Studies on the Allelopathic effects of two Social Forestry trees On *Triticum aestivum* var Lok1

Latha Sadanandan*[1] and Mammen Daniel[2]
[1]*Department of Botany, Sree Narayana College, Kollam, Kerala, India*
[2]*Dr. Daniel's Laboratories, Vadodara, Gujarat, India*

ABSTRACT

Both the social forestry trees, Cassia siamea Lam and Melia azedarach Linn, promoted the later stages of growth of wheat (T. aestivum var. Lok1), though the leaves of M.azedarach tree inhibited germination and seedling growth. Flowering and fruiting of the crop also were promoted by leaves of both trees. Of the two groups of allelochemicals, flavonoids and phenolic acids, former was more in leaves of Melia and latter in Cassia. Melia leaves decomposed faster and left more residual phenolics in soil especially in summer. Leaf manure of both Cassia and Melia can be used for wheat cultivation, though the latter plant has to be avoided in the early stages of growth.

Key words: Allelopathy, Social forestry, *Cassia siamea, Melia azadarach, Triticum aestivum* var. Lok1

INTRODUCTION

Cassia siamea Lam. is an introduced species from Indonesia with good adaptability in India [1]. It is commonly planted in avenues and gardens. The allelopathic effect of this tree is evident in that it is used to eradicate *Lantana* sp. and *Impereta cylindrica* Beauv. [2]. *Melia azedarach* Linn. is native to West Asia and naturalized throughout the warm countries. It is well suited for afforestation purposes. The leaves of both *C.siamea* and *M.azedarach* are used as green manure. The leaves of *C.siamea* are rich in sennosides, quinones, alkaloids, triterpenoids, flavonoids and phenolic acids, while that of *M.azedarach* are rich in limonoids, peptides and carotenoids. They may either promote the overall growth of the crops nearby or may have negative impact on them. With this context in mind, the present work is envisaged to (1) to study the allelopathic effects of leaves of *C.siamea* and *M.azedarach* on test crop wheat using laboratory and pot studies, (2) To analyse the phytochemicals in the leaves of the trees to screen for their allelochemicals, (3) To study the rate of release of total phenolics from leaves of the trees studied and (4) To analyse the residual phenolics in the organic soil below the canopy of the trees and their rhizosphere.

MATERIALS AND METHODS

Allelopathic study: Laboratory experiments

Leaf leachates of *C.siamea* and *M.azedarach* were prepared by keeping 5g of the dried powdered leaves in 100ml distilled water for 48 h and filtering. This filtrate is diluted in distilled water to make desired concentrations of 20%, 40%, 60% and 100%. 500 g of soil, washed free of organic matter were irrigated with desired amount of the leaf leachates of different concentrations separately. The soil samples containing distilled water were maintained as the control. Three replicates were maintained for each treatment. Certified seeds of wheat (*Triticum aestivum* var Lok1) procured from Gujarat State Seeds Corporation, Vadodara, Gujarat were surface-sterilized in 0.1% mercuric chloride for 15 minutes and washed thoroughly in distilled water. Ten seeds were sown in each replicate separately. They were incubated at room temperature for 14 days. Results were taken on the 15th day after incubation. The parameters studied were percentage of seed germination, root length, shoot length and dry weight of the seedlings. The experimental data were subjected to ANOVA and multiple range test using LSD procedure.

Study on chemical characteristics of soil used for pot experiments

Before pot experiments, the soil samples collected for pot experiments were analysed for their pH, water holding capacity and Electrical conductivity [3], macronutrients such as organic carbon [4], available nitrogen [5], available phosphorus [6], available potassium [7], available sulphur [8], micronutrients such as Zinc, manganese, iron, and copper [9] and total residual phenolics [10].

Allelopathic study – pot experiments

Two kilograms of soil, free from stones and plant debris were collected in earthern pots. The seeds of the crop were surface-sterilized in 0.1% mercuric chloride for 15 minutes and washed thoroughly in distilled water. Two days before the sowing the seeds, the soil were amended separately with powdered leaf material of the trees. For each treatment, two concentrations were maintained, one containing 25g of powdered leaf material and the other with 50g. The soil samples without the leaf material served as the control. The treatments were irrigated regularly. The treatment of the samples with the respective leaf materials continued at an interval of 30 days for three months from the sowing date. The parameters studied were shoot length, number of spikes per plant, length of the spikes, number of grains per spike and average weight of the grains per spike. The experimental data were analysed using ANOVA and multiple range test using LSD procedure.

Phytochemical studies of the leaves of the trees

Screening for allelochemicals in the leaves of the trees selected were done by extracting 50g of dried, powdered material with methanol in Soxhlet's apparatus for 48h. Aglycones obtained after acid hydrolysis of the crude extract were separated in ethyl acetate. The fraction was analysed for phenolic acids [11], for flavonoids [12]. The identities of both phenolic acids and flavonoids were confirmed by co-chromatography with authentic samples. Analysis of minor components such as alkaloids, tannin, anthocyanin, sugars and amino acids were carried out using standard procedures [13],[14].

Studies on the rate of release of total phenolics to soil from the leaf litter of the trees using pot experiments

25g and 50 g of leaf litter (neither chopped nor ground) of selected trees were added to two kilograms of organic soil collected in earthern pots. They were soaked with 12 liters of water every 7th day, in order to maintain moisture in the soil and to ensure release of leaf leachates to the soil. Unamended soil samples (2 kg) soaked with same amount of water served as control. Three replicates were used for each of the treated and control samples. Estimation of total phenols in the soil samples were carried out at intervals of 15, 30 and 45 days using Swain and Hillis method (1959). The experiment was accompanied by analysis of pH value also at regular intervals of 15, 30 and 45 days.

Estimation of residual phenolics in organic soil below the canopy of the trees and their rhizosphere

Estimation of residual phenolics were carried out following Swain and Hillis, 1959. Soil samples (1 kg) were collected from below the trees at a distance of one meter from the base of the tree trunk and at a depth of 10cm from the ground level. Samples collected from three different directions around the base of the trunk served as replicates. These samples collected from below each tree were packed in separate sterilized polythene bags. The soil samples thus collected were air-dried, crushed and passed through 2mm sieve. They were treated with double amount (2000 ml) of distilled water and incubated at room temperature (30 ± 2 ºC) for 48 hours with occasional stirring. The solution were then filtered and the filtrate was concentrated and made upto 10ml in a volumetric flask with distilled water. 5ml each of these stock solutions were pipette out into separate test tubes. To this, 0.5ml of Folin – Ciocalteau's reagent was added. The solutions were incubated at room temperature for one hour. Absorbance was read at 720nm using spectrophotometer. The corresponding concentration of the solutions was calculated from a previously prepared standard graph of gallotannin. The concentrations of the total residual phenolics were calculated in terms of g per 100g of the soil collected.

RESULTS AND DISCUSSION

Allelopathic study: Laboratory experiments

The germination of wheat seeds was promoted by leaf leachate of *C.siamea*, though insignificantly. Except for 60% concentration, all other concentrations of the leachate promoted germination. The shoot growth of wheat seedlings was promoted at low concentration of the leachate while at higher concentration, there was inhibition, though insignificant. The root growth of the crop was significantly inhibited by the leachate at all concentrations. A maximum inhibition was observed at 100% concentration of the leachate. All concentrations of the leachate promoted biomass.The germination of seeds and the root growth of the seedlings were significantly inhibited with increase in concentration of leaf leachate of *M.azedarach*. The leachate significantly promoted the shoot growth of the seedling at 100% concentration (Table 1). The dry weight of the wheat seedlings was significantly reduced at 100% concentration of the leachate.

TABLE 1. ALLELOPATHIC EFFECTS OF LEAF LEACHATES OF *C.SIAMEA* AND *M.AZEDARACH* ON GERMINATION AND SEEDLING GROWTH OF *TRITICUM AESTIVUM* VAR. LOK1

Host trees	Dilution (%)	Germination percentage		Hypocotyl growth (cm)		Epicotyl growth (cm)		Dry weight (g)	
		Mean (±SD)	% increase(+) /decrease(-)	Mean (±SD)	% increase(+) /decrease(-)	Mean (±SD)	% increase(+) /decrease(-)	Mean (±SD)	% increase(+) /decrease(-)
C.siamea	Control	3.55 (0.09)	-	8.58 (0.06)	-	12.5 0.06	-	0.09 (0.01)	-
	20	4.00 (0.08)	+12.4	5.39* (0.02)	-37.1	14.1 0.06	+12.2	0.09 (0.02)	-
	40	4.88 (0.07)	+37.4	4.64* (0.09)	-45.8	13.4 0.09	+7.26	0.10 (0.04)	+2.1
	60	3.44 (0.08)	-3.1	3.33* (0.05)	-61.1	12.7 0.06	+1.75	0.10 (0.02)	+2.6
	100	4.89 (0.06)	-37.0	2.77* (0.02)	-67.7	11.6 0.02	-7.18	0.10 (0.03)	+2.7
M. azedarach	Control	6.08 (0.02)	-	12.3 (0.12)	-	11.1 (0.09)	-	0.09 (0.04)	-
	20	5.08 (0.05)	- 16.4	4.77* (0.17)	-65.1	14.1* (0.08)	+ 26.9	0.07 (0.03)	-15.7
	40	4.00* (0.04)	- 34.2	3.02* (0.08)	-75.4	13.9 * (0.04)	+25.3	0.06 (0.04)	-34.4
	60	4.00* (0.08)	- 34.2	2.16* (0.09)	-82.3	14.3* (0.04)	+28.9	0.07 (0.03)	-23.9
	100	2.33* (0.02)	- 61.6	1.94* (0.09)	-84.1	15.9* (0.06)	+43.3	0.04* (0.04)	-53.8

*Significant at $p \leq 0.05$

Chemical characteristics of unamended organic soil used for pot experiments

Characterization of soil for physical factors such as pH, EC and WHC were found to be normal. Macronutrients were excess, micronutrient Zn was excess while sulphur was less and others in medium range. Residual phenolics was also in normal range (Table 2).

TABLE 2: CHEMICAL CHARACTERISTICS OF UNAMENDED ORGANIC SOIL USED FOR POT EXPERIMENTS

Sl. No.	Soil Characteristics	Quantity ± SD
1	pH	7.61 ± 0.05
2	Electrical Conductivity (mmho/cm)	0.31 ± 0.30
3	Water Holding Capacity (%)	45.0 ± 0.12
4	Organic carbon (%)	0.88 ± 0.43
5	Total nitrogen (Kg/Acre)	758.56 ± 0.50
6	Available phosphorus P_2O_5 (Kg/Acre)	53.0 ± 0.20
7	Available potassium K_2O (Kg/Acre)	165.0 ± 0.84
8	Zn (ppm)	6.18 ± 0.01
9	Fe (ppm)	5.44 ± 0.60
10	Mn (ppm)	5.36 ± 0.15
11	Cu (ppm)	2.86 ± 0.005
12	S (ppm)	5.30 ± 0.03
13	Total residual phenolics (mg/100g)	0.53 ± 0.15

Allelopathic study – pot experiments

The shoot growth of wheat plants was significantly promoted by the leaves of *C.siamea* and *M.azedarach* at both concentrations of 25g and 50g. Leaves of *Cassia* reduced the number of spikes per plant , though insignificantly. But 50g of *Melia* leaves significantly promoted the parameter. The length of spikes increased significantly at high concentration of *C.siamea* leaves. and both concentrations of *M. azedarach* leaves The yield of the crop in terms of the number of grains per spike and the average weight of grains per spike increased significantly at both concentrations of leaves of both *C.siamea* and *M.azedarach* (Table 3).

TABLE 3: ALLELOPATHIC EFFECTS OF LEAVES OF *C.SIAMEA* AND *M.AZEDARACH* ON VEGETATIVE AND REPRODUCTIVE GROWTH OF *TRITICUM AESTIVUM* VAR. LOK1

Parameters studied	Values obtained	Control	Treatments (Powdered leaves)			
			C. siamea		*M. azedarach*	
			25g	50g	25g	50g
Shoot growth (cm)	Mean ± SD	46.3±2.6	60.0*±2.7	60.1*±2.5	51.4±3.0	58.7*±2.2
	% increase(+) /decrease(-)	-	+ 29.5	+29.7	+ 11.0	+26.8
No. of spikes / plant	Mean ± SD	3.0±2.1		2.8±2.0		5.2*±2.4
	% increase(+) /decrease (-)	-		-3.7		+74.0
Length (cm) of spikes / plant	Mean ± SD	6.0±0.9	6.4±0.6	7.5*±1.1	7.5*±0.7	7.5*±1.0
	% increase(+) /decrease (-)	-	+5.5	+24.1	+24.1	+24.1
No. of grains /spike	Mean ± SD	17.6±3.3	23.1*±2.6	31.2*±3.3	27.8*±3.5	30.8*±3.4
	% increase(+) /decrease (-)	-	+31.2	+77.2	+57.9	+75.0
Weight(g) of grains / spike	Mean ± SD	0.67±0.3	1.02*±0.2	1.08*±0.3	0.81*±0.3	1.17*±0.3
	% increase(+) /decrease (-)	-	+52.8	+61.4	+20.9	+74.5

*Significant at $p \leq 0.05$

Phytochemical studies of the leaves of *C.siamea* and *M.azedarach*

The compounds identified from leaves of *C.siamea* were apigenin, vanillic acid, syringic acid, p-coumaric acid, cis and trans-ferulic acid, gallic acids, alkaloids (in large amounts), sugar alcohols, arabinose, alanine and glutamic acid.

Melia leaves yielded 3',4'-diOMe quercetin and 4'-OMe kaempferol, vanillic acid, syringic acid, ferulic acid, gentisic acid, a simple phenol, saponin, sugar alcohols, galactose and threonine.

Comparison between the phenolic components of the fresh mature leaves and old fallen leaves of the trees showed that many of the compounds underwent decomposition with ageing. Moreover, presence of newly formed compounds were also observed. Mature leaves of *C.siamea* had less flavonoids but more phenolic acids. Melia leaves were rich in flavonoids and phenolic acids in mature fresh leaves while old fallen leaves of both *C.siamea* and *M.azedarach* contained only syringic acid. The quantity of total phenolics (mg/g) were more in *C.siamea* when compared to those in *M. azedarach* (Table 4).

TABLE 4: PHENOLIC COMPOUNDS IDENTIFIED IN THE LEAVES OF *C.SIAMEA* AND *M.AZEDARACH*

Host trees	Flavonoids		Phenolic acids		Total phenolics (mg/g)	
	Mature fresh leaves	Old fallen leaves	Mature fresh leaves	Old fallen leaves	Mature fresh leaves	Old fallen leaves
C. siamea	1. apigenin	2.isoflavone	1. Vanillic, 2. Syringic, 3. ferulic, 4. Gallic, 5. *p*-coumaric	1. syringic	56.5 ± 0.5	48.4 ± 0.2
M.azedarach	1. 3',4'-diOMe quercetin, 2. 4'-OMe kaempferol	1.Quercetin, 2. 4'-OMe quercetin, 3. 3',4'-diOMe quercetin	1. Vanillic, 2. Syringic, 3. ferulic	1. syringic	26.0 ± 0.6	36.4 ± 0.2

Rate of release of phenolics from leaf litter of the host trees to soil

Leaves of *C.siamea* released less phenolics during initial days of 15 to 30 days when compared to *M.azedarach*, that gradually increased by 45th day. Leaf litter of *M.azedarach* released more phenolics during 15 to 30 days which decreased by 45th day (Table 5). pH of the soil before amendment with leaf litters was 7.0 and it was in the range of 6.0 to 7.0 throughout the treatment period.

TABLE 5: TOTAL PHENOLICS (µG/100G) RELEASED AT VARIOUS INTERVALS IN SOIL SAMPLES TREATED WITH LEAF LITTER OF HOST TREES

Treatments	15th day Mean ± SD	30th day Mean ± SD	45th day Mean ± SD
Control	54 ± 1.5	36 ± 2.0	20 ± 2.3
Cassia leaf (25 g)	180* ± 1.4	380* ± 0.9	590* ± 0.6
Cassia leaf (50 g)	446* ± 0.8	586* ± 1.1	980* ± 0.7
Melia leaf (25 g)	354* ± 1.5	350* ± 2.0	290* ± 1.4
Melia leaf (50 g)	630* ± 1.6	610* ± 2.1	580* ± 1.9

*Significant at $p \leq 0.05$

Estimation of residual phenolics and identification of phenolic components in organic soil below the canopy of the trees and their rhizosphere soil

Total phenolics (TP) were estimated in April and December to find out the phenolic content variation in the two seasons. The TP content below the canopy of *Melia* and *Cassia* were higher in summer than in winter (Table 6). The soil samples collected from below *Melia* showed the presence of syringic acid while that from *Cassia* did not show the presence of any phenolic acids.

The soil samples collected from rhizosphere of *Melia* showed more TP when compared to that of *Cassia*. Phenolic acid viz Syringic acid was present in the soil samples collected from the zrhizosphere of both the trees in addition to other unidentified compounds.

TABLE 6: SEASONAL VARIATION OF TOTAL RESIDUAL PHENOLICS (µG/100G) IN ORGANIC SOIL BELOW THE CANOPY OF *C.SIAMEA* AND *M.AZEDARACH*

Place of collection	April Mean ± SD	December Mean ± SD
Control (barren land)	17 ± 0.6	20 ± 0.2
Cassia siamea	700* ± 0.1	563* ± 0.01
Melia azedarach	1110* ± 0.03	245* ± 0.8

*Significant at $p \leq 0.05$

The present study on the allelopathic effects of *Cassia siamea* and *Melia azedarach* on *Triticum aestivum* showed that leaves of *M.azedarach* had an inhibitory effect on the germination of the seeds, root growth and dry weight of the seedlings while the reproductive growth of the crop was significantly promoted. This result is in accordance to the reports of Maroua and co-workers [15] against radish and on green gram and black chickpea [16] where *M.azedarach* inhibited germination and seedling growth of the test crops but promoted their reproductive growth. The result showed that mulching of soil with leaves of *M.azedarach* may be avoided in the initial stages but added after seedling stage to promote reproductive growth and yield of wheat plant. Leaves of *Cassia siamea* promoted the germination and seedling growth and vegetative growth of the test crop. The yield of the crop was significantly promoted. This is contrary to the inhibitory allelopathic effect of *C.siamea* on maize [17] and other herbaceous species [18]. Phytochemical studies of the leaves of the trees showed the presence of phenolic allelochemicals that underwent transformation with ageing and showed seasonal variations. Total phenolics being more in summer than in winter. Many phenolics and terpenoids are known to be degraded or transformed by the abiotic and biotic factors to yield phenolic compounds [19]. The rate of release of phenolics into the soil revealed the fast decomposition of leaves of *M.azedarach* when compared to *C.siamea*. This may be due to the papery texture of the former when compared to the leathery texture of the latter. The more amount of soil phenolics from under the canopy of the trees also revealed the faster decomposition of *Melia* leaves when compared to that of *C.siamea*. Many workers have isolated allelopathic phenolic acids from soil [20].

SUMMARY AND CONCLUSION

Allelopathy is a chemical interaction occurring often between exotic plant species with native plants leading to the loss of biodiversity of local community. It also leads to economic loss in agriculture. Therefore, the study aims to analyse the allelopathic effect of leaves of two social forestry trees viz, *Cassia siamea* and *Melia azedarach* that are often cultivated on agricultural lands, against a common staple crop *viz Triticum aestivum* var Lok 1. The study revealed that *M. azedarach* significantly inhibited the seedling growth of the crop except for epicotyl growth, while *C.siamea* insignificantly promoted the seedling growth of the test crop except for hypocotyl growth of the seedling. Further vegetative growth of wheat was significantly promoted by leaves of both the trees. The reproductive growth in terms of number and length of spikes per plant were significantly promoted by *Melia* leaves and insignificantly promoted by *Cassia* leaves. The yield of the crop in terms of number of grains and weight of grains per spike were significantly promoted by the leaves of both the host trees. Phytochemical analysis of the leaves of the trees revealed the presence of phenolic acids and flavonoids that are potent allelochemicals. Variety of phenolic acids were more and flavonoids were less in *C.siamea* while *M.azedarach* showed reverse composition. The rate of decomposition of the leaves in terms of release of phenolics revealed that leaves of *M. azedarach* released more phenolics in the initial days from 15th day to 30th day that later decreased indicating their faster decomposition than the leaves of *C.siamea* whose release of phenolics was comparatively slow and increased by 45th day. The organic soil below the canopy of *M.azedarach* showed more amount of residual phenolics than that below *C. siamea* that indicates the constant supply of phenolics to soil due to fast decomposition of leaves of *M. azedarach*. The total residual phenolics in organic soil below the canopy of both the trees were more in summer than in winter.

The study reveals the positive allelopathic effect of leaves of both the trees selected on the test crop wheat, though leaves of *M.azedarach* may be avoided during germination and seedling stage of the test crop. But leaves of *Melia azedarach* exhibited more significant promotory effect on the reproductive growth of the test crop than the leaves of *Cassia siamea.* Hence, the study recommends the amendment of leaves of both *Melia azedarach* and *Cassia siamea* as green manure for the vegetative and reproductive growth of *Triticum aestivum* var. Lok1 during their cultivation after seedling stage of the crop.

Acknowledgements
The first author gratefully acknowledges the financial support rendered by the UGC, New Delhi.

Conflict of Interest

The authors declares no competing or conflict of interest.

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