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ALLELOPATHIC EFFECTS OF FOUR EUCALYPTUS SPECIES ON REDGRAM *(CAJANUS CAJAN* L.)

K. Sasikumar, C.Vijayalakshmi and K.T. Parthiban
Forest College and Research Institute, Mettupalayam 641 301, Tamil Nadu, India

Abstract: Investigations to identify the allelopathic compounds in the leachates of bark, fresh leaves and leaf litter of *Eucalyptus tereticornis, E. camaldulensis, E. polycarpa* and *E. microtheca* using paper and as chromatography showed the presence of coumaric, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids and catechol. The influence of identified phenolics as well as leachates on the germination, seedling length, dry matter production, vigour index and nitrogenase activity of redgram (CO.5) was studied. Germination was inhibited by each individual compounds tested while vigour index was significantly affected by catechol, ferulic, gallic and syringic acids, compared to control. Bioassay with leachates revealed significant reduction in germination over control in all the cased, 7 days after sowing. Dry matter production was affected by *E. camaldulensis* and *E. microtheca.* Meanwhile, vigour index was affected by *E. camaldulensis, E. polycarpa* and *E. microtheca.* Seedling length was affected in all the cases except *E. camaldulensis,* 37 days after sowing. Simultaneously, reduction in vigour index and nitrogenase activity was also noted in all the cases, compared to control.

Key words: Allelopathy, dry matter production, *Eucalyptus,* germination, nitrogenase activity, phenolic compounds, vigour index.

INTRODUCTION

Agroforestry, which involves combining woody plants with annual or perennial crops or livestock, increases the biophysical and/or socio-economic productivity of an agricultural enterprise (Bentlay, 1985). However, farmers have voiced concern about the harmful effects of trees on cultivated lands and standing crops. Although allelopathy the direct or indirect deleterious effect of one plant upon another through the production of chemical inhibitors is by no means a universal explanation of this failure or poor crop growth, yet an increasing body of literature imputes these phenomena to phyto-toxins exuded by the tree crops (Suresh and Rai, 1987). Thus, Baker (1966) reported that *Eucalyptus globulus* produces volatile materials that inhibit the root and hypocotyl growth of cucumber seedlings. A similar lack of herbaceous species under *E. globulus* and *E. camaldulensis* due to their allelopathic effects was also reported by del Moral and Muller (1969). *E.microtheca* compared to *Casuarina cunninghamiana* was reported to posses a poor understorey due to allelopathic effects in central Iraq (Al-Mousawi and Al-Naib, 1976). So, *Eucalyptus* though a potential industrial crop is not being recommended as an intercrop in agroforestry systems (Bansal, 1988; Suresh and Rai, 1987), presumably due to the release of inhibitory compounds from the trees (Lisanework and Michelson, 1993). The release of phenolic compounds adversely affects the germination and growth of plants through their interference in energy metabolism, cell division, mineral uptake and biosynthetic processes (Rice, 1984). Leachates from stemflow and litterfall are responsible for such an effect (Molina *et al.,* 1991). The rains result in periodical release and accumulation of allelo-chemicals in plantations. Hence, an attempt was made to analyze the leachates for its chemical composition, followed by the effect of the compounds on individual and mixture of test crops.

MATERIALS ANS METHODS

Collection of samples

Bark, fresh leaves and leaf litter samples of *Eucalyptus tereticornis* Sm., *E.camaldulensis* Dehnh., *E.polycarpa* F. Muell and *E. microtheca* F. Muell were collected from 10 year old plantations growing at the Forest College and Research Institute, Mettupalayam, Coimbatore, Tamil Nadu, India.

Extraction ofphenolic compounds

Samples of bark, fresh leaf and leaf litter (5 g) were shaken with 100 ml water in 500 ml conical flask at 100 rpm for 24 h. The leachate was filtered and the filtrate acidified to pH 2.0. Phenolic acids from the filtrate were extracted with an equal volume of peroxide free ether. The extract was then air dried and dissolved in a minimal volume (100 µl to 250 µl) of dioxane (Whitehead *et al,* 1983).

Identification of phenolic compounds

Ascending paper chromatography was performed using Whatman No.1 chromatogram papers of 30 x 20 cm dimension with isopropanol-ammonia-water (20:1:2 v/v) as solvent and diazotized sulphanilic acid as spraying reagent to detect the separated phenolics. Using sigma chromatographic standards, the unknowns were identified by co-chromatography (Mahadevan and Sridar, 1982).

One hundred µl of the sample was derivatized by the addition of 20 µl of pyridine and 100 µl of trimethyl silyl acetamide (TMSA) and incubated for 2 h at 35°C. One µl of the derivatized sample was injected into the column (DB-5; film thickness 0.25 urn; 30 m x 0.025 mm) fitted to the gas chromatograph (Shimadzu, GC-14B, Japan) connected to a flame ionization detector. Nitrogen gas was used as the carrier (40 ml/min). Hydrogen and oxygen were used for flame (40 ml/min). Oven temperature was maintained at 200°C and 240°C respectively.

Bioassays with identified allelopathic compounds and leachates on redgram

The identified compounds were tested individually and as mixture at 1 mM and 2 mM concentrations for their effect on the germination and vigour index of redgram (CO.5, a high yielding variety released by Tamil Nadu Agricultural University and popular among the farmers of Tamil Nadu). Besides, leachates from different parts of *Eucalyptus* species (10 per cent of bark, fresh leaves and leaf litter) were tested for their effect on germination, seedling length, dry matter production, vigour index and nitrogenase activity of redgram (Agarwal, 1980; Abdul-Baki and Anderson, 1973; Turner and Gibson, 1980). Germination test was conducted under laboratory conditions wherein 50 seeds were placed over the germination paper kept in a petridish, treated with leachates and observations recorded. Seedlings grown in potculture under nursery conditions, regularly irrigated with leachates from different plant parts, were used for testing other parameters. Three replications were maintained and completely randomized design was followed for statistical analysis (Panse and Sukhatme, 1989).

Nitrogenase activity

Redgram roots (two numbers) were detached without disturbing the nodules, thoroughly washed with water and air-dried. Roots along with nodules were put into 20 ml vials. Vials were sealed with rubber septum (serum cup). One ml of air was sucked from the vials and 1 ml of acetylene was injected. Nodulated roots were incubated for 1 h with acetylene. One hundred µl of gas, sucked from vials was injected in a gas chromatograph fitted with Poropak Q column. Acetylene and ethylene were used as standards. Nitrogenase activity (nm g^{-1} h^{-1}) was measured by using the formula proposed by Turner and Gibson (1980).

$$X = \frac{[\text{Area count x gas volume of the flask x } 0.0006] \div [\text{Volume of gas sample injected into GC x hours of incubation x g dry weight of nodules}]}{}$$

Here, unit dry weight may be g dry weight nodules or mg dry weight cell or mg protein (Turner and Gibson, 1980).

RESULTS AND DISCUSSION

Both paper and gas chromatographic analysis showed the presence of phenolic acids as the major constituent of the leachates. The phenolic acids identified were coumaric, ferulic, gallic, hydroxybenzoic, syringic and vanillic acids, apart from catechol (Table. 1). Vaughan and Ord (1990) reported that most of the phenolics released from plant parts were benzoic and cinnamic acid derivatives. Jayakumar *et al.* (1990) identified the presence of chlorogenic, coumaric, caffeic and gallic acids from *E. globulus.* The presence of gentisic, ellagic, sinapic and caffeic acids, phenolic aglycons, glycosides and terpenoides from *E.baxteri* was also reported (Waller, 1987). Sivagurunathan *et al.* (1997) identified and quantified the phenolics viz., caffeic, coumaric, ferulic, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids and catechol present in bark, fresh leaves, litter, root and seed leachates of *E. citriodora, E.globulus* and *E.tereticornis,* at 0.02 mM and 2.45 mM concentrations.

The mixture of allelopathic compounds (catechol, coumaric, ferulic, gallic, gentisic, hydroxybenzoic, syringic and vanillic acids) in

Table 1. Phenolic comounds identified from different parts of four *Eucalyptus* species by gas chromatography

Compounds	*E. tereticornis*			*E. camaldulensis*			*E. polycarpa*			*E. microtheca*		
	B	FL	LL	B	FL	LL	B	FL	LL	B	FL	LL
Catechol	+	+	-	-	-	-	+	+	+	-	-	+
Coumaric acid	+	-	+	+	-	+	-	-	+	-	+	+
Ferulic acid	-	-	-	+	-	+	+	+	-	-	+	+
Gallic acid	-	+	-	-	+	-	-	+	+	-	-	+
Gentisic acid	+	-	+	-	-	-	-	-	+	-	-	-
Hydroxybenzoic acid	+	+	+	-	+	-	-	-	-	+	-	-
Syringic acid	-	-	-	+	-	-	-	-	-	+	-	-
Vanillic acid	+	-	-	+	+	-	-	-	+	-	+	-

B = Bark, FL = Fresh leaves, LL = Leaf litter, + = Detected, - = Not detected

Table 2. Effect if identified phenolic compounds at 1mM and 2 mM concentrations on germination and vigour index of redgram, 7 days after sowing

Compounds	Germination %		Vigour index	
	1 mM	2mM	1 mM	2mM
Control	100 (88.7)	100 (88.7)	1558	1558
Catechol	96.6 (79.4)	96.6 (79.4)	940	1058
Coumaric acid	100 (88.7)	93.3 (75.1)	1911	2313
Ferulic acid	96.6 (79.4)	90.0 (71.6)	1130	1884
Gallic acid	96.6 (79.7)	93.3 (73.5)	375	1816
Gentisic acid	93.3 (75.0)	93.3 (75.0)	1212	1788
Hydroxybenzoic acid	100 (88.7)	93.3 (75.0)	1368	2018
Syringic acid	93.3 (75.0)	86.6 (68.5)	1022	1914
Vanillic acid	96.6 (79.4)	100 (88.7)	1228	1825
Mixture	90.0 (71.6)	86.6 (68.5)	298	860
CD (0.05)	Germination		Vigour index	
1 mM	4.184		79.09	
2 mM	3.461		81.67	

(Values in parentheses are sin transformed values)

bioassays showed pronounced inhibition on germination and vigour index of redgram, both at 1 mM and 2 mM concentrations. However, the specific compounds also had either stimulatory or inhibitory influence (Table. 2). Germination was inhibited by all the other compounds except coumaric and hydroxybenzoic acids at 1 mM concentration and vanillic acid at 2 mM concentration, compared to control. Vigour index was inhibited by catechol, ferulic, gallic, hydroxybenzoic, syringic and vanillic acids at 1 mM concentration whereas only catechol had inhibitory influence at 2 mM concentration. Coumaric acid at 1 mM concentration and all the other compounds except catechol at 2 mM concentration shown stimulatory effect on vigour index of redgram, compared to control. Vaughan and Ord (1990) also observed that caffeic, hydroxybenzoic, vanillic and syringic acids inhibited the root elongation in wheat, rye and mungbean.

Bioassay with leachates revealed significant reduction in germination over control, in all the cases, 7 days after sowing (Table. 3). Dry matter production was affected by *E. camaldulensis* and *E. microtheca.* Meanwhile,

Table 3. Effect of leachates of different parts of four *Eucalyptus* species on germination, dry matter production and vigour index of redgram, 7 days after sowing.

	Germination %			Dry matter produation (mg / 10 seedlings)			Vigour index		
	B	FL	LL	B	FL	LL	B	FL	LL
Control	100 (88.7)	100 (88.7)	100 (88.7)	0.46	0.46	00.46	480	480	480
E. tereticornis	92 (73.6)	100 (88.7)	88 (88.7)	-	-	-	-	-	-
E. camaldulensis	84 (66.4)	96 (78.6)	88 (69.8)	0.44	-	-	281	-	-
E. polycarpa	92 (73.6)	92 (73.6)	96 (78.6)	0.45	0.51	0.20	-	439	168
E. microtheca	84 (66.4)	80 (63.4)	92 (73.6)	-	0.23	0.82	-	186	821
CD (0.05)	Germination			Dry matter production			Vigour index		
B	1.232			0.031			31.08		
FL	1.180			0.036			29.13		
LL	1.213			0.034			32.87		

(Values in parentheses are sin transformed values)
B = Bark, FL = Fresh leaves, LL = Leaf litter, - = data not recorded

Table 4. Effect of leachates of bark. fresh leaves and leaf litter on seedling length, dry matter productions, vigour index of redgram, 37 days after sowing

	Seedling length (cm plant^{-1})			Dry matter production (mg / 10 seedlings)			Vigour index			Nitrogenase activity (nm g^{-1} h^{-1})		
	B	FL	LL	B	FL	LL	B	FL	LL	B	FL	LL
Control	64.5	64.5	64.5	1.15	1.15	1.15	6450	6450	6450	1153	1153	1153
E.tereticornis	51.7	76.5	55.0	0.64	1.05	0.81	4756	7650	4840	1130	109	1105
E. camaldulensis	60.0	60.0	55.5	0.90	1.13	0.87	5040	5760	4884	287	470	76
E polycarpa	62.0	66.5	61.0	1.06	1.73	1.21	5704	6118	5856	838	244	798
E microtheca	60.5	53.0	59.5	1.42	0.95	1.12	5082	4240	5474	967	982	1005
CD (0.05)	Seedling length			Dry matter production			Vigour index			Nitrogenase activity		
B	1.387			0.129			28.34			36.35		
FL	1.201			0.090			31.26			32.18		
LL	1.290			0.110			34.35			35.65		

B = Bark, FL = Fresh leaves, LL = Leaf litter

vigour index was affected by *E. camaldulensis, E. polycarpa* and *E. microtheca.* Seedling length was affected in all the cases except *E. camaldulensis,* 37 days after sowing (Table. 4). Simultaneously, reduction in vigour index and nitrogenase activity was also noted in all the cases, compared to control (Table. 4). Suresh and Rai (1987) have also observed similar inhibition of germination, root length and dry matter production in some field crops treated with the aqueous extracts of leaves of *Casuarina equisetifolia, E.tereticornis* and *Leucaena leucocephala.* Jayakumar *et al.,* (1990) observed reduction in chlorophyll content on crops treated with extracts of abscised leaves of *Eucalyptus,* which hints the possibility of poor photosynthesis and in turn poor plant growth. Intervention of phenolic acids in the metabolic process and thereby inhibition of normal growth has also been reported (Moorland and Novitsky, 1987). Reduction in nitrogenase activity might be due to reduction in the beneficial role, played by rhizobia under allelopathic conditions (Duhan *et al.,* 1994).

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