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Physiological Responses of *Eucalyptus globulus* Leaf Leachate on Seedling Physiology of Rice, Sorghum and Blackgram

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ABSTRACT

Leaf leachate of *Eucalyptus globulus* was evaluated for alleopathic effect on rice, sorghum and blackgram. Leaf leachate was tried at 5, 10 and 20% concentrations. Seed germination, shoot length, seedling dry matter and vigour index were significantly reduced by leaf leachate and highest inhibition was observed in 20% concentration. The magnitude of inhibition followed the order of blackgram > rice > sorghum. Germination and root length was inhibited to a tune of 18.6 and 75.8, and 14.7 and 60.0% for blackgram and sorghum, respectively at 20% concentration. The leaf leachate application also influenced the metabolism of seedlings *viz*., chlorophyll *a*, *b* and total chlorophyll, soluble protein, proline and phenol content. In general, leaf leachate increased the proline and phenol content, and decreased the chlorophyll and soluble protein contents, in all the test crops over control. Among the parameters studied, proline was found to be associated with higher seedling growth of rice (200% increase over control), blackgram (94% increase over control) and sorghum (183% increase over control).

Key Words: Allelopathy; Seedling; Rice; Sorghum; Blackgram; *Eucalyptus globulus*

INTRODUCTION

Agroforestry is the integration of agriculture and forestry to increase the farm productivity and sustainability of farming systems. *Eucalyptus globulus* is a major species in Agroforestry system (AFS) in Tamil Nadu due to its good timber value and coping ability. However, farmers have voiced concern about its harmful effects on crops (Djanaguiraman *et al*., 2002). The inhibition of one plant by another through the release of allelochemicals is well known (Jadhav & Gaynar, 1995). The leaf, stem and roots extracts or leachates from different *E. globulus* inhibited the germination and seedling growth of cereals (wheat, maize), millets (sorghum, pearl millet), legumes (chickpea, pigeonpea) etc. (Djanaguiraman *et al*., 2002; Sasikumar *et al*., 2002; Pawar & Chavan, 2004). Therefore, *Eucalyptus* though a potential industrial crop is not recommended as an intercrop in agroforestry systems. The effects of ferulic and p-coumaric acids isolated from the leaves of *Eucalyptus* have been studied on some crops. The allelopathic influence of *Eucalyptus* has been attributed to the production of several volatile terpenes (del Moral *et al.,* 1978)**.** Allelochemicals were reported to be highest in the foliage of many plants; these chemicals were found to be released in to the soil ecosystem through volatilization, root exudation, and leaching from the foliage (Fisher, 1980). However, information on allelopathic effect of *Eucalyptus globulus* on rice, sorghum and blackgram is scanty. Hence, the present study was conducted to determine the influence of aqueous leachates of *E. globulus* leaves on seed germination, seedling growth, chlorophyll content, soluble protein, proline and phenol contents of rice, sorghum and blackgram.

MATERIALS AND METHODS

The bioassay study was conducted at Coimbatore (11^o02 N latitude and 76^0 77 E longitude and at an altitude of 426.7 m above MSL), South India during 2003-2004. It consisted of two factors viz., (i) four leachate concentrations (0, 5, 10 and 20%) and (ii) three test crops (rice 'IR 20', sorghum 'CO1' and blackgram 'KM2'). The treatments were replicated five times in completely randomized design. Fresh leaves of *Eucalyptus globulus* were collected from the seven years old trees in Farm at senescent stage (90-100 days) for preparation of leaf leachates. The leachates from the leaves were prepared by immersing 200 g leaves in 1.0 L double distilled water in a flask for 24 h at room temperature (30±2^{o}C) (Djanaguiraman *et al*., 2002). Then these were filtered through Whatman No. 1 filter paper. These leachates were of 20% concentration and were further diluted to 5 and 10% concentrations. Twenty five seeds of test crops viz., rice, sorghum and blackgram were treated with 0.1% mercuric chloride, washed thrice with distilled water and dried on an absorbent to eliminate fungal attack. The seeds were germinated on filter paper soaked in the aqueous leaf leachates of different concentrations at 30±2^{o}C in lab, while distilled water was used for control treatment. The petri plates of 14.0 cm diameter were irrigated with 10 mL leachate on alternate days. Germination, shoot length, seedling dry matter, vigour index, chlorophyll *a, b* and total chlorophyll, soluble protein, proline and phenol contents were recorded at 10 days after sowing in all the test crops.

Chlorophylls content were estimated according to Yoshida *et al.* (1976), by extracting the chlorophylls in 80% acetone and expressed as mg g^{-1} FW. Soluble protein was extracted on the 10^{th} day after sowing from the leaf samples of the test crops and quantified by the method of Lowry *et al.* (1951), and expressed as mg g^{-1} FW. Proline was extracted in 3% sulfosalicylic acid, estimated by using acid ninhydrin reagent and measuring the absorbency of the toluene chromophore at 520 nm (Bates *et al*., 1973), and expressed as µM g^{-1} DW. Total phenol content was assayed according to Swain and Hillis (1959), and expressed as µg g^{-1} DW. The data were analysed statistically according to Sukhatme and Amble (1985). Significance between control and treatment was compared at 0.05 probability levels.

RESULTS AND DISCUSSION

The leaf leachates of *E. globulus* inhibited the seed germination of blackgram, rice and sorghum (Table I). The inhibitory effects were observed in all the test crops however, highest inhibition (41%) was observed in concentrated leaf leachates (20%). The magnitude of inhibition from leachates followed the order: blackgram > rice > sorghum. Among the concentrations, maximum inhibition was observed in 20%, followed by 10 and 5%. The allelopathic effect of eucalyptus may be attributed to the water soluble inhibitors present in its leaves (Al-Mousawi & Al-Naib, 1975). The inhibition of germination is dependent on the concentration of the extract; perhaps it may be due to the entry of water soluble allelochemicals into the seed, which retards the germination and growth (Suseelamma & Venkataraju, 1994). These results are in conformity with John and Nair (1998). Likewise, Djanaguiraman *et al*. (2002) observed that the aqueous extracts of *E. globulus* caused inhibition of seed germination in greengram, blackgram and cowpea.

The leachates of *E. globulus* also caused significant reduction in seedlings growth of blackgram, rice and sorghum (Table I). The inhibition of shoot 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 blackgram, 20, 10 and 5% leaf leachates concentration, exhibited 84, 58 and 28% inhibition in shoot length, respectively. Likewise, the reduction in rice and sorghum was 57, 46 and 28% and 36, 30 and 17%, respectively. Our findings correlate with those of Suseelamma and Venkata raju (1994) in groundnut, Beres and Kazinczi (2000) in field crops and Sasikumar *et al*. (2002) in pulse crops.

The inhibition of shoot length by *E. globulus* may be due to the presence of higher amount of volatile chemicals (α-pinene, β-pinene, α-phellandrene and cineole) or phenols like ellagic, chlorogenic, p-coumaryl quinic, gentistic and gallic acid (del Moral & Muller, 1970). 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).

Dry matter per plant directly affects the final yield. *E. globulus* leachates decreased the dry matter in all the test crops and the magnitude of reduction was maximum in blackgram followed by rice. The magnitude of reduction in all the crops was proportionate with the leachates concentration. 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; 2000). A reduction of 19% in dry matter of sorghum by leaf leachates of eucalyptus (20%) had been reported and the dry matter of rice and blackgram was further reduced (37 and 75%, respectively). These indicate, among the test crops sorghum was comparatively tolerant to growth suppression by eucalyptus. A close parallel relationship between concentration of leaf leachates and inhibition of dry matter production on pulses, rice and sorghum was reported by John and Nair (1998), Sasikumar *et al.* (2002), Djanaguiraman *et al.* (2002), Karthiyayini *et al*. (2003) and Singh and Rao (2003). Similarly, the vigour index was also reduced with corresponding increase in concentration, maximum being 173 and 150% in blackgram and rice, respectively, in 20% concentration as compared to control.

Porwal and Mundra (1993) reported similar allelopathic effect of purple nut sedge and barnyard grass on germination and seedling growth of rice and blackgram. Djanaguiraman *et al.* (2002) found a similar type of result, that *E. globulus* reduced the vigour index in greengram, blackgram and cowpea. A similar inhibitory effect of *Digera muricata* on sorghum was reported by Karthiyayini *et al.* (2002). The reduction in vigour index in all the test crops may be due to reduced germination and shoot length, as vigour index is the product of germination and seedling length.

Contents of chlorophylls were also reduced significantly in all the treatments (Table I). Among the test crops, blackgram and rice showed a maximum decrease of chlorophyll *b* than chlorophyll *a*, whereas, sorghum showed a reverse trend in all the concentrations assayed. In blackgram, chlorophyll *a* was reduced by 50%, chlorophyll *b* by 138% and total chlorophyll by 71 in 20% concentrated leaf leachates. However, rice and sorghum recorded a decrease of 49, 41, 85, 23 and 60, 30%, respectively, over control. The results of the present study are line with the finding of Singh and Rao (2003) in rice. The reduction in chlorophyll contents observed in all the concentrations might be due to (a) degradation of chlorophyll pigments or reduction in their synthesis and (b) the action of flavanoids, trepenoids or other phytochemicals present in leaf leachates (Tripathi *et al.,* 1999, 2000). The more reduction of chlorophyll *b* than chlorophyll *a*, indicates its susceptibility to stress (Djanaguiraman *et al*., 2003). During stress situation, in tolerant species conversion of chlorophyll *b* to chlorophyll *a* may occur (Djanaguiraman *et al*., 2003). Hence, the increase in chlorophyll *a* in sorghum is justified.

Reduction in chlorophylls may decrease the photosynthesis and thereby substantially decrease all the metabolites viz., total sugars, proteins and soluble amino acids (Singh & Rao, 2003). In the present study, maximum reduction of soluble protein was observed in blackgram (113%), rice (105%) and sorghum (36%) at 20% leaf leachates concentration (Table II). Perhaps, the phytochemicals might have decreased the protein biosynthetic process, which reduced the content of soluble protein. This was also confirmed by Singh and Rao (2003) in rice.

The free proline content in blackgram, rice and sorghum seedlings is increased due to leaf leachates of *Eucalyptus* (Table II). Twenty per cent leaf leachate was highly effective in increasing proline content. Highest accumulation (214%) of proline was observed in sorghum in comparison to rice (193%) and blackgram (94%) at 20% leaf leachates concentration. Pawar and Chavan (2004) also observed the similar type of result in sorghum. The proline accumulation under stress conditions is mainly attributed to increased synthesis from glutamate. Besides, in germinating seeds increased proteolysis can also lead to increase in free proline along with other amino acid (Pawar & Chavan, 2004). Proline protects proteins from denaturation by maintaining the hydration level. As proline is involved in stabilization of protein it has a role as a protector of enzymes of plant metabolism (Schobert & Tschesche, 1978). It also plays a role in osmoregulation and provides a store of nitrogen and carbon for subsequent utilization during post stress recovery (Barnett & Naylor, 1966).

The total phenols concentration was increased in all the concentrations of *E. globulus* leaf leachates as compared to control (Table II). Maximum increment of phenols (257%) was observed in blackgram and minimum enhancement (112%) was observed in sorghum at concentrated leaf leachates. Increase in phenol contents was also responsible for reducing the seedling growth. Bansal (1997) also observed increase in phenolic contents in wheat seedlings due to allelopathic effect of *Ranunculus arvensis*. The increase in phenol contents was correlated with reduction in seed germination and seedling growth of blackgram. The maximum enhancement of phenols contents in concentrated leaf leachates (20%) suggested that the concentration of phytotoxic allelochemicals inhibitory to growth was greater in the 20% concentration as compared to the 10 and 5% leaf leachates concentration.

These results revealed that the leaf leachates of *E. globulus* inhibited the germination, seedling growth, vigour index, chlorophylls and soluble protein content with a concomitant increase in proline and phenol contents in all the test crops at all concentrations. The inhibitory effects of *E. globulus* on blackgram, rice and sorghum may be due to the presence of allelochemicals in the leachates.

Table I. Effect of *E. globulus* leaf leachates on seed germination, seedling growth, chlorophyll *a* and *b* and total chlorophyll of blackgram, rice and sorghum at 10 days

Leachate concent-ration (%)	Germination (%)			Shoot length (cm)			Dry matter (g plant^{-1})			Vigour Index			Chlorophyll a (mg g^{-1} FW)			Chlorophyll b (mg g^{-1} FW)			Total Chlorophyll (mg g^{-1} FW)		
	BG	R	S	BG	R	S	BG	R	S	BG	R	S	BG	R	S	BG	R	S	BG	R	S
0	98.1	87.0	88.8	13.1	6.3	8.5	1.63	0.48	0.54	1863.5	991.8	1258.4	1.23	1.52	1.58	0.86	0.98	1.12	2.26	2.62	2.82
5	93.0	74.2	82.0	10.2	4.9	6.2	1.32	0.42	0.46	1227.6	606.8	787.2	1.00	1.27	1.38	0.61	0.72	1.02	1.84	2.22	2.63
	(5)	(17)	(8)	(28)	(28)	(37)	(23)	(14)	(17)	(51)	(63)	(59)	(23)	(19)	(14)	(41)	(36)	(10)	(22)	(18)	(7)
10	87.0	70.4	80.2	8.3	4.3	5.2	1.02	0.40	0.44	904.8	511.0	616.0	0.87	1.11	1.24	0.52	0.61	0.97	1.51	1.85	2.35
	(13)	(23)	(11)	(58)	(46)	(63)	(60)	(20)	(23)	(106)	(93)	(104)	(41)	(37)	(27)	(65)	(61)	(15)	(50)	(42)	(20)
20	80.2	62.0	75.0	7.1	4.0	4.8	0.93	0.35	0.41	681.7	396.8	512.5	0.82	1.02	1.12	0.36	0.53	0.91	1.32	1.64	2.17
	(22)	(40)	(18)	(84)	(57)	(77)	(75)	(37)	(31)	(173)	(150)	(145)	(50)	(49)	(41)	(138)	(85)	(23)	(71)	(60)	(30)
Mean	89.5	73.4	81.5	9.7	4.9	6.1	1.23	0.41	0.46	1169.4	626.6	768.5	0.96	1.23	1.33	0.58	0.71	1.01	1.68	2.08	2.49
CD (5%)	2.5	1.6	1.0	0.56	0.12	0.25	0.03	0.02	0.02	41.3	56.9	38.9	0.03	0.02	0.08	0.09	0.06	0.04	0.12	0.14	0.16

BG – Blackgram R – Rice S – Sorghum * - value in paranthesis indicate % decrease over control

Table II. Effect of *E. globulus* leaf leachates on soluble protein, proline and phenol content of blackgram, rice and sorghum at 10 days after sowing

Leachate concen-tration (%)	Soluble protein (mg g^{-1} FW)*			Proline (μM g^{-1} FW)$			Phenol (μg g^{-1} FW)$		
	BG	R	S	BG	R	S	BG	R	S
0	9.32	10.52	11.24	35.23	32.76	37.45	137.2	157.5	148.7
5	7.15 (30)	8.32 (26)	11.18 (0.5)	41.25 (15)	54.48 (66)	65.69 (96)	340.9 (148)	248.6 (58)	225.9 (52)
10	5.86 (59)	6.63 (59)	10.24 (10)	60.15 (70)	72.26 (120)	87.46 (161)	385.8 (181)	379.8 (141)	300.8 (102)
20	4.37 (113)	5.12 (105)	8.24 (36)	68.46 (94)	96.25 (193)	105.11 (214)	490.2 (257)	480.3 (204)	315.4 (112)
Mean	6.43	7.65	10.23	51.27	63.94	73.93	338.5	316.6	247.7
CD (5%)	0.46	0.32	0.56	2.1	2.9	1.4	2.4	1.3	2.8

BG – Blackgram R – Rice S – Sorghum $ - value in paranthesis indicate % increase over control * - value in paranthesis indicate % decrease over control

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