Journal of Chemical Ecology, Vol. 19, No. 8, 1993

ALLELOPATHIC COMPOUNDS IN LEAVES OF *Gliricidia sepium* (JACQ.) KUNTH EX WALP. AND ITS EFFECT ON *Sorghum vulgare* L.

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(Received September 16, 1991; accepted March 19, 1993)

Abstract—Allelochemicals from *Gliricidia sepium* were extracted, identified, and quantified using HPLC. Fifteen toxic compounds, namely gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, gentisic acid, β-resorcyclic acid, vanillic acid, syringic acid, *p*-coumaric acid, *m*-coumaric acid, *o*-coumaric acid, ferulic acid, sinapinic acid (*trans* and *cis* forms), coumarin, and myricetin were identified and quantified. These compounds from the plant extracts were tested on the seeds of the crop plant, *Sorghum vulgare*. Rate of germination of the seeds and root elongation were found to be inhibited by the various compounds of the extract. Different quantities of *Gliricidia* leaf mulch, viz., 400, 800, and 1200 g/m^2 applied to the *Sorghum* grown fields, were found to effectively control weeds. Mulching improved the total yield of *Sorghum*. Leaf manuring and mulching showed better crop yield when applied up to 800 g of *Gliricidia* leaf/m^2. Crop yield was better in mulch-applied fields when compared to the manure-applied ones.

Key Words—Allelochemicals, *Gliricidia sepium*, *Sorghum vulgare*, HPLC, aglycone, phenolic acids, weed control, root biomass, shoot biomass, inflorescence biomass, grain yield, weed biomass.

INTRODUCTION

Gliricidia sepium leaves are widely used for mulching purposes in agricultural practice (Wilson et al., 1986). The performance of leaf mulch of *Gliricidia sepium* is more effective in weed control than the leaf mulch of *Leucaena leucocephala* (Budelman, 1988). *Gliricidia sepium* leaf mulch improved the yield

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0098-0331/93/0800-1691$07.00/0

of maize and promoted the soil fertility in degraded alfisol soil, and at the successive crop seasons, application of *Gliricidia* prunings as mulch did not affect the yield (Atta-krah and Sumberg, 1987).

Using a paper chromatography technique, Griffiths (1962) identified nine phenolic acids and a yellow fluorescing flavonol in the young and senescent leaves of *Gliricidia sepium*, which were allelopathic in nature (Rice, 1984). Protocatechuic acid was tentatively identified in *Gliricidia sepium* by Inostrosa and Fournier (1982). They studied the allelopathic effects of *Gliricidia sepium* on seed germination of a weed (*Bidens pilosa*) and tomato and found inhibition of germination.

Since *Gliricidia sepium* leaves are used as mulch and manure in dry-land agricultural practices, an attempt was made to identify and quantify the various allelochemicals present in the leaves of *Gliricidia sepium*. The biological activity of these allelochemicals present in the leaf extracts was tested on the seeds of the crop plant *Sorghum vulgare* (variety MSH 37). The impact of *Gliricidia* leaf mulch and manure on the yield of the crop plant *Sorghum vulgare* and weed control was tested under field conditions.

METHODS AND MATERIALS

Leaves of *Gliricidia* were collected from the Biomass Research Center of Madurai Kamaraj University, Madurai. Leaf extract was prepared according to the procedure of Singh et al. (1989). A crude extract was prepared by soaking 100 g of dried leaf material in 1000 ml of distilled water for 48 hr. Extract was freed from debris by centrifugation and filtration. Lipids and aglycones were removed by fractionation of crude extract with hexane (fraction A) and diethyl ether (fraction B), respectively. The remaining aqueous fraction was subjected to strong alkaline hydrolysis under nitrogen. The alkaline hydrolysate was acidified and fractionated with diethyl ether (fraction C). All extractions with solvents (100 ml) were repeated three times. The extracted fractions A, B, and C were subjected to bioassay with *Sorghum vulgare* seeds.

Bioassay

Three sets of Petri plates with filter papers were wetted evenly with fractions of A, B, and C separately (concentrations equivalent to 500 μl of fraction per dish). Excess of pure extraction solvent was added to the Petri dishes to ensure the uniform dispersal of compounds, and the dishes were allowed to dry completely. A fourth set, which received only the pure solvent used for fractionation, was used as control. Twenty seeds of *Sorghum vulgare* were placed in each Petri plate. The filter papers were saturated with double-distilled water. The assay was carried out by counting the seed germination for three days.

Identification and Quantification of Allelochemicals

Based on the bioassay results, identification of allelopathic compounds was done in the inhibitory fractions using a high-performance liquid chromatograph (LKB 2158, Uvicord SD HPLC equipped with LKB 2150 HPLC pump, LKB 2154 valve injector and LKB 2210 recorder). The solvent diethyl ether in the fractions was evaporated separately, and the residues were dissolved in the same volume of the mobile-phase liquid used in the HPLC. The columns (LKB 2113 Lichrosorb RP 18, 4 × 250 mm, 5 μM column) were eluted isocratically with 40% methanol in 1% acetic acid at a flow rate of 2 ml/min and monitored with a UV detector at 254 nm for detecting phenolic acids in fraction C (Cheng and Rimer, 1989). Acetonitrile and water were used as the mobile phase for aglycone in fraction B; the UV detection range was set at 280 nm (Singh et al., 1989). Authentic standards were used to identify the allelopathic compounds by comparing their retention time. Identified samples were spiked with authentic standards to reconfirm the identity.

Field Experiments. In southern India, *Sorghum vulgare* is cultivated in the semiarid zone. In Madurai (09′52°N, 74′10°E) the crop season falls between July and October. The field experiments in the present study were carried out between July and September 1991.

The trial area was divided into small experimental units of 2 × 3 m. A completely randomized block design of four treatments with 10 replicates was adopted. Irrigation channels (12 in. wide) divided the plots and acted as the buffer zone. The trial plots were saturated with water before transplantation. Eighteen-day-old uniformly grown *Sorghum* seedlings (variety MSH 37) were transplanted in trial plots in four rows with 45-cm intervals between the rows, and 15-cm intervals between the plants in the rows. Seven days after transplantation, a thorough weeding was done in the trial plots. The dry matter of *Gliricidia* leaves mulch (rachis included) was applied at the rate of 400 g (T1), 800 g (T2), and 1200 g (T3)/m^2. Mulching was excluded in the control plots. The plots were irrigated once in 10 days.

Weed sampling and crop plant samples were taken once in 20 days after the mulch layer was applied. A single weed sample per plot was taken using a 50 × 50-cm square iron quadrat. Weed frequency, weed density, weed abundance, and total weed biomass were calculated (Phillips, 1959). Five randomly selected sorghum plants from each plot for every treatment were uprooted, dried, and taken for analysis. Total root biomass (RB), shoot biomass (SB), inflorescence biomass (IB), and grain yield (GY) per plant were calculated for treated plants and compared with control plants.

The above experimental design was adopted for *Gliricidia* leaf-manuring experiments. Leaf manure were applied 20 days before *Sorghum* seedling transplantation at the rate of 400 g (t1), 800 g (t2), and 1200 g (t3)/m^2, and thoroughly mixed with the trial plot soil. Thorough weeding was done at 15 days and 45 days after the transplantation of *Sorghum* plants in the manure fields. Weed sampling was excluded in the manure fields. Plant sampling was done as that of mulch trials.

Statistical Analysis

Pair comparisons were done by using least significant difference analysis (LSD program, SPSS/PC IBM computer).

RESULTS

Bioassay of extracts B and C on the germination index of *Sorghum* seeds showed marked differences (Table 1). Extracts B and C (500 μl/Petri plate) showed inhibition of the root growth in *Sorghum vulgare*, of which extract C was comparatively more inhibitory than extract B. Extract A did not show any marked effect on the germination or elongation of root compared with control. Phenolic acids and aglycone in fractions B and C were identified and quantified in terms of percentage present in 10 μl of fraction. Since extract A did not have any effect on the germination of *Sorghum* seedlings, it was excluded in the present investigation. Fifteen allelochemicals were identified and quantified (Table 2), of which 13 compounds were phenolic acids. The flavonol identified in the leaves of *Gliricidia sepium* was myricetin. Coumarin and myricetin are the aglycones present in fraction B with trace amounts of other phenolics identified in fraction C. Some unknown peaks are also noted in the fractions B and C.

Effect of Leaf Mulch in Sorghum *Total Biomass and Weed Control*

The yields of control, T1, T2, and T3 plants were calculated at regular intervals and the results are shown in Table 3.

Root Biomass. The RB in T1 and T2 plants did not show any significant

TABLE 1. EFFECT OF VARIOUS OF LEAF EXTRACTS OF *Gliricidia sepium* ON GROWTH AND GERMINATION IN *Sorghum vulgare*[a]

Treatment 500 μl/dish	Germination (%) after 72 hr	Radicle length (cm)
Control	100	5.54
Fraction A	98	5.70 NS
Fraction B	80	1.32**
Fraction C	62	2.49**

[a]Levels of significance (compared to control): NS = not significant, ** = significant at 1% level.

TABLE 2. MAJOR ALLELOCHEMICAL COMPONENTS IN FRACTIONS OF *Gliricidia sepium* LEAF EXTRACT[a]

Allelochemical	Fraction B[b]	Fraction C[b]	% contribution in the total allelochemical content of the leaf
1. Gallic acid	−	+	1.79
2. Protocatechuic acid	−	+	1.65
3. *p*-Hydroxybenzoic acid	+	+	2.23
4. Gentisic acid	−	+	1.85
5. β-Resorcyclic acid	−	+	1.48
6. Vanillic acid	−	−	1.25
7. Syringic acid	−	+	1.45
8. *p*-Coumaric acid	+	+	18.55
9. *m*-Coumaric acid	−	+	14.91
10. *o*-Coumaric acid	+	+	20.09
11. Ferulic acid	+	+	5.19
12. *trans*-Sinapinic acid	+	+	14.47
13. *cis*-Sinapinic acid	+	+	6.48
14. Myricetin	+	+	4.93
15. Coumarin	+	+	3.68

[a]Total allelochemicals present in leaves of gliricidia = 5.4 mg/g matured leaf.
[b]Not detectable, −; present, +.

reduction when compared with the control RB. Initially T3 plants showed a reduction in RB, but RB soon recovered in later harvests.

Shoot Biomass. No significant difference was noted in the SB between control and treatments (T1, T2, and T3).

Inflorescence Biomass and Grain Yield. There was a conspicuous increase in the IB and GY in T2 and T3 mulch-treated plants compared to control. In mulch treatments, the total biomass increased more in T2 than others (Table 3). The higher quantity of mulch in T3 did not improve the GY/plant compared with T2, which received a lower quantity of mulch. The GY per plant showed no significant difference between control and T1 plants.

Weed Biomass. All the mulch treatments controlled total WB (Tables 4 and 5). Frequency percentage, abundance, density, and percentage of contribution to total weed biomass are shown in Table 4. Seven species of weeds were identified in the trial plots. In the treated plots, except *Cyperus difformis* and *Isachne dispar*, all other existing weed species (*Dactylotenium aegyptenium*, *Paspalidium flavidum*, *Amaranthus Spinosa*, *Boerhavia diffusa*, and *Phyllanthus nirurii*) are poor in percentage frequency and density compared to control. *Cyperus difformis* survived in all the mulch-treated plots. *Cyperus difformis* was more frequent (i.e., percentage frequency 100) in all four harvests in control,

TABLE 3. EFFECT OF MANURE AND MULCH ON BIOMASS OF *Sorghum vulgare*[a]

Treatment	Root (g)				Shoot (g)				Inflorescence (g)			Grain yield plant (g)
	I	II	III	IV	I	II	III	IV	I	II	III	
Control	0.28	2.03	2.39	2.41	1.32	16.6	43.5	47.5	0	2.21	7.58	7.92
4 tons (mulch)	0.18**	1.48*	2.35NS	2.43NS	1.74**	16.8NS	44.5*	48.6*	0	1.64NS	8.66**	7.99NS
8 tons (mulch)	0.21**	1.69NS	2.44NS	2.39NS	1.77**	17.2NS	44.6*	49.9**	0	2.95*	15.10**	11.87**
12 tons (mulch)	0.14**	1.62NS	1.89*	2.36NS	2.34**	16.7NS	44.0*	48.2*	0	2.68NS	10.23**	10.04**
Control	0.27	1.95	2.44	2.52	1.84	17.9	44.1	48.3	0	2.71	7.04	7.42
4 tons (manure)	0.62**	2.17NS	2.07**	2.21*	3.30**	19.5**	44.0NS	47.6NS	0	3.67*	7.31NS	8.47*
8 tons (manure)	0.59**	1.67NS	1.87**	2.01*	3.29**	19.4**	45.5**	49.2NS	0	2.97*	8.91*	10.72**
12 tons (manure)	0.23*	1.11**	1.26**	1.88*	3.06**	12.4**	43.2*	45.2*	0	1.10*	7.09NS	8.21*

[a]Levels of significance (compared to control): NS = not significant, * = significant at 5% level, ** = significant at 1% level. I, first harvest; II, second harvest; III, third harvest; IV, fourth harvest.

TABLE 4. EFFECT OF *Gliricidia* MULCHING ON WEED DISTRIBUTION[a]

Treatment	Abundance				Frequency (%)				Density				Contribution (%) to total weed biomass			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Cyperus difformis																
Control	7.3	19.3	26.5	24.2	100	100	100	100	7.3	19.3	26.5	28.2	88	83	68	40
T1	6.2	15.3	17.9	26.2	100	100	100	100	6.3	15.3	17.9	16.5	100	88	83	40
T2	4.3	5.2	11.6	13.3	100	100	100	100	4.3	5.2	11.6	12.4	100	68	71	74
T3	1.0	2.5	2.4	2.5	20	60	50	90	0.2	1.5	1.2	0.9	100	100	100	100
Isachne dispar																
Control	8.0	8.2	8.6	8.6	100	100	100	100	8.0	8.2	8.6	9.2	10	12	9	34
T1	0.0	3.2	7.6	7.2	0	90	90	100	0.0	2.9	6.9	11.2	0	6	8	30
T2	0.0	2.4	3.4	3.0	0	70	90	100	0.0	2.2	2.4	2.4	0	30	21	15
T3	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0
Dactylotenium aegyptium																
Control	7.3	19.3	26.5	6.0	30	100	100	100	0.4	2.4	6.6	6.2	0.5	2.7	10.6	12
T1	6.2	15.3	17.9	6.8	0	90	90	100	0.0	2.9	6.9	8.1	0.0	5.3	5.5	19
T2	4.3	5.2	11.6	2.8	0	0	0	100	0.0	1.0	6.3	0.4	0.0	0.0	0.0	3
T3	1.0	2.5	2.4	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Paspalidium flavidum																
Control	0	0	0	0	0	0	7	0	0.0	0.0	1.0	0.0	0	0	7	0
T1	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0
T2	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0
T3	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0	0	0	0

TABLE 4. CONTINUED

Treatment	Abundance				Frequency (%)				Density				Contribution (%) to total weed biomass			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Amaranthus spinosa																
Control	1.0	1.0	1.0	1.3	20	20	30	60	0.2	0.2	0.3	0.3	0.4	1.0	1.7	7
T1	0.0	1.0	1.5	1.8	0	10	20	40	0.0	0.1	0.1	0.1	0.0	0.4	0.0	4
T2	0.0	1.0	1.5	1.0	0	10	20	20	0.0	0.1	0.0	1.0	0.0	0.8	0.0	3
T3	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
Boerhavia diffusa																
Control	1.0	1.0	1.0	1.0	10	30	20	60	0.1	0.3	0.2	0.7	0.9	0.3	2.2	5.6
T1	0.0	1.0	1.5	1.3	0	10	20	70	0.0	0.1	0.3	0.9	0.0	0.3	3.0	6.4
T2	0.0	1.0	1.5	1.0	0	10	20	10	0.0	0.1	0.3	0.2	0.0	0.8	7.3	3.7
T3	0.0	0.0	0.0	0.0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phyllanthus nirurii																
Control	1	1	2	1	10	10	30	10	0.1	0.1	0.2	0.1	0.2	1.1	1.5	1.4
T1	0	0	1	1	0	0	20	30	0.0	0.0	0.1	0.1	0.0	0.0	0.5	0.6
T2	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
T3	0	0	0	0	0	0	0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 5. WEED BIOMASS IN *Gliricidia* MULCH-APPLIED TRIAL PLOTS HARVESTED AT 20-DAY INTERVALS[a]

Treatment	Harvest I ($g/0.5\ m^2$)	Harvest II ($g/0.5\ m^2$)	Harvest III ($g/0.5\ m^2$)	Harvest IV ($g/0.5\ m^2$)
Control	5.56	10.74	22.45	34.72
T1	3.33*	8.54*	17.92**	39.92*
T2	1.80**	4.04**	5.01**	12.03**
T3	0.08**	0.50*	0.54**	1.20**

[a]Levels of significance (compared to control): NS = not significant, * = significant at 5% level, ** = significant at 1% level.

T1, and T2 plots; however, in T3 the frequency percentage of *Cyperus difformis* gradually increased (Table 4) from the first harvest to the fourth harvest. Other species of weeds slowly emerged in successive stages of the mulch decomposition (Table 5). There is a proportional relationship between mulch quantity and weed control. Weed control was found to be highest in T3, where the amount of mulch was 1200 g/m^2.

Effect of Leaf Manure on Sorghum

Root Biomass. Total biomass of *Sorghum* was estimated at successive intervals in the control and manure-applied plots, and the results are shown in Table 3. In the first harvest, there was a significant increase in RB of manure-applied plants; later the RB decreased significantly in all manure-treated plants. Heavy dose of leaf manure adversely affected the RB.

Shoot Biomass. SB increased significantly in first, second, and third harvests of all treated plants. In the final harvest there was no difference among the SB contents of control, t1, and t2, whereas the t3 showed a significant reduction in SB.

Inflorescence Biomass and Grain Yield. During second harvest, IB was increased in t1 and t2 plants, whereas there was a significant decrease in t3 plants. In the third harvest, only t2 showed a significant increase in IB compared to control. The GY significantly increased in all the manured plants and particularly in t2, which showed more GY than others.

DISCUSSION

Inhibitory effects of certain phenolic compounds on germination have been shown in *Sorghum* (Rasmussen and Einhellig, 1977; Einhellig et al., 1982). Phenolic compounds are known to be growth inhibitory and are allelopathic in nature (Rice, 1984; Kuiters and Sarink, 1987; Weidenhamer et al., 1989). In the present study also there was an inhibition of seed germination and elongation of radicle in *Sorghum* due to the allelochemicals found in the leaves of *Gliricidia sepium* (Table 1).

Beneficial Effects

The performance of the mulching practice was better than manuring in the experimental plots. Mulch treatment of 800 g/m^2 was optimum for *Sorghum* under experimental conditions, and there was better performance under field trials. Obando (1987) has reported that *Gliricidia sepium* leaf mulches controlled certain weed species without affecting crops such as corn or beans. In the present study mulching controls weeds as well as nourishes the crop plant *Sorghum vulgare*.

Allelopathic Effects

The thick mulch layer of *Gliricidia* leaves applied in the plots retarded seed germination and establishment of the weeds by the potential allelopathic compounds. In the leaf-manure-treated plots, there was a reduction in the RB owing to the allelochemicals present in the rhizosphere of the sorghum plants of t1, t2, and t3. Heavy doses of leaf manure or mulch (above 800 g/m^2) did not promote the biomass yield as the optimal dose, possibly due to much accumulation of allelochemical, i.e., in the soil above optimal levels.

The farmers in the semiarid zones of Madurai District (Tamil Nadu, South India) apply only organic manure for most of the crops. *Gliricidia* leaf mulch could be a substitute for physical methods such as hand weeding, which are time-consuming and costlier. In dry land, crops are in need of moisture, and mulch conserves moisture. The traditional practices of dry-land agriculture in India provide the best base to develop an effective production system with minimum inputs. Haider and Martin (1975) reported that 42–98% of specifically labeled phenolic acids decomposed in 12 weeks in a green-field sandy loam topsoil obtained from a citrus grove. The soil samples were maintained in ideal conditions for microbial action, and therefore it is difficult to extrapolate to field conditions (Rice, 1979). *Gliricidia sepium* leaf mulch can be used as a cheap source of nourishment for sorghum grown in dry-land agroecosystems following the optimal application levels. It is necessary to test the effectiveness of *Gliricidia* leaf mulching on various other crop plants and weeds in the field condition.

Acknowledgments—We wish to acknowledge Dr. P. Ramesh, and Mr. C.R. Yuvarajan for providing some phenolic standards and for their technical advice. We thank Mr. N. Sankar for his conscientious help in operating HPLC. Financial support by the Department of Non-Conventional Energy Sources, Government of India to Dr. K.P. is gratefully acknowledged. We thank anonymous referees for their valuable suggestions.

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