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ALLELOPATHIC EFFECT OF EUCALYPTUS CAMALDULENSIS LEAF EXTRACT ON GERMINATION AND GROWTH OF MUNGBEAN

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Abstract

Allelopathic effect of *Eucalyptus* leaf extract on mungbean was studied for making a good compatible tree-crop association; two separate experiments were conducted during 2009. The first experiment on germination of mungbean seeds was conducted in growth chamber of the Department of Wood and Forest Sciences, Laboratory of the Centre for Forest Research (CFR), Universite Laval, Quebec, Canada. The second pot experiment was conducted in the field of the Department of Agroforestry and Environment, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh to observe the growth performance of mungbean. The study consisted of seven treatments having different aqueous extracts i.e. 0% (control), 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 % of eucalyptus leaves. In seed germination experiment, both rate of germination and radicle growth in lower concentration treatments showed better performance than in higher concentration treatments, while no significant variation was observed between 0% and 2.5% aqueous extract solutions. In growth study, all parameters of mungbean performed better in lower aqueous concentration treatment and the performances were found gradually poor with increasing the concentrations. Regarding weed growth, the highest weed biomass (0.42 g pot^{-1}) was recorded in control (0%) treatment, which was decreased drastically when leaf extract solution was applied. Interestingly, no weed was grown at 15.0% aqueous extract solution. The study suggest that Eucalyptus leaf extract at lower concentration may not have negative effect on mungbean germination and growth, while higher concentration might have great potential for use in organic weed control strategies.

Key words: Allelopathic effect, *Eucalyptus*, Mungbean.

Introduction

Allelopathy also may be one of several attributes which enable a plant to establish in a new ecosystem (Callaway and Aschehoug, 2000; Callaway and Ridenour, 2004). It is well known that allelopathic interaction by a plant is possible through leaching, volatilization from aerial parts, decay of fallen parts and/or exudation in the rhizosphere (Rice 1984). Allelochemicals include terpenoids, phenolic compounds, phenylpropane derivatives, flavonoids, organic cyanides, long-chain fatty acids, and others. Potential allelopathic plants are most often connected with the total phenol content (Ben-Hammouda *et al.* 2001; Lee *et al.* 2004).

Tree-crop association popularly known as 'agroforestry' is a modern tool to develop sustainable land use and to increase food production. To optimize the gains of agroforestry, selection of suitable agroforestry species is very important, and such selection should be based on a number of important characters including allelopathic effect between crop & tree species. Eucalyptus native to Australia (Dawar *et al.*, 2007), widely grown in Bangladesh including in crop field since last couple of decades because of fast growing, wide range of climatic edaphic adaptability.

Allelopathic compounds play important roles in determination of plant diversity, dominance, succession, and climax of natural vegetation and in plant productivity of agroecosystems. Since, *Eucalyptus spp.* is widely grown in association with the different field crops in Bangladesh it is essential to examine its allelopathic effect on the major associated crops. There are strong evidences that *Eucalyptus spp.* have allelopathic effects due to the presence of toxins in its plants (del Moral and Muller, 1970; Singh *et al.*, 1992; Florentine and Fox, 2003). Iqbal *et al.* (2003) found 16 components in essential oil of E. camaldulensis L., out of which five compounds (α-pinene, 3Δ-carene, β-phellandrene, 1-8 cineole and p-cymene) were identified as allelopathy compounds.

Mungbean, *Vigna radiata* (L.) Wilczek, is becoming an important agroforestry crop because of its short duration, nutritional superiority and nitrogen fixing character. Two characteristics of mungbean i.e. adding nutrient (especially N) to the soil and improving soil structure are particular importance for developing countries where agricultural production is often limited by an economical supply of N fertilizer (Mansoor, 2007). The compatibility of this crop with *Eucalyptus spp.* is very important.

Weeds constitute one of the major problems in agriculture. These days, growers spend much money on weed control. About 6 million ton of herbicides were sold in the world in 2006 and herbicides are the most used pesticides; they form approximately 38% of the total amount of pesticides

(FAO 2006). In Bangladesh, in the year 2006-2007, the amount of imported pesticides was 19185 metric tons and their value was 3262.74 million taka of which herbicide was 17% (Anonymous, 2009). Joye and Dubey (2001) reported that several allelo-chemicals have a potential for use as herbicides and have provided structural models for herbicide development.

Advanced studies are accomplishing across the world to explode the potentiality of allelochemicals in agriculture. Unfortunately, no systematic research yet been reported in Bangladesh in this context. Considering the importance of *Eucalyptus spp* and mungbean association, this investigation was undertaken to study the effect of *Eucalyptus* leaf extract on the germination and growth of mungbean.

Materials and Method

Place of Study

Seed germination and radical growth study was conducted in the growth chamber of the Department of Wood and Forest Sciences, Laboratory of the Center for Forest Research (CFR), Université Laval, Quebec, Canada. Plant growth study was conducted in pots at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. The studies were conducted from September to December 2009.

Preparation of aqueous extract for germination Study

Mature leaves of *Eucalyptus camaldulensis* species were sundried on a threshing floor for seven days. The dried leaves were then grinded and made into powder using an electrical grinder.

The study consisted of seven treatments having different aqueous extracts. Aqueous extracts were prepared with distilled water and shaken for four hours. Before making the aqueous extracts, beakers of 2 liter capacity were sterilized/autoclaved. For making sufficient extracts, 20 times of leaves powder i.e., 50, 100, 150, 200, 250 and 300 g of *Eucalyptus* leaves powder were added to the 2 liter of distilled water representing 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% extracts, respectively. For controlled treatment (0% extract), same amount of water without leaf powder was used. All mixtures (powder + water) were then shaken for about five hours for mixing thoroughly. After that, filtration was done using Buchner funnel containing Whatman No. 1 filter paper. The extracts with different concentrations were poured in Conical Flasks and covered by Aluminum Foil. The prepared aqueous extracts were then stored in a cool place (4°C) and used when necessary.

Seed germination Study

This experiment was conducted in sterilized petri dishes (size of 150 x 20 mm) in September, 2009. Seeds of mungbean (var. BU Mung 4) were surface sterilized with 0.2% (w/v) mercuric chloride and rinsed several times with distilled water (Singh and Singh, 2003). Then the three layers sterilized Whatman No. 1 filter papers were placed in petri dishes and saturated with 10 ml extracts of each concentration, while in case of 0% concentration, only the water was used. Forty sterilized seeds of mungbean were then placed in each petri-dish and sealed off with the foil paper. The petri-dishes were then place in growth chamber at dark condition and maintained 25°C temperature and 80% humidity throughout the growing period. The seeds were monitored everyday and germination percent and length of radical were recorded after seven days.

Plant growth Study

This experiment was conducted in pot during October to December, 2009. Mungbean seeds of same variety (var. BU Mung 4) were sterilized with vitavex and sown on 21 October 2009. The experiment was laid out in a Completely Randomized Design (CRD) with five replications. There were seven treatments i.e. 0% (control, distilled water), 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% aqueous extracts solutions of Eucalyptus leaf. Leaf extract solutions were applied as irrigation water. Irrigation was applied twice in a week. The pot size was 6.5"x8.5", which was filled with 3 kg of soil (2:1 = Soil: Compost). Five seeds were sown in each pot and one healthy seedling was kept after one week. Nutrients were supplied as per recommendation of the test crop variety. Pesticides viz. marshal @2ml/litre and tilt@0.5ml/liter were applied to control insects and fungus at 15 day after sowing. Data on plant height, number of leaves per plant, leaf SPAD (soil plant analysis development) value and soil moisture content were collected at one week interval started from two week after sowing. SPAD value and soil moisture were recorded by SPAD meter (SPAD-502, Minolta, Japan) and soil moisture meter (PMS-714), respectively.

Data analysis

Data were analyzed by MSTAT computer software and mean separation was done following least significance difference (LSD) test at 5% level of significance.

Results and Discussion

Rate of germination of mungbean seed

Germination of mungbean seeds was significantly influenced by different concentrations of Eucalyptus leaf extracts (Fig. 1). The highest germination rate (81%) was noted in distilled water (0%) treatment, which was statistically similar with 2.5% aqueous extract solution treatment (79%). Seed germination rate, however, was decreased with the increasing concentrations of leaf extract and the lowest germination rate (56%) was observed in the highest concentration i.e. 15% aqueous extract solution treatment.

The inhibition of germination in Eucalyptus leaf extract treated seeds might be due to presence of inhibiting allelochemicals (Nandal *et al.* 1992). These allelochemicals might inhibit the synthesis of gibberellins and indole acetic acid. Besides, allelochemicals in aqueous leaf leachate might inhibit some physiological process responsible for seed germination (Singh and Singh, 2003). The leaves of Eucalyptus contain flavonoids, triterpenes, such as ursolic acid, arjunolic acid, 2-α-hydroxyursolic acid and essential oil (Choudhury *et al.*, 1987; Padhy *et al.*, 2000). The terpenes presents in the leachates of the leaves might have strongly inhibited the seed germination.

Radicle growth

A distinct variation was found in producing radicle growth of mungbean seeds as influenced by different leaf extract concentrations (Fig. 2). The longest radicle (3.89 cm) was recorded in control (0%) treatment, which was statistically similar with 2.5% aqueous extract solution treatment (3.81 cm), but radicle growth decreased significantly with further increase of

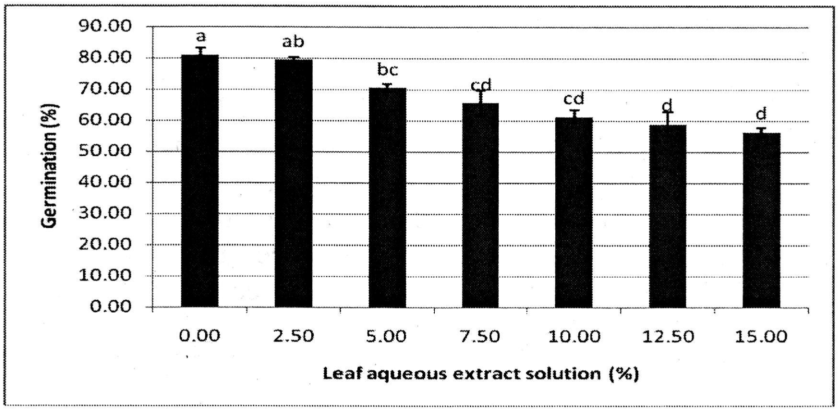

Fig. 1. Effect of Eucalyptus leaf aqueous extracts on seed germination of mungbean. (Columns followed by common letters are not significantly different from each other at 5% level of significance, Error bars are standard errors of means).

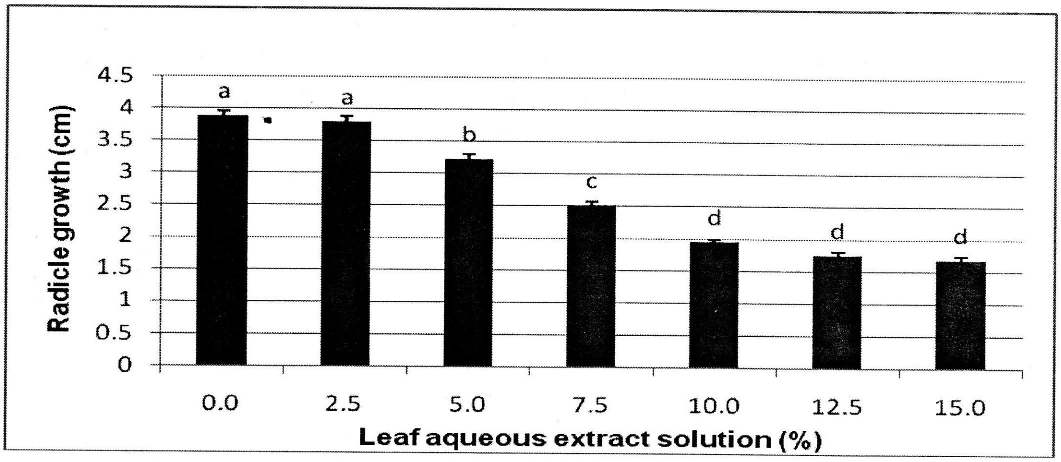

Fig. 2. Effect of Eucalyptus leaf aqueous extracts on radicle growth of mungbean. (Columns followed by common letters are not significantly different from each other at 5% level of significance, Error bars are standard errors of means).

concentrations i.e. in 5.0% (3.23 cm) and 7.5% (2.52 cm) aqueous extract solution treatments. The smallest radicle (1.69 cm) was recorded in 15% aqueous extract solution treatment, while these values were statistically similar with 12.5% (1.76 cm) and 10% (1.96 cm) aqueous extract solution treatments. The study revealed that there was inhibition of radicle growth by Eucalyptus leaf extract treatments having higher concentrations. This might be due to the inhibitory effect of allelochemical on cell division and elongation of mungbean seeds (Nandal *et al.*, 1992). A number of water soluble phytotoxins were determined from Eucalyptus leaves by bio-assay and paper chromatographic methods (Hills, 1996; Kholi, 1990) those showed adverse effects on seedling growth of various crops (Bedi and Kholi, 1989; Singh *et al.*, 1992).

Plant height

Plant height of mungbean crop was measured weekly interval started from 14 days after sowing (DAS) and continued up to 56 DAS. At all the measuring dates, the tallest and shortest plants were noted in control (0%) and 15% aqueous extract solution treatments, respectively, except at first measuring time i.e., 14 DAS. At 14 DAS, the shorter plant was produced by control solution treatment, but at this stage, variation in plant height was insignificant from 0% to 10% aqueous extract solutions. In general, plant height was decreased with increasing leaf extract concentrations. The variation among the leaf extract

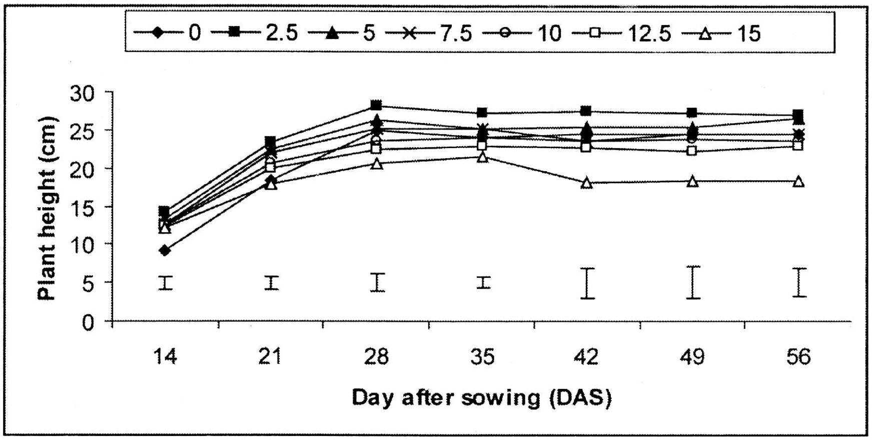

Fig. 3. Effect of Eucalyptus leaf aqueous extracts (%) on plant height of mungbean at different day after sowing (DAS). (Vertical bars indicate the LSD values at 5% level of significance).

concentrations in plant height was more distinct from 35 DAS. Plant height of mungbean at control and 2.5% extract solution were found statistically similar up to 42 DAS. However, plant height of mungbean grown in 15% aqueous extract solution was significantly decreased after 35 DAS and continued up to the end of the measurement (56 DAS). At the final measuring date (56 DAS), plant height was significantly shorter in 15% aqueous extract solution, considering other treatments plant height were statistically similar (Fig. 3).

Leaf number per plant

Leaf number per plant was significantly influenced by leaf extract concentrations measured at different DAS. The maximum and minimum numbers of leaves per plant were recorded in control and 15% aqueous extract solution, respectively, regardless of measuring dates. The trend of leaf number per plant increased up to 28 DAS for all the treatments and it was found statistically higher in control condition, after that leaf/plant decrease regardless of treatments which might be due to chill effect at the onward of 42 DAS (Fig. 4). At 35 DAS, leaf number of control (12.40) to 7.5% (11.20) aqueous extract solution did not vary significantly. From 42 DAS to 56 DAS leaf no/plant statistically alike at plant of control and 2.5% extract solution.

Leaf chlorophyll content

The chlorophyll content of leaf was measured and expressed as SPAD value. Generally, SPAD value indicates

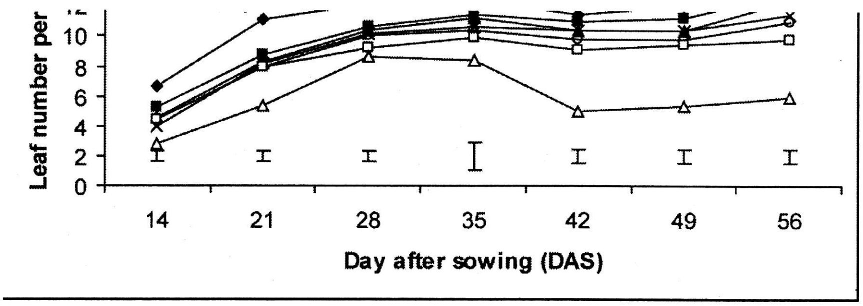

Fig. 4. Effect of Eucalyptus leaf aqueous extracts (%) on leaf number per plant of mungbean at different day after sowing (DAS). (Vertical bars indicate the LSD values at 5% level of significance).

the healthiness and higher chlorophyll content of leaf. A distinct variation was observed in case of SPAD value of leaf (Fig. 5). At all the measuring dates, significantly the highest SPAD values were recorded in control condition, which was decreased with increasing leaf extract concentrations of *Eucalyptus* plant. Up to 42 DAS, the variation of leaf SPAD among the plants of all treatments were less, while the variation was more from 49 DAS to 56 DAS. In general, SPAD value was decreased with increasing concentration of extract. Higher SPAD value indicates higher chlorophyll content. From this study, it was revealed that different concentrations of leaf extract considerably hinders the synthesis of chlorophyll which influenced the metabolism of carbohydrate, protein and nucleic acids. The inhibition of chlorophyll synthesis might be due to interaction of phenolic compounds and phytotoxins present in leachate with phosphorylation pathways or inhibition of activation of $Mg^{2}+$ and ATPase activity (Moreland and Novitzky, 1988). The present findings corroborate with the findings of several studies in other plants (Daizy and Kholi, 1991; Moreland and Novitzky, 1988; Singh *et al.*, 1992).

Weed suppression

Weed dry weight was remarkably influenced by leaf extract concentrations. Distinctly higher weed biomass (0.42 g pot^{-1}) was recorded in control treatment. However, weed biomass drastically decreased when Eucalyptus leaf extract was applied

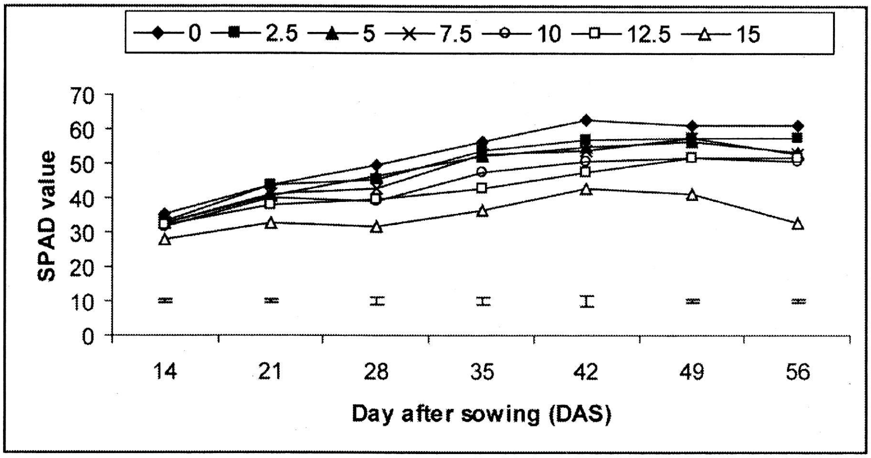

Fig. 5. Effect of Eucalyptus leaf aqueous extracts (%) on leaf SPAD value of mungbean at different day after sowing (DAS). (Vertical bars indicate the LSD values at 5% level of significance).

along with irrigation water even at minimum concentration. Weed biomass noted in 2.5, 5.0 and 7.5% aqueous extract solution treatments were 0.15, 0.13 and 0.10 g pot^{-1}, respectively. In treatments of 10.0% and 12.5% aqueous extract solution, weed biomass decreased remarkably and only 0.03g pot^{-1} weed were found in both treatments. Interestingly, no weed was observed in 15% aqueous extract solution treatment (Fig. 6).

Eucalyptus leaves release phenolic and volatile compounds (Al-Nabi and Al-Mousawi, 1976), which may inhibit the growth of weeds (May and Ash, 1990). Khan *et al.* (2005) reported that leaf aqueous extracts of *E. camaldulensis* and *P. juliflora* have inhibitory effect on germination percentage, seedling length and biomass yield of A. tenuifolius, Brassica campestris, Ipomoea sp., and *Triticum aestivum.* Among them, *A. tenuifolius* and *Brassica campestris* were badly affected. He also suggested that *P. juliflora and E. camaldulensis* can be exploited as bioherbicides for sustainable weed management. Kohli (1990) and Kohli *et al.* (1990) reported that volatile terpenes from *Eucalyptus spp.* particularly the fractions of citronellol and cineole are significant for controlling noxious weed *Parthenium hysterophorus* and *Lantana camara.* However, the allelopathic effect of *Eucalyptus* leaves was not much more harmful for mungbean growth. Thus, allelopathy could play an important role in weed management in

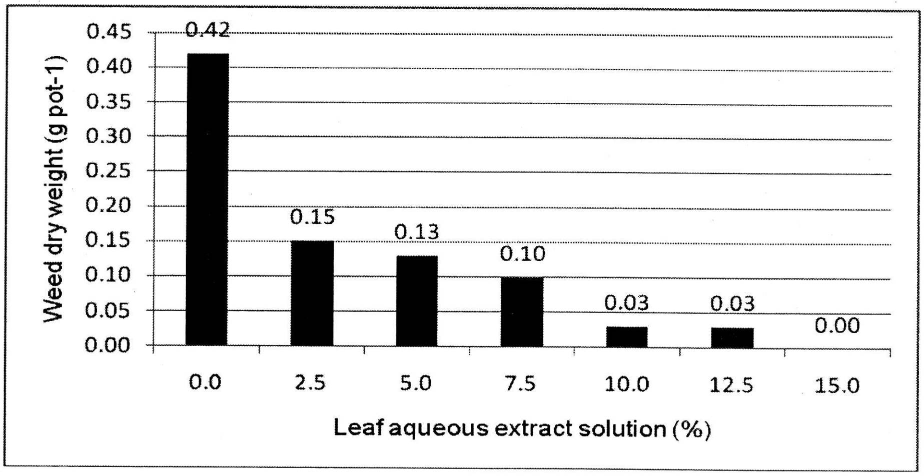

Fig. 6. Effect of Eucalyptus leaf aqueous extracts on weed dry weight in mungbean growth experiment.

organic agriculture which could be one of the most important aspects of organic farming.

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