2002). The forestry plantation residue of *Eucalyptus* (leaf and branch) suppressed seed germination and early seedling growth of the dicotyledonous species (Schumann *et al.*, 1995). Ziaebrahimi and Khavari-Nejad (2007) in pot experiment, using three wheat cultivars. Aqueous extracts of *E. camadulensis* leaves had inhibitory effects on germination of seed especially in high concentrations (40 and 50%). Singh and Ranjana (2003) found that the aqueous leaf leachates of *E. citrodora* inhibited the germination and seedling growth of *Vigna radiate*, *V. mungo* and *Arachis hypogaea*. A large number of studies confirmed that *Eucalyptus* sp. leachates contained phenolic compounds such as coumaric, gallic, gentisic, catechol, hydroxybenzoic syringic and vanillic acid. Germination of cereals depends on α-amylase activity that regulates starch break down, necessary for supplying substrates to respiratory metabolism. Eucalyptus *(Eucaliptus globosus)* leaf leachates decreased α-amylase activity in seeds of finger milet (*Eleusine coracanta)*, resulting in inhibition of germination (Padhy *et al.*, 2000). Similardata were obtained in the case of cress (*Lepidium sativum*) seeds in the presence of 6-methoxy-2-benz-oxazolinone (MBOA) commonly occurring in cereals (Kato-Noguchi and Macias, 2004). Phenolic compounds extracted from soils covered by beech (*Fagus sylvatica)* and pine markedly inhibited germination of pine seeds (Muscolo *et al.*, 2001). Tested phenolic compounds lowered the activity of glucose-6-phosphate dehydrogenase (G6PDH), glucose phosphate isomerase and aldolase enzymes involved in glycolysis and Oxidative Pentose Phosphate Pathway (OPPP), which ensure the seed supply with sufficient level of reducing power, ATP and carbon skeletons for biosynthesis. Additionally, it was suggested that the observed decrease in enzymatic activity is a secondary effect of allelochemicals, related to protein damage (Muscolo *et al.*, 2001).

Therefore, effects of allelochemicals on seed germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage of organelles. Reserve mobilization, a process which usually takes place rapidly during early stages of seed germination seems to be delayed or decreased under allelopathy stress conditions (Gniazowska and Bogatek, 2005). The leachates of *E. camaldulensis* also caused significant reduction in seedlings growth of sorghum and Kidney-bean (Fig. 1b-d). The inhibition of shoot and length is concentration dependent. The magnitude of inhibition from leachates followed the order: 20>10>5%. This trend was similar in all the test crops. In sorghum 20, 10 and 5% leaf leachates concentration, exhibited 75, 63 and 37% inhibition in shoot length and 90, 74 and 35% in root length, respectively. Likewise, the reduction in

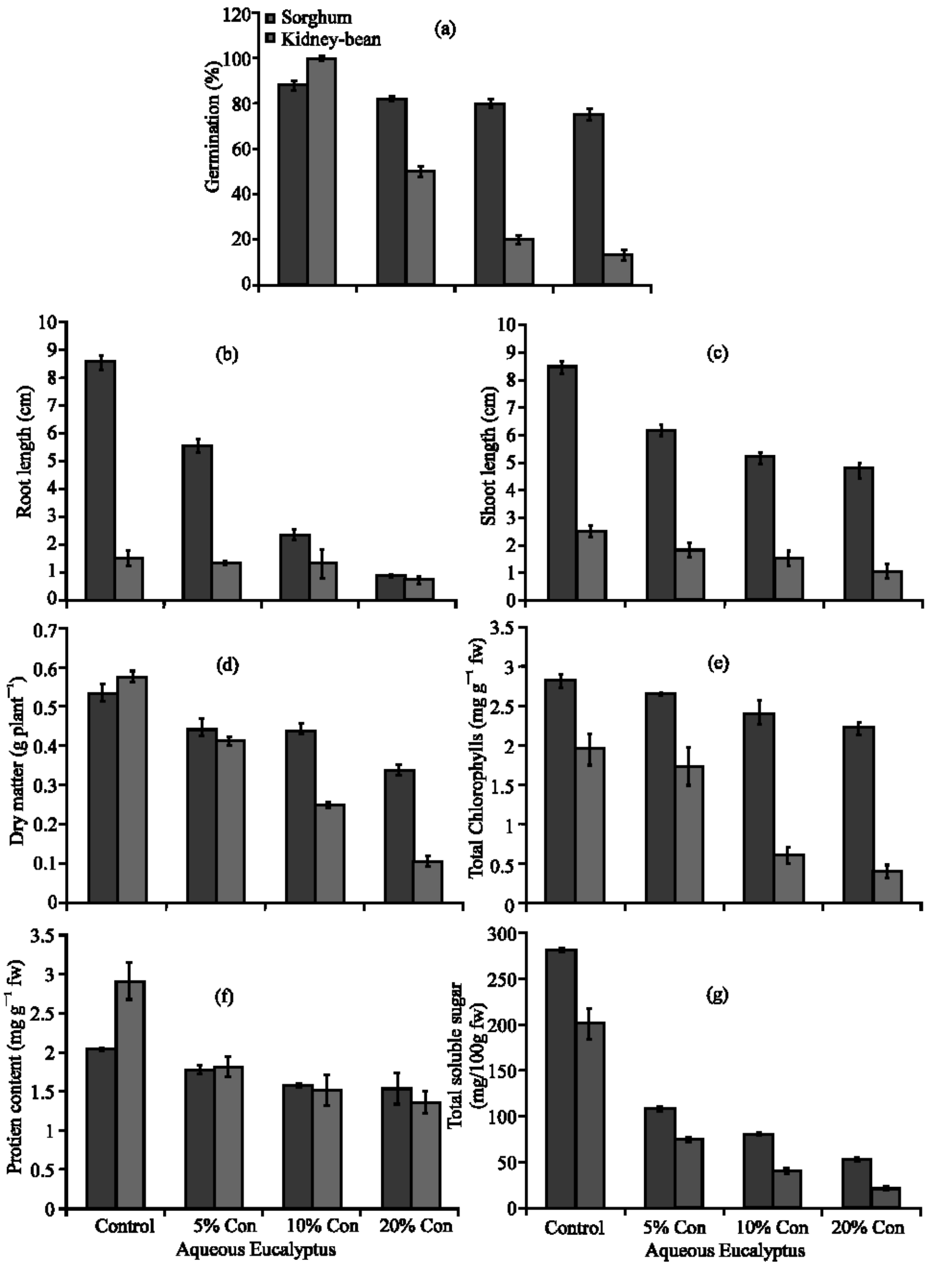

Fig. 1: The Effects of aqueous Eucalyptus (*E. camadulensis* Labill) extracts on seed germination, root and shoot length, total chlorophyll content, protein and total soluble sugar of *Phaseolus vulgaris* and *Sorghum bicolor*

kidney-bean was 58, 37 and 33% in shoot length and 60, 36 and 33% in root length, respectively. The findings correlate with those of Suseelamma and Venkataraju (1994) in groundnut, Beres and Kazinezi (2000) in field crops and in pulse crops (Sasikumar *et al*., 2002). The inhibition of shoot length by *Eucalyptus* may be due to the presence of higher amount of phenols like chlorogenic, p-coumaryl quinic, gentistic and gallic acid (Del Moral *et al*., 1978). These phenolic compounds might have interfered with the phosphorylation pathway or inhibiting the activation of Mg^{2+} and ATPase activity or might be due to decreased synthesis of total carbohydrates, proteins and nucleic acids (DNA and RNA) or interference in cell division, mineral uptake and biosynthetic processes (Sasikumar *et al*., 2002). The reduction of biomass was correlated with reduced seedling growth. The reduction in biomass may be due to stunted and reduced seedlings growth (Tripathi *et al*., 1999). A reduction of 31% in dry matter of sorghum by leaf leachates of eucalyptus (20%) had been reported and the dry matter of kidney-bean was further reduced (81%). These indicate, sorghum was comparatively tolerant to growth suppression by *Eucalyptus*. There is also much data on the effect of allelochemicals on membrane bound enzymes e.g. proton pumping ATPase localized in plasma membrane (H+-ATPase). H^+-ATPase is responsible for generation of proton electrochemical gradient (Michelet and Boutry, 1995) and thereby providing the driving force for the up take and efflux of ions and metabolites across the plasma membrane (Palmgren, 2001). H^+-ATPase inhibition results in reduction in mineral and water up take by roots and as a consequence leads to strong effect on essential plant functions such as photosynthesis, respiration or protein synthesis leading finally to reduction of growth. The total chlorophyll and consequently soluble sugar and protein content of sorghum and kidney-bean were reduced due to the application of Eucalyptus leaf leachates.

Contents of chlorophylls were also reduced significantly in all the treatments (Fig. 1e). The reduction of chlorophyll content in both sorghum and kidney-bean in 20 and 10% leaf leachates concentration was significant but in 5% leaf leachates concentration was insignificant. Among the test crops, kidney-bean showed a maximum decrease of total chlorophyll, whereas, sorghum showed a reverse trend in all the concentrations assayed. In sorghum, total chlorophyll was reduced by 21 in 20% concentrated leaf leachates. However, kidney-bean recorded a decrease of 80, 69, 15%, respectively, over control. The results of the present study are line with the finding of Singh and Ranjana (2003) in rice. The reduction in chlorophyll contents observed in all the concentrations might be due to degradation of chlorophyll pigments or reduction in their synthesis and the action of flavanoids, trepenoids or other phytochemicals present in leaf leachates (Tripathi *et al*., 1999).

Reduction in chlorophylls may decrease the photosynthesis and thereby substantially decrease all the metabolites viz., total sugars, proteins and soluble amino acids (Singh and Ranjana, 2003). In the present study, maximum reduction of soluble protein was observed in kidney-bean (55%) and sorghum (28%) at 20% leaf leachates concentration (Fig. 1f). The reduction of soluble sugar content in both sorghum and kidney-bean in all treatment was significant. In sorghum, 20, 10 and 5% leaf leachates concentration, exhibited 81, 71 and 63% inhibition and in kidney-bean, 90, 80 and 62% inhibition, in sugar content (Fig. 1g). Baziramakenga *et al*. (1997) reported that many phenolic acids reduced the incorporation of certain amino acid into proteins and thus, reduced the rate of protein synthesis. Phenolic acids have been shown to be toxic toactivities of many enzymes (Hopkins, 1999). Moreover, increase of pre-soaking time of seeds in Eucalyptus leachates well as increase of leachates concentration decreased the respiration rate and catalase and α-amylase activities. Pandey *et al*. (1993) concluded that the inhibitors that leached out of the plants research through changes in macromolecules (protein, lipid and nucleic acids), resulting in root dysfunction and other inhibitory activities both in roots and shoots. Present results indicated that the monocot plants (sorghum) is more tolerant than the dicote one (kidney-bean). We can suggest that the allelopatic chemicals of Eucalyptus may have the potential as either herbicide.

CONCLUSION

In this study, the allelopatic chemicals of Eucalyptus may have the potential as either herbicide. And the inhibitory effect of leaf leachate on germination, growth, morphological and physiological parameters in dicot (*Phaseolus vulgaris*) plant was more than monocot (*Sorghum discolor*) plant.

REFERENCES

Alexander, M.J., 1989. The long term effect of *Eucalyptus* plantation on tinmine spoil and its implication for reclamation. Landscape Urban Plann., 17: 47-60.

Arnon, D.I., 1949. Copper enzymes is isolated chloroplasts pollyphenol oxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.

Baziramakenga, R., G.D. Levoux, R.R. Simard and P. Nadeau, 1997. Allelopathic effects of phenolic acids on nucleic acid and protein levels in soybean seedlings. Can. J. Bot., 75: 445-450.

Beres, I. and G. Kazinezi, 2000. Allelopathic effect of shoot extracts and residues of weeds on field crops. Allelopathy J., 7: 93-98.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 248-254.

Chon, S.U., S.K. Choi, S.D. Jung and H.G. Jang, 2002. Effects of alfa alfa leaf extracts and phenolic allelochemical on early seedling growth and root morphology of alfa alfa and barnyard grass. Crop Prot., 5: 1077-1082.

Chou, H.C., 1980. Allelopathic researches in the subtropical vegetation in Taiwan. Phys. Ecol., 5: 222-234.

Del Moral, R., R.J. Willis and D.H. Ashton, 1978. Suppression of coastalheath vegetation by Eucalyptus baxteri. Aust. J. Bot., 26: 203-219.

Einhellig, F.A., 2002. The Physiology of Allelochemical Action: Clues and Views. In: Allelopathy from Molecules to Ecosystems, Reigosa, M.J. and N. Pedrol (Eds.). Science Publi., Enfield, New Hampshire.

El-Khawas, A.S. and M.M. Shehata, 2005. The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and Dicot (*Phaseolus vulgaris* L.) plants. Biotechnology, 4: 23-34.

Gniazowska, A. and R. Bogatek, 2005. Allelopathic interactions between plants. Multi site action of allelochemicals. Acta Physiol. Plantarum, 27: 395-407.

Homme, P.M., B. Gonalez and J. Billard, 1992. Carbohydrate content, fructan and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Lolium perenue* L.) as affected by source and sink modification after cutting. J. Plant Phys., 140: 282-291.

Hopkins, W.G., 1999. Introduction to Plant Physiology. 2nd Edn., John Wiley and Sons, Inc., New York.

Kato-Noguchi, H. and F.A. Macias, 2004. Mode of action of MBOA on inhibition of plant germination. Proceedings of the 2nd European Allelopathy Symposium, Allelopathy from Under Standing to Application. http://seas.iung.pulawy.pl/pdf/str96.pdf.

Khan, M.A., I. Hussain and E.A. Khan, 2008. Suppressing effect of *Eucalyptus camaldulensis* L. on germination and seedling growth of six weeds. Pak. J. Weed Sci., 14: 201-207.

Mata, R., R. Bye, E. Linares, M. Macias, I.R. Cruz, O. Perez and B.N. Timmermann, 2003. Phytotoxic compounds from *Flourensia cernua*. Phytochemistry, 64: 285-291.

Michelet, B. and M. Boutry, 1995. The plasma mem brane H^+-ATPase. A highly regulated enzyme with multiply physiological functions. Plant Physiol, 108: 1-6.

Muscolo, A., M.R. Panucio and M. Sidari, 2001. The effect of plant phenols on respiratory enzymes in seed germination respiratory enzyme activities during germination of *Pinus larico* seeds treated with phenols extracted from different forest soils. Plant Growth Regul., 35: 31-35.

Padhy, B.B., P.K. Patanaik and A.K. Tripathy, 2000. Allelopathic potential of *Eucalyptus* leaf litter leachates on germination and seedling growth of fingermillet. Allel. J., 7: 69-78.

Palmgren, M.G., 2001. Plant plasma membrane H^+-ATPases: Powerhouses for nutrient uptake. Ann. Rev. Plant Physiol. Plant Mol. Biol., 52: 817-845.

Pandey, D., L. Kauraw and V. Bhan, 1993. The inhibitory effect of Partenium hysterophorus residue on growth of Eichhornita crassipes. Chem. Ecol., 19: 2651-2662.

Patil, R.H., C.S. Hunshal and C.I. Itnal, 2002. Influence of bund planted *Eucalyptus* trees row on winter Wheat. Allelopathy J., 10: 21-28.

Sasikumar, K., C. Vijayalakshmi and K.T. Parthiban, 2002. Alleopathic effects of *Eucalyptus* on blackgram (*Phaseolus mungo* L.). Allelopathy J., 9: 205-214.

Schumann, A.W., K.M. Little and N.S. Eccles, 1995. Suppression of seed germination and early seedling growth by plantation harvest residues. South Afr. J. Plant Soil, 12: 170-172.

Singh, N.B. and S. Ranjana, 2003. Effect of leaf leachate of *Eucalyptus* on germination,growth and metabolism of green gram, black gram and peanut. Allel. J., 11: 43-52.

Suseelamma, M. and R.R. Venkataraju, 1994. Effect of *Digera muricata* (L.) Mart. extracts on the germination and seedling growth of groundnut. Allelopathy J., 1: 53-57.

Tripathi, S., A. Tripathi and D.C. Kori, 1999. Allelopathic evaluation of *Tectona grandis* leaf root and soil aqueous extracts on soybean. Indian J. For., 22: 366-374.

Weir, T.L., S.W. Park and J.M. Vivanco, 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opin. Plant Biol., 7: 472-479.

Ziaebrahimi, L., R.A. Khavari-Nejad, H. Fahimi and T. Nejadsatari, 2007. Effects of aqueous eucalyptus extracts on seed germination, seedling growth and activities of peroxidase and polyphenoloxidase in three wheat cultivar seedlings (*Triticum aestivum* L.). Pak. J. Biol. Sci., 10: 3415-3419.