PJWSR. 1988, Vol. 1, No. 1.

Allelopathic Effects of Pakistani Weed *Cynodon dactylon* (L) Pers

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ABSTRACT

Cynodon dactylon (L) Pers is widespread cosmopolitan weed, including Pakistan. Its allelopathic potential was tested against wheat, barley and maize varieties 'Sarhad white' and 'Sarhad Yellow'. The shoot and root litter or their aqueous extracts and soil collected from beneath *Cynodon dactylon* patches significantly reduced either the germination, early growth, biomass, moisture or chlorophyll contents of the susceptible species in various laboratory experiments. Paper chromatography indicated the presence of ferulic, p-coumaric, vanillic, p-hydroxybenzoic and syringic acids in the shoots and roots while gas chromatography revealed the last three and benzoic and caffeic acids. The toxicity was related to species and variety used, parts assayed and physiological parameters measured. The findings suggest that *C. dactylon* litter must be removed from the field due to allelopathic effects. These effects are, however, modified by other environmental factors.

INTRODUCTION

In agricultural fields weed litter is allowed to stay to improve the fertility, water holding capacity and structure and porosity of the soil. However, not all the litter from weeds exhibit these expected benefits due to interference with biological processes. Putnum and Duke (1978), Hussain (1983) and Rice (1984) emphasized the role of allelopathy in agroecosystems. The allelopathic effects of some Pakistani weeds has been reported (Dirvi and Hussain, 1979; Hussain *et al.*, 1984 a; 1985, 1987) *Euphorbia supina*, *Euphorbia corollata* and *E. marginata* (Rice 1965) and *E. escula* (Steenhagen and Zimdahl, 1979) exhibit allelopathy against the cultivated species. Hussain (1980) demonstrated the allelopathic effects of *E. granulata*. *Lolium multiflorum* also exhibits allelopathy against the agricultural crops (Naqvi and Muller, 1975).

Cynodon dactylon (L) Pers, is one of the most common perennial prostrate weed throughout Pakistan. Hussain *et al.* (1982, 1984 b) reported its distribution in agricultural fields. However, no reference exists on its allelopathy against the cultivated species. The present investigation was, therefore, conducted to envisage its allelopathic behaviour and to identify the toxic principals. The findings will add to our present knowledge of allelopathy by weeds.

MATERIALS AND METHODS

Mature plants of *Cynodon dactylon* along with runners and roots were collected and air-dried at room temperature (25-30°C). Glassware was sterilized at 170° C for at least four hours. Filter papers, liquid and other heat labile substances, were autoclaved. Wheat, barley, maize varieties 'Sarhad Yellow' and 'Sarhad White' were used as the test species. The

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seeds were incubated at 25°C for 96 hours. Ten replicates, each with 10 seeds were used. Germination, radicle, plumule growth and number of seminal roots were measured. The fresh and dry weight of 20 randomly selected seedlings were determined in each treatment. The seedlings were dried at 65°C for 72 hours. The results were statistically analysed using "Z" and "t" tests. This standard procedure was followed throughout this investigation.

AQUEOUS EXTRACT BIOASSAY

Five grams dried shoots (leaves, stems and runners) and roots (roots and underground parts) were separately soaked for 6, 12 and 24 hours at room temperature and filtered. The extracts were stored at 5-10°C when not used. Seeds of the aforesaid test species were placed on twice-folded Whatman. No.1 filter paper seedbeds. Tests were made by moistening the filter papers with the respective extracts. Distilled water was used as the control. The dishes were incubated following standard procedure

LITTER BED BIOASSAY

Five grams finely crushed dried shoots and roots were separately spread in a petri dish and topped with a single sheet of filter paper. To each dish, 5 ml distilled water was added. Control was similar to the test except the litter was replaced with fine pieces of filter papers. Seeds of the test species were placed on the top of the filter papers in the dishes which were incubated as before.

SOIL RESIDUAL TOXICITY

Soil samples were collected from places with or without *Cynodon dactylon* and separately air-dried, sieved through 2 mm mesh and used in soil-bed bioassay following the standard procedures against the aforementioned test species (Hussain, 1980; Hussain *et al*, 1979, 1984).

MULCHING EXPERIMENT

Equal volumes of dried sand, sterilized at 170°C for 4 hours, was taken in 16x15 cm plastic glasses. Ten grams crushed fresh roots and shoots were incorporated separately into the top layers. Ten seeds of the test species were sown in each of the glasses. There were four replicates for each species. Control was similarly made by replacing shoots and roots with fine pieces of filter papers. The plastic glasses were incubated at 25° C. Each glass was provided with equal volumes of Hoagland's solution to avoid nutrient and moisture defeciency. Germination was recorded after four days incubation and the seedlings thinned to six uniform healthy equi-distant individuals per glass. The number of leaves and height of seedlings was determined after another 10 days. The plants were then uprooted to determine fresh and dry mass of roots and shoots. The plants were oven dried at 65°C for 72 hours. Chlorophll contents were determined spectrophoto-meterically using Spectronic-20 after Harborn (1964).

IDENTIFICATION OF PHYTOTOXINS

Ten percent hot water extracts of roots or shoots were separately concentrated to 1/3 of their original volume. It was acidified to pH 2.5 and extracted three times, each with 60 ml ether by vigorous reflux shaking till the caesation of air bubbles. The mixture was allowed to stand for an overnight for the separation of

etherial and aqueous layers. The aqueous fractions were ultimately discarded. The three etherial fractions were mixed and evaporated in rotavapor at 30°C under reduced pressure to get the residue. The residue was dissolved in 2.5 ml of 95 percent ethanol and used for spotting Whatman No.1 filter paper at 2.5 cm distance from the base and dried. These were run in 6 percent AA, (6:94 v/v, acetic acid: water) followed by BAW (63:10:27: v/v/v, n-butanol: acetic acid: water) solvent systems following Naqvi (1976) and Lodhi (1975). The chromatograms were inspected under short (2537 A) UV light. They were sprayed with diazotized p-nitroaniline, diazotized sulfanilic acid and potassium fericyanide-ferric chloride reagents (Naqvi, 1976; Lodhi, 1975). The Rf, UV florescence and colours with the spraying reagents were recorded and compared with the standard markers which were simultaneously run using the same procedure. Gas chromatography was carried out on a Perkin Elmer GC Model N. 3920 equipped with FID and SCOT Capillary Column, 20 x 0.5 m (inner dia). The flow rate of the carrier gas (nitrogen) was 3.5 ml/min., air 500 ml/min and hydrogen 40 ml/min. The temperature of the injector and dectector was respectively 200° and 250°C. The column temperature was programmed from 150°C (2 min)-200°C (8 min) with a rise of 4°C/min. The chart speed was 5 mm/min. Authentic samples were co-chromatographed.

RESULTS AND DISCUSSION

AQUEOUS EXTRACT BIOASSAY

The germination of wheat in six hours shoot extract reduced to 84.44 percent while other species were unaffected (Tabel 1). The radical growth of maize Sarhad White in 6 and 12 hours root and shoots extracts decreased while th remaining test species were unaffecte (Table 1). The plumule growth of barley i 6 and 12 hours root and 12-24 hour shoots; maize varieties 'Sarhad Yellow' i all the root and shoot extracts, except 1 hours shoots; maize 'Sarhad White' in and 12 hours roots and shoots extract were significantly retarded under the tes conditions (Table 1).

The seminal roots of wheat in hours shoots and 12 hours roots extrac of barley in 6 hours root and that maize varieties 'Sarhad White' in all th treatments except 24 hours roots extract were significantly reduced by the extract (Table 1).

The fresh mass of wheat in 12 hour roots and shoots and 24 hours shoot ex tracts, barley in all the extracts except 2 hours roots, maize varieties 'Sarhad Yel low' in the extracts except 6 and 24 hou root extracts and maize 'Sarhad White in all the treatment except 6 hours shoo and 24 hours shoot and root extracts wer severely decreased in the test condition (Table 1).

The dry mass of wheat in 12 hou root and 24 hours shoot extracts, maiz 'Sarhad Yellow' in 12 hour and 24 hou root and shoot extracts decrease (Table 2).

The moisture contents of barley in a the treatments, except 24 hours root maize 'Sarhad Yellow' in 12 hours roo and shoots and of maize 'Sarhad White in 6 hours and 12 hours roots and 24 hour shoots significantly decreased in the tes condition (Table 2).

LITTER BED BIOASSAY

The germination of barley reduced t 78 percent in shoot litter while othe

Table 1 Effect of aqueous extracts of *Cynodon dactylon* on the germination and early growth of test species.

Test species	Extract Soaking Time (Hours)					
	6		12		24	
	Root	Shoot	Root	Shoot	Root	Shoot
Germination						
Wheat	100.00	84.44	91.11	93.33	97.78	102.22
Barley	90.48	95.24	95.24	88.10	76.19	90.48
Maize (S. Yellow)	100.00	104.17	102.08	102.08	104.17	102.08
Maize (S. White)	102.04	97.96	100.00	90.00	102.04	92.00
Radicle Growth						
Wheat	109.69	101.33	90.33	101.39	101.68	96.62
Barley	97.15	105.69	105.65	80.24	102.64	91.52
Maize (S. Yellow)	94.34	129.14	109.45	132.45	98.66	77.78
Maize (S. White)	60.06	53.70	57.34*	33.28**	109.09	122.40
Plumule Growth						
Wheat	109.62	99.26	92.19	98.05	98.59	105.79
Barley	89.72	112.44	102.08	69.96	87.37	59.27
Maize (S. Yellow)	74.71	85.59	86.45	112.32	75.45	42.11*
Maize (S. White)	57.12	74.24*	53.02*	25.12**	186.93	195.85
No. of Seminal Roots						
Wheat	95.34	84.20*	85.49*	98.44	105.18	108.81
Barley	88.89	103.70	98.41	85.19	96.30	96.65
Maize (S. Yellow)	97.07	110.87	109.62	110.46	106.09	96.23
Maize (S. White)	72.88*	78.39*	73.73*	52.52**	103.81	91.46

*Significantly different from control at P = 0.05
**Significantly different from control at P = 0.01

All values, expressed as percent of control, are means of 10 replicates, each with 10 seeds

Table 2 Effect of aqueous extracts of *Cynodon dactylon* on the biomass and moisture contents of test species.

Test Species	Extract Soaking Time (Hours) 6 Root	6 Shoot	12 Root	12 Shoot	24 Root	24 Shoot
Fresh Weight						
Wheat	105.00	96.33	80.00*	89.33*	96.66	79.00
Barley	64.64	79.16	78.63	53.30	128.76	53.83*
Maize (S. Yellow)	75.41*	96.99	78.85	87.29	90.75	83.61*
Maize (S. White)	89.14	93.14	84.29	59.50	97.86	96.86
Dry Weight						
Wheat	101.69	96.61	76.27**	100.00	100.00	71.18
Barley	105.08	104.62	90.77	106.15	104.62	98.46
Maize (S. Yellow)	89.59	91.75	90.00	70.79*	58.10	67.30
Maize (S. White)	94.68	97.87	91.07	75.74*	86.32	101.70
Moisture Contents						
Wheat	103.05	97.05	106.09	86.72	95.85	113.66
Barley	54.99**	70.62	83.86	39.90	127.86	45.28
Maize (S. Yellow)	98.59	102.77	88.46*	70.99*	224.47	178.98
Maize (S. White)	68.53	98.60	79.42	123.58	107.82	72.48**

* Significantly different from control at P = 0.05
** Significantly different from control at P = 0.01
All values expressed as percent of control, are means of 10 replicates each with 20 seedlings.

species were unaffected in their germination (Table 3).

The root and shoot litter significantly reduced the radicle growth of all the test species. The growth varied from 23.53 per cent (wheat) to 57.92 percent (maize 'Sarhad White') by shoots. In root-beds it was 56.04 percent (wheat) to 83.05 percent (maize 'Sarhad White') among the species. The plumule growht of all the test species decreased when they grew upon the shoot litter-beds (Table 3). The range of inhibition varied from 30.81 percent (wheat) to 53.17 percent (barley) in shoot litter and from 56.51 percent (maize 'Sarhad Yellow') to 85.04 percent (barley) in root litter among the species. The seminal roots of all the species in shoots and those of maize in root litter significantly decreased (Table 3). The fresh wieght of wheat and barley in shoot litter and of barley in root litter also decreased under the test conditions (Table 3). Drymass, however, dit not reduce in any of the species. The moisture contents of all the test species, except maize 'Sarhad Yellow' in shoots and wheat in roots, reduced severely (Table 3).

SOIL BED BIOASSAY

There was no effect on the germination whereas radicle and plumule growth of wheat and maize 'Sarhad Yellow'; seminal roots of maize 'Sarhad Yellow'; fresh weight of wheat, dry weight of maize 'Sarhad Yellow' and moisture contents of maize 'Sarhad White decreased because of their grwoth upon the *Cynodon* affected soil. There was no difference in the growth of seedlings and germination in distilled

Table 3. Effect of litter mulching and soil from *Cynodon dactylon* on the germination, early growth, biomass and moisture contents of test species.

Observation	Test Species			
	Wheat	Barley	Maize S. Yellow	Maize S. White
Shoot Litter bed				
Germination	97.50	78.00*	100.00	98.00
Radicle Growth	23.53**	33.67**	56.88 *	57.92 **
Plumule growth	30.61**	53.17**	51.99**	40.72**
Seminal roots	73.96*	71.12*	74.55*	76.37*
Fresh weight	78.45*	52.82**	133.52	94.28
Dry weight	92.68	142.41	139.70	115.37
Moisture contents	76.25*	38.53**	93.40	65.65*
Root Litter bed				
Germination	97.50	100.00	97.96	98.00
Radicle growth	56.04**	81.02*	61.43*	83.05*
Plumule growth	69.28*	85.04*	56.51**	60.19*
Seminal roots	96.45	100.86	75.46*	86.81*
Fresh weight	100.00	65.37*	102.30	106.46
Dry weight	101.22	129.31	140.45	112.76
Moisture contents	98.14	42.40**	59.44*	89.40
Soil bed Bioassay				
Germination	104.76	97.87	95.74	100.00
Radicle growth	67.22	99.15	57.94**	98.48
Plumule growth	58.96**	95.60	56.23**	98.14
Seminal roots	106.45	97.12	84.26	99.00
Fresh weight	77.36	102.44	97.32	109.79
Dry weight	113.56	89.71	103.06	72.96*
Moisture contents	64.13*	117.02	117.46	130.55

* and ** significantly different from control at P = 0.05 and 0.01
All values are expressed as percent of control

water and control soil.

MULCHING EXPERINENT

The germination of wheat in shoots and roots and of maize 'Sarhad White' in shoots mulch reduced. The height of barley and maize 'Sarhad Yellow' in both shoots and roots litter decreased singificantly. The fresh weight of wheat shoots in shoot litter and fresh mass of barley roots in shoot litter mulch dwindled. There was no effect on the drymass. The moisture contents of shoots of wheat, maize 'Sarhad Yellow' and barley in shoot mulch and of later two species in root mulch severly declined in the test conditions. The fresh weight of roots of maize 'Sarhad White' and Sarhad Yellow' and wheat in shoots mulch and of the latter two species in roots; dry weight of roots of wheat and maize 'Sarhad White' in shoots and roots mulches decreased in the test conditions.The root moisture contents of barley and maize 'Sarhad Yellow' in shoot mulch and of both the maize varieties in root mulch significantly decreased (Table 4).

The total chlorophyll contents and chlorophyll *a* and *b* of wheat and maize

Table 4. Effect of *Cynodon dactylon* Litter mulch on the germination, early growth, biomass, moisture and chlorophll contents of test species.

Test Species	Germination	Shoot				Root			Chlorophyll		
		Length	Fresh weight	Dry weight	Moisture contents	Fresh weight	Dry weight	Moisture contents	Total	a	b
Root Litter											
Wheat	84.21*	79.62*	75.5*	90.90	79.70*	62.00*	67.44*	93.90	71.59*	80.20*	64.51*
Barley	97.87	64.30*	97.04	107.41	87.93	134.58	95.00	151.24	91.90	63.63*	120.39*
Maize (S Yellow)	104.26	61.66*	117.86	100.91	119.78	95.46	111.63	82.95*	137.07	197.32	145.49*
Maize (S White)	126.32	112.80	98.84	104.85	93.39	58.11**	65.10*	85.62*	58.64*	76.69*	51.40**
Shoot Litter											
Wheat	89.47	102.26	50.5*,*	96.96	42.61**	50.50**	44.18**	118.20	46.93	49.66*	50.00**
Barley	102.13	70.69*	82.22*	103.70	74.10*	103.74	235.00	31.26	280.00	209.74**	110.19
Maize (S Yellow)	104.26	84.29*	91.69	106.42	83.69*	78.99**	107.75	68.47*	223.57	188.62**	218.80**
Maize (S. White)	73.68*	95.14	115.53	104.85	111.78	89.55*	39.22	272.00	16.32**	19.77**	22.13*

and ** Significantly different from control at P=0.05 and 0.01 respectively
All values are expressed as percentages

varieties 'Sarhad White' in shoot and root litters and chlorophyll *a* of barley in root litter were significantly retarded (Table 4).

IDENTIFICATION OF PHYTOTOXINS

Ferulic, vanillic, p-hydroxybenzoic, syringic and p-coumaric acids were identified with the help of paper chromatograph (Table 5) while all the four except p-coumaric acid two additional benzoic and caffeic acids were identified by gas chromatography (Fig.1) as some of the inhibitors in shoots and roots. All of them are proven allelopathic agents and, therefore, relying upon early workers (Naqvi, 1976; Lodhi, 1975; Rice, 1984) were not further assayed for their phytotoxicity.

In nature the plant or their parts might release water-soluble phytotoxins into the environment which accumulate to the extent of toxicity to affect the species occupying simultaneously or sequentially the same habitat. The *Cynodon dactylon* litter generally remains in the fields either after the completion of the life cycle and/or weeding which is soaked by rain, dew, soil moisture or irrigation water to release water soluble toxins as demonstrated in the present study.

The aqueous extracts from all parts retarded the germination and subsequent seedling growth to reduce the biomass of the susceptible species. Dirvi and Hussain (1979), Hussain (1980), Naqavi and Muller (1975) and Hussain *et al* (1984) also reported the reduction of germination and early growth of many crop species. As such our findings agree with these. Rice (1984) reported aqueous extracts of many weeds to be strongly inhibitory aginst the susceptible crops. The phytotoxicity of *Cynodon dactylon* was related to species/variety used and parts assayed. It was interesting to note that both the varieties of maize had an independent susceptibility to the same extract or treatment. The variety-specificity of extracts has been reported by Rice (1984), Naqvi and Muller (1975), Putnam and Duke (1978) and Tukey (1969) which support these findings.

The test species exhibited more retarded germination and growth when they came directly in contact with the *Cynodon* litter suggesting that crop seeds or seedlings coming in direct contact with the litter might be affected severly than the situation where no such contact is established in the nature. The phytotoxins could spread in the adjacent soil to render it unfavourable for growth at least for the susceptible species. The same phenomenon was demonstrated by the inhibited germination and growth of susceptible species when they grew upon *Cynodon* affected soil. The potentially nontoxic otherwise favourable soil acquires toxicity due to the growth of *Cynodon dactylon* in a manner demonstrated in the laboratory. However, the effectiveness of added toxins mainly depends on the accumulation and retention in the soil which in turn depends upon a number of other ecological factors (Rice, 1984, Hussain *et al*, 1984 a). The incorporated mulch and litter from *Cynodon* strongly reduced not only the germination but also the growth, moisture and chlorophyll contents of test species in the sterilized and nutrient rich growth medium. Dirvi and Hussain (1979), Hussain *et al* (1984 a) and Naqvi and Muller (1975) have earlier demonstrated a similar soil-plant phytotoxicity and their data support these findings.

The moisture contents of susceptible species decreased in the test condition due to some physiological disorders in water absorption mechanism by roots or

Table 5. Chromatorgaphic identification of phytotoxins from *Cynodon dactylon* (L.) Pers.

Compounds	Rf on		UV		Colours with		
	6 %AA	BAW	Short 2540 A°	Long 3660 A°	FFC	DAS	DPA
Standerd p-coumaric acid	.70	.96	Absorbed	Absorbed	Blue	Brick red	Off white
Suspected p-coumaric acid	.68	.97	Absorbed	Absorbed	Blue	Brick red	Off white
Standerd p-OH-benzoic acid	.34	.80	Light blue	Absorbed	Blue	Orange red	Off white
Suspected p-OH-benzoic acid	.34	.81	Light blue	Absorbed	Blue	Orange red	Off white
Standerd vanillic acid	.82	.90	Light blue	Absorbed	Blue	Faint orange	Faint black
Suspected vanillic acid	.82	.90	Light blue	Absorbed	Blue	Faint orange	Faint black
Standerd ferulic aicd	.42	.87	Light blue	Bright blue	Blue	Brick red	Off white
Suspected ferulic acid	.43	.87	Blue	Bright blue	Blue	Brick red	Off white
Standerd syringic acid	.44	.90	Blue	–	Blue	Faint	Voilet
Suspected syringic acid	.46	.89	Blue	–	Blue	Faint	Voilet

Key:- 6 % AA (6:94 V/V, acetic acid: water); BAW (63:10:27, V/V/V, n-butanol: acetic acid water), FFC: feric chlorid-potasium fericyanide; DAS diazotized sulfanillic acid; DPA: diazotized p-nitro-aniline. NV – n

there might have been a physiological drought, hampering the availability of water. Dirvi and Hussain (1979), Begum and Hussain (1980) and Rice (1984) reported similar reduced moisture contents of plants due to allelopathy. The chlorophyll contents of susceptible species also decreased under the test conditions. Rice (1984) and Hussain *et al* (1987) reported reduced chlorophyll contents of the susceptible test species due to allelopathy. A plant with reduced moisture and chlorophyll contents will lag behind in growth and exhibit poor biomass due to food starvation. The identification of ferulic, p-coumaric, vanillic, p-hydroxybenzoic and syringic acids confirm the allelopathic nature of *Cynodon dactylon*. All of them are water extractable, strong and recognized allelopathic agents (Naqvi 1976; Lodhi, 1975; Rice, 1984; Hussain and Ilahi, 1985) and capable of suppressing growth, germination, chlorophyll and moisture contents of plants (Rice, 1984).

The present finding, therefore, reveal that *Cynodon dactylon* is strongly allelopathic at least against the species tested in the present study. The phytotoxic effects are, however, related to the part assayed, test species used and depend upon the growth parameter measured. It is, therefore, suggested that the litter from *Cynodon dactylon* may not furnish its benefits as organic matter owing to allelopathy. Moreover, its strong competitive capacity will further enhance its allelopathic potential against the crop species occupying the habitat simultaneously or sequentially. However, the character of soil, availability of water, time of precipitation, amount of litter and other agronomic practices might alter the allelopathic behaviour.

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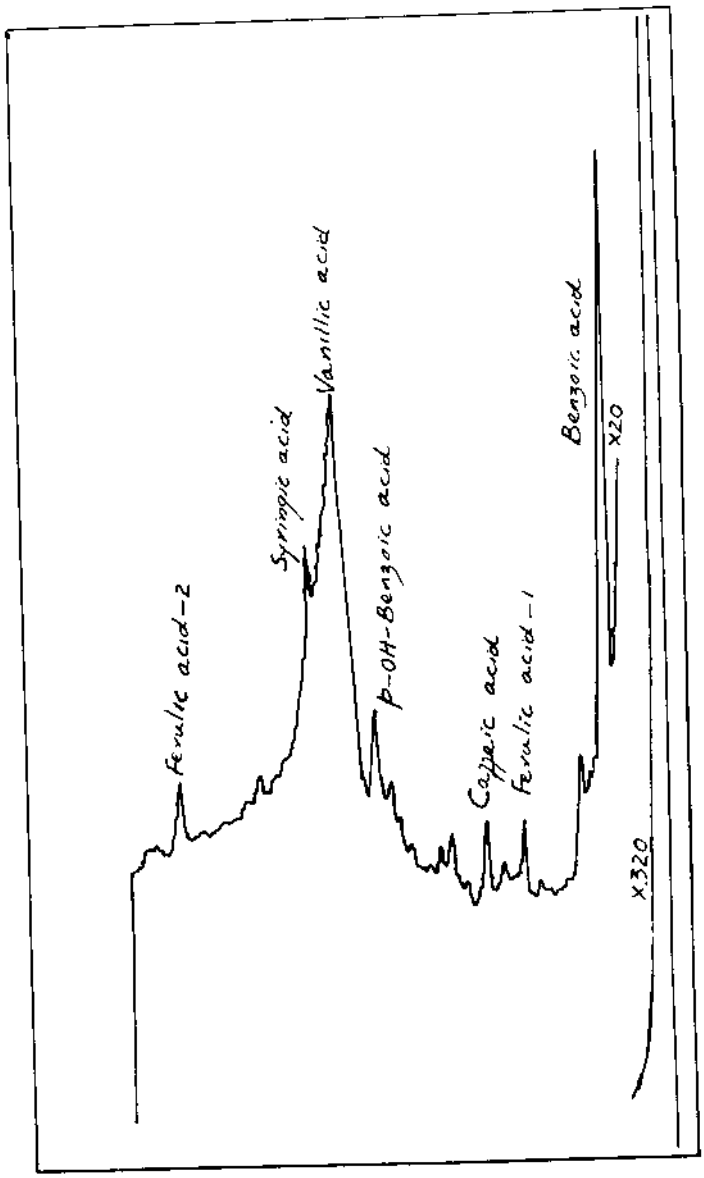

Fig 1. Gas chromatogram of Cynodon dactylon shoot Extract.