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Inhibitory Effects of Allelopathic Substances from Floral Parts of *Delonix regia* (Boj) Raf.

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The various floral parts such as sepals, petals, androecium and gynoecium of *Delonix regia* (Boj) Raf. were examined to find out the different allelopathics and their phytotoxicity on the germination, root length and shoot length of *Vigna mungo* (L) Hepper. The results indicated that the hexane extract from gynoecium was very phytotoxic. GC-MS studies revealed the presence of various allelochemicals and 1,2 Benzene dicarboxylic acid was present in all the four floral parts. The significance of these findings are discussed.

Key Words: Allelopathy, Delonix regia, *Vigna mungo*, Benzene dicarboxylic acid

Introduction

The term allelopathy refers to the detrimental effects of higher plant of one species on the germination, growth, or development of plants of another species. The impact of allelopathy in agro-ecosystem has been recognised since ancient times. Allelopathic interactions have been the concern of many ecologists and agronomists in recent years. Allelochemicals are reportedly present in virtually all plant tissues and are released in a variety of ways such as volatilisation, leaching, root exudation and decomposition of the plant residues. Continued discovery of agrochemically active compounds from natural sources supports the notion that new herbicides, insecticides and antimicrobials may be developed from templates discovered among natural products (Cardellina 1988)

Delonix regia (Boj) Raf., an ornamental tree, is a deciduous tropical tree distributed in India, Philippines, Indonesia, Africa and many other parts of the world. Systematic and pathologic investigation of *D. regia* have been carried out in the past (Ather & Mahmood 1980, Gill & Husaini 1982, Hodges & Tenorio 1984). Biochemical studies and allelopathic interaction studies are scarce although much allelopathic studies on leguminous plants have been carried out in many parts of the world (Chou & Kuo 1986, Walter 1987, Chou & Walter 1989).

Sung and Fowden (1989) isolated azetidine-2-carboxylic acid from seedlings of *D. regia* which was found to interfere with the biosynthesis of proline. Chou and Leu (1992) reported the presence of 4-hydroxybenzoic, chlorogenic, 3,4-dihydroxybenzoic, gallic 3,4 dihydroxy

cinnamic, 3,5-dinitro benzoic, L-azetidine-2-carboxylic acid and 3,4-dihydroxy benzaldehyde in leaves, flowers and twigs of *D. regia*. Some of these biologically active natural products may be developed into commercial herbicides that may be more environmentally acceptable than the present herbicides (Duke 1986).

The present study was carried out with a view to identify some of the allelopathic substances that are present in different floral parts of *D. regia* and to assess the level of phytotoxicity.

Materials and Methods

Flowers were collected from the trees of *D. regia* in our campus. Fully opened fresh flowers were selected, washed with distilled water and air dried. The different parts of flowers were separated; 5 g of each floral part was soaked in 25 ml hexane in separate containers for 2 hr. The containers were subjected to intermittent shaking. The extract was filtered and allowed to evaporate under reduced pressure at room temperature; the crude fraction was weighed and diluted with acetone and the diluted extracts were used to treat the seeds. 1%, 2% and 3% solutions were used. Seeds of *Vigna mungo* var TMV 3 obtained from the Agricultural Office, Manikandam, were used for the experiment. Ten seeds were soaked in 10 replicates in respective extracts of various floral parts for 8, 12 and 24 hr. After the treatment, the soaked seeds were germinated in petri plates covered with Whatman No. 1 filter paper moistened with distilled water. After 72 hr, the percentage of germination, the length of the root and shoot were recorded. The known plant growth inhibitor dl-2-aminoadipic acid was used as the standard and control for measure of phytotoxicity in the same concentrations of 1%, 2% and 3%. The method of Elakovich and Wooten (1995) was followed for the bioassay tests.

Another series of extracts was prepared by adding 5 g of air-dried floral material to 25 ml of hexane. The containers were constantly shaken for 20 hrs. and the extract was used for GC-MS analysis through Hewlett-Packard Gas Chromatography (Model No. 5890) equipped with a mass selective detector model 5971 A and an IIP-1 fused silica capillary column (25m x 0.25 mm ID). Using NIST library, we identified the compounds matching the peaks and any compound showing a matching of 95% was considered to be pure.

Results and Discussion

We wanted to observe the inhibitory effects of only non-polar compounds which reported to have allelopathic effects in nature and hexane was used in elution since it selectively elutes non-polar compounds. The germination, root lengths and shoot lengths were reduced both with the increase in concentration of the extract and the duration of the treatment (table 1). Allelochemicals from the gynoecium showed the maximum effect. The responsible allelopathic substances present in hexane extracts of different floral parts of *D. regia* were identified by GC-MS. The total ion chromatograms of different components are in figures 1-4. 1,2 benzene dicarboxylic acid was abundant in all the four floral

Table 1 The effect of allelochemicals from different floral parts of *D. regia* on germination, root length and shoot length of *Vigna mungo* (L.) Hepper

Extract	Time	Percentage Germination			Root length After 72 hrs			Shoot length After 72 hrs		
Concentration		1%	2%	3%	1%	2%	3%	1%	2%	3%
Water	8	90 ± 2	-	-	4.8 ± 0.7	-	-	6.9 ± 0.8	-	-
(control)	12	90 ± 4	-	-	4.3 ± 0.8	-	-	7.0 ± 0.9	-	-
	24	90 ± 3	-*	-	4.4 ± 0.7	-	-	*6.7 ± 0.7	-	-
Hexane	8	82 ± 4	-	-	4.7 ± 0.8	-	-	6.9 ± 0.9	-	-
(control)	12	82 ± 3	-	-	4.5 ± 0.7	-	-	6.5 ± 0.8	-	-
	24	81 ± 4	-	-	4.2 ± 0.8	-	-	6.1 ± 0.8	-	-
Sepal	8	61 ± 4	59 ± 3	58 ± 5	4.6 ± 0.8	4.3 ± 0.6	4.0 ± 0.7	6.8 ± 0.9	6.3 ± 0.8	6.1 ± 0.7
	12	52 ± 3	50 ± 4	49 ± 3	3.7 ± 0.9	3.5 ± 0.7	3.4 ± 0.8	5.9 ± 0.8	5.4 ± 0.9	5.0 ± 0.9
	24	43 ± 3	41 ± 5	38 ± 4	2.9 ± 0.8	2.6 ± 0.5	2.2 ± 0.7	3.2 ± 0.9	2.9 ± 0.8	2.1 ± 0.8
Petal	8	64 ± 3	61 ± 4	60 ± 3	4.8 ± 0.7	4.5 ± 0.6	4.2 ± 0.7	6.4 ± 0.9	6.0 ± 0.8	5.7 ± 0.8
	12	57 ± 6	55 ± 3	50 ± 5	4.3 ± 0.8	4.2 ± 0.7	4.0 ± 0.8	5.8 ± 0.9	5.3 ± 0.7	5.0 ± 0.9
	24	55 ± 4	50 ± 6	46 ± 4	3.8 ± 0.9	3.6 ± 0.8	3.5 ± 0.9	5.3 ± 0.9	5.0 ± 0.8	4.6 ± 0.7
Stamen	8	72 ± 4	65 ± 3	63 ± 4	4.0 ± 0.7	3.8 ± 0.7	3.7 ± 0.8	6.3 ± 0.8	5.9 ± 0.7	5.6 ± 0.8
	12	61 ± 5	53 ± 4	49 ± 3	3.8 ± 0.6	3.5 ± 0.6	3.2 ± 0.4	5.5 ± 0.6	5.3 ± 0.6	5.1 ± 0.7
	24	50 ± 3	41 ± 3	38 ± 5	3.5 ± 0.8	3.2 ± 0.7	2.9 ± 0.5	5.1 ± 0.5	4.8 ± 0.7	4.6 ± 0.6
Gynoecium	8	68 ± 4	64 ± 4	60 ± 2	2.2 ± 0.8	2.0 ± 0.7	1.9 ± 0.6	3.3 ± 0.8	3.1 ± 0.6	2.9 ± 0.7
	12	57 ± 3	52 ± 5	48 ± 4	2.1 ± 0.7	1.9 ± 0.8	1.6 ± 0.7	3.1 ± 0.6	2.8 ± 0.7	2.7 ± 0.6
	24	50 ± 4	46 ± 3	41 ± 3	2.0 ± 0.6	1.7 ± 0.5	1.3 ± 0.8	2.9 ± 0.7	2.6 ± 0.8	2.4 ± 0.6

* Value expressed as mean and standard deviation

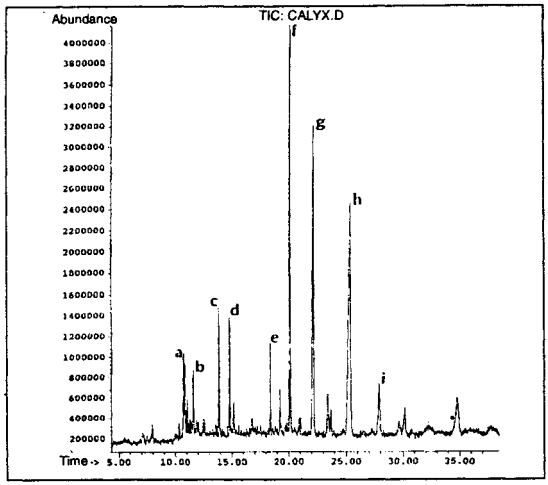

Figure 1 GC profile of the hexane extract from sepals; **a**, 2-aminopyrimidin-1-oxide; **b**, 3-methylbutylidene-cyclopentane; **c**, 1,2-benzenedicarboxylic acid butyl 2-methylpropyl ester; **d**, 1,2-benzenedicarboxylic acid bis (4-methypentyl) ester; **e**, 4,8-dimethyl-tridecane; **f**, 3-methyl-hexadecane; **g**, Heptacosane; **h**, 3-methyl-nonadecane; **i**, dihydro-3-methylene-4-octyl-, [3aR-(3a.alpha., 4.alpha., 6a.alpha)]-Furo[3,4-b]furan-2,6(3H,4H)-dione

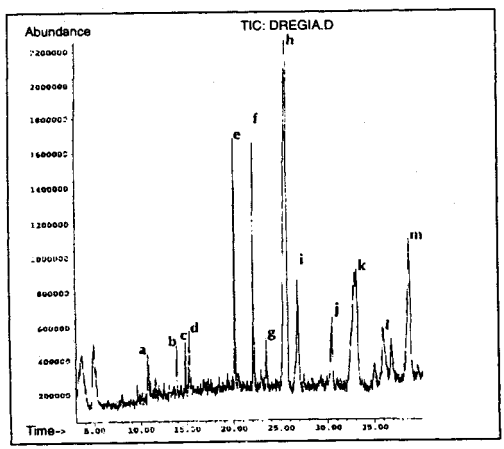

Figure 2 GC profile from hexane extract of petals; **a**, 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester; **b**, 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester; **c**, 1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester; **d**, Decanoic acid; **e**, 1,2-Benzenedicarboxylic acid, isodecyl octyl ester; **f**, Hexatriacontane; **g**, 9-methyl nonadecane; **h**, Pentacosane; **i**, (2-pentadecyl-1,3-dioxolan-4yl) Hexadecanoic acid methyl ester; **j**, Heptacosane; **k**, 1,1a,4,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, (1a.alpha., 7.alpha., 7a.alpha., 7b.alpha.)-2H-Cyclopropa[a]napthalen-2-one; **m**, N-[(3,4-Dimethoxyphenyl) [2-(1-Ethyl-2-Oxopropyl)-4,5-Dimethoxyphenyl] Methylene] Acetohydrazide

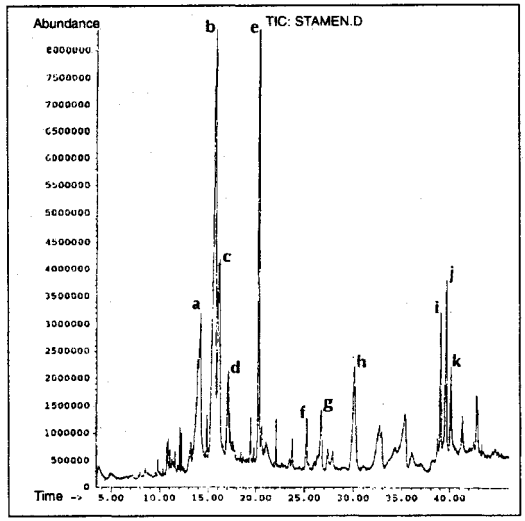

Figure 3 GC profile from hexane extracts of stamens; **a,** 2,3-Benzenedicarboxylic acid bis (4-methylpenty) ester; **b,** Hexadecanoic acid; **c,** cis-p-Menth-8(10)-en-9-ol; **d,** N.N-dimethyl-Methanethioamide; **e,** 1,2-Benzenedicarboxylic acid disiooctyl ester; **f,** 2,6,10,15-tetramethyl heptadecane; **g,** (2-pentadecyl-1, 3-dioxolan-4-yl) methyl ester; **h,** Vitamin E acetate; **i,** 2,4,5-T isooctyl ester; **j,** cis 2-(1-octadecenyloxy) myristic acid ethyl ester; **k,** 6-(3,3-dimethylbutyl)-cycloheptanol;

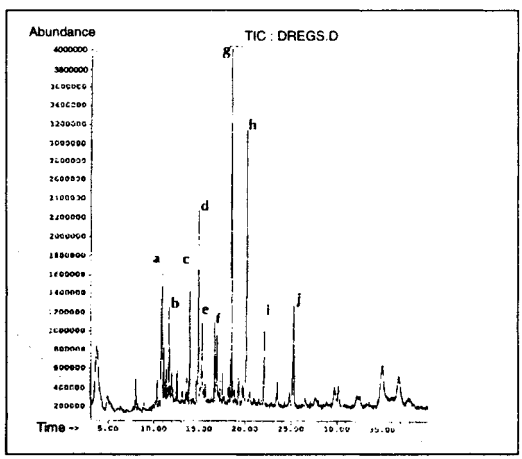

Figure 4 GC profile from hexane extract of gynoecium; **a,** Trans 3-methyl-6-(1-methylethenyl)-Cyclohexene; **b,** 6-methyl-5-(1-methylethyl)-Cyclohexene **c,** 1,2-Benzenedicarboxylic acid butyl 2 methylpropiy ester; **d,** 1,2-Benzenedicarboxylic acid butyl cyclohexyl ester; **e,** Hexadecanoic acid; **f,** Nonacosane; **g,** 3-methyl-heptadecane; **h,** 1,2-Benzenedicarboxylic acid bis (8-methylnonyl) ester; **i,** Heptacosane; **j,** Pentacosane

parts tested, while pentacosane was present in sepals, petals and gynoecium and Vitamin E in androecium. A few other allelochemicals such as amyrin and myristic acid were also present. In order to compare the activity of known allelopathic compound in our assay system, we used commercially available dl-2-aminoadipic acid. It exhibited progressive inhibitory effect. From our results it is clear that the various extracts strongly inhibited the germination, root lengths and shoot lengths; the inhibitory effect was more than 10 times that of dl-2-aminoadipic acid. Root appeared to be more sensitive than shoot.

Heisey (1980) indicated that most Simarubaceae plants possess water-borne phytotoxins that are methonol-soluble quassinoids. Chou and Leu (1992) identified four water-soluble phytotoxins in *D. regia* leaves, flowers and twigs, which are identical to our findings eventhough water soluble compounds may be different from hexane soluble compounds. The findings of this study are in agreement with these reports. The different allelochemicals present in the floral parts of *D. regia* were soluble in hexane and they exhibited inhibitory effects. Stobiecki *et al.* (1993) reported the synergistic effect of different phenolic acids isolated from bitter lupine seeds. Pandey (1994a, b) reported the inhibitory effect of *Parthenium* leaf extract on the growth of *Salvinia* and paddy. Our findings are in agreement with these reports. The flower parts contained a complexity of different allelochemicals which contributed to their allelopathic nature. We cannot clearly say that all the compounds present in the extract are phytotoxic. Further experiments are to be carried out with the isoloted individual compounds to ascertain this.

The presence of n-alkane like pentacosane, nonacosane, hexatriacontane etc. in the different floral part is not surprising since they are important components of surface waxes of plant tissues. 1-2 benzene dicarboxylic acid too is widely reported from various plant tissues but their functional groups vary in the different parts as seen in the GC-MS analysis.

The finding indicates the possibility of using some of the allelopathic compounds in biological control.

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