

Effect of *Acacia tortilis* on Seedling Metabolites of Clusterbean

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Acacia tortilis Forsk., locally known as Israeli Babool, was shown to have allelopathic effects on pearl millet, sesame and clusterbean (Sundaramoorthy & Kalra 1991). The present study reports results of further investigations into the allelopathic effects of *A. tortilis* on seedling metabolites of clusterbean.

Aqueous methanolic (10%) extract was prepared from leaves (1:4 ratio, weight/volume) of *A. tortilis*, using soxhlet apparatus for 48 h. The solution, thus obtained was considered as stock solution, and diluted to various concentrations, viz. 0, 25, 75, 100%. Twenty five seeds of clusterbean (var. Durgapura safed) were germinated in triplicate for each concentration in controlled growth room conditions ($28 \pm 2^\circ\text{C}$; 100 lux). Five-day old seedlings were separated into cotyledons and shoot axis, analysed individually for soluble protein (Bradford 1976), amino acids as glycine equivalent (Moore & Stein 1953). To identify the chemical nature of substances, leaves and soil beneath *A. tortilis* were extracted using methanol in soxhlet. The extract was condensed and a part was used for organic acids identification through paper chromatogram (Harborne 1973). The other part of condensed extract was hydrolysed using 7% hydrochloric acid. The process of hydrolysis was continued for 7-8 h with reflux condenser at $80-100^\circ\text{C}$ and fractionated after hydrolysis using petroleum ether. The petroleum ether fraction was condensed and the compounds were separated through paper and thin layer chromatogram (Harborne 1973, Daniel & Sabnis 1977). Phenols and sterols were identified, based on presence and R_f values obtained from standard chemicals. No co-chromatogram study was made. The chromatographic studies were done in controlled growth room conditions.

The soluble proteins in cotyledons and shoot axis decreased with increased concentration of aqueous extract. Free amino acids are below detec-

table level even at 50% concentration of the extract. In contrast to protein, soluble and insoluble sugars increased with the increased concentrations (Table 1). The preliminary analysis revealed the presence of four organic acids with R_f values of 0.986, 0.969, 0.185, 0.176 and three phenolic compounds. The phenolic compounds have the R_f values of 0.587/0.448, 0.614/0.551 and 0.809/0.424 in two dimension paper chromatography using benzene, acetic acid and water as first solvent and sodium formate, the second solvent. They are coumaric, vanillic, phloretic acids. The same phenolics were also identified in soils.

Cinnamic acid derivatives are known to be present ubiquitously in the plant kingdom (Putnam 1988). Ferulic and p-coumaric acids were among the inhibitors found in the residues of corn, oats, sorghum and wheat, as well as in the soils in which these crops were grown (Guenzi & Mc Calla 1966). Although numerous phenolic, benzoic and phenyl propane derivatives have been isolated from plants and soils, the more persistent ones appear to be caffeic, ferulic, p-coumaric, p-hydroxybenzoic and vanillic acids (Lodhi 1976, Putnam 1988). In the present study also coumaric, vanillic and phloretic acids were identified from plant and soil extracts, in addition to four unidentified organic acids. However, the identified cinnamic acid derivatives are known to cause metabolic changes and act as germination inhibitors (Rice 1974). We have studied only the synergistic effects of the allelochemicals of *A. tortilis*. And thus, it is possible that individual inhibitors may have lesser effect than cumulative.

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Table 1 Effect of different concentrations of aqueous methanolic extract of *A. tortilis* leaves on protein, free amino acids and sugars in the seedlings of clusterbean

Conc entra tion (%)	Seeding part	Proteins ($\mu\text{g g}^{-1}$ FW)	Amino acids (mg g^{-1} FW)	Sugars	
				Soluble (mg g^{-1} FW)	Insoluble
0	Cotyledon	2160 \pm 0.00	10.0 \pm 1.4	7.22 \pm 0.19	12.13 \pm 1.89
	Shoot	2160 \pm 0.00	6.6 \pm 0.4	20.72 \pm 1.61	7.53 \pm 0.81
25	Cotyledon	2100 \pm 0.00	5.7 \pm 0.6	7.50 \pm 0.00	12.78 \pm 0.95
	Shoot	2100 \pm 0.00	—	21.94 \pm 0.75	7.56 \pm 0.13
50	Cotyledon	2085 \pm 9.50	—	7.63 \pm 0.14	13.19 \pm 0.22
	Shoot	2070 \pm 8.60	—	22.63 \pm 0.87	8.06 \pm 1.13
75	Cotyledon	2010 \pm 6.30	—	14.66 \pm 0.19	14.38 \pm 0.00
	Shoot	2040 \pm 9.90	—	25.00 \pm 0.85	14.48 \pm 0.22
100	Cotyledon	—	—	—	—
	Shoot	—	—	—	—

FW = Fresh weight

— = Data not available

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