POST-INFECTIONAL COMPOUNDS OF <u>ANOGEISSUS LATIFOLIA</u> WALL. AND <u>MADHUCA INDICA</u> GMEL.

CHAPTER VIII

POST-INFECTIONAL COMPOUNDS OF *ANOGEISSUS LATIFOLIA* WALL. AND *MADHUCA INDICA* GMEL.

Anogeissus latifolia wall. and *Madhuca indica* Gmel are two economically important forest trees of Gujarat. An attempt has been made here to study the post infectional changes and the phytoalexin response in these two trees.

ANOGEISSUS LATIFOLIA

A.latifolia is commonly found in the deciduous forests of Gujarat, Madhya Pradesh and Southern parts of India. The tree is 10-25 m tall with greyish white or ash-coloured, smooth bark, peeling off in scales. Leaves 2.5-4 cm, ovate or oblong, elliptic oblong or obovate, glabrous and coriaceous. Flowers yellow, 0.4-0.5 cm across, in axillary pedunculate heads. Fruits, 0.4-0.7 cm across flat,orbicular, glabrous, samara. Seeds pale-brown, ellipsoid and glabrous.

Anogeissus is one of the important timber trees of Gujarat. It is also the source of a gum, gum ghatti, which is used as an adhesive in calicoprinting. Gum ghatti is also used in medicine (Chopra *et al.*, 1956). The leaves and twigs of *A.latifolia* being tanniferous are used in the leather industry.

Reddy et al.,(1964) reported the presence of gallotannins, corilagen and traces of ellagic acid from the leaves of A. latifolia. Chaturvedi and Saxena (1985) isolated quercetin -3-O - β -D- galactopyranosyl (1 $\rightarrow$ 4)-O- α - L - rhamnopyranoside from roots of A. latifolia.

MADHUCA INDICA

M.indica is a large deciduous tree usually with a short bole and a large rounded crown. Bark is dark coloured and longitudinally fissured. Leaves, clustered near the ends of branches, elliptic or elliptic-oblong, 7.5 -23 cm x 3.8-11.5 cm, coriaceous, pubescent when young, almost glabrous when mature, flowers in dense fasicles near ends of branches, many, small, Calyx coriaceous; corolla tubular, fleshy, cream coloured; berries ovoid upto 5 cm long, greenish turning reddish yellow or orange when ripe; seeds 1-4, brown ovoid, smooth, shining 2.5-3.7 cm long.

The timber of M.indica is used for making cabinet frames, doors, beams and for other construction purposes. The flowers of the tree form an article of diet for villagers in several parts of India. When dried flowers are properly soaked in water and allowed to ferment, it produces a spirit on distillation which has many applications in India. The seeds yield a fatty oil known in commerce as mahua butter, mowra fat, illepe butter or bassia fat. Over 33,000,000 kg are exported to Europe for use as a margarine, chocalate fat and for soap manufacturing. Mahua oil is also used for edible and cooking purposes in some rural areas. Refined oil finds use in the manufacture of lubricating greases and fatty alcohols. The oil is also used as a batching oil in jute industry and as a raw material for the production of stearic acid. Mahua oil has emolliant properties and is used in skin diseases, rheumatism and headache. It is also used as a laxative and is considered useful in habitual constipation, piles and haemorrhoids. Oil cake is used as a manure either alone or in mixture with other cakes and ammonium sulphuate. It also possesses insecticidal and pesticidal properties. Two sterols, mp. 117° and 22°C respectively were isolated from the unsaponifiable matter of mahua oil (Bhargawa and Singh, 1958).

In the present work, post infectional changes in the leaves of A.latifolia and M.indica affected by the leaf spot disease were studied. Spores of the pathogenic fungus and that of a nonpathogen Fusarium solani (Mart.) Sacc. were used to test the phytoalexin response in both the trees using the drop diffusate technique and the facilitated diffusion technique.

MATERIALS AND METHODS

Healthy and infected leaves of A.latifolia and M.indica were collected from Botanical Garden, M.S.University of Baroda, Baroda. Fresh healthy leaves for the studies on phytoalexin response also were collected from the same trees.

The procedures described in chapter II are followed for the extraction, isolation and identification of compounds, pathogenicity test, drop diffusate and facilitated diffusion techniques.

RESULTS

Alternaria alternata (Fr.) Kiesseler and Colletotrichum gleosporoides (Penzig) Penzig and Sacc. were the fungi isolated from the infected leaves of A.latifolia and M.indica respectively. Pathogenicity tests confirmed that A.alternata and C.gleosporoides were pathogenic on the respective host plants from where they were isolated (Figs. 36 and 37).

The leaf spot disease in A.latifolia was found to occur in the months of August and September. The diseased lesions developed 5-6 days after inoculation with the pathogen. The symptoms were visible as small brownish spots. The spots were present only on the upper surface of the leaf.

In M.indica the leaf spot disease was noted during November and December. The first signs of diseased lesions were seen 4-5 days after inoculation with C. gleosporoides. The symptoms seen as small blackish patches, grew to form bigger patches, covering a major portion of the leaf blade. The diseased lesions were seen on both the surfaces of the leaf, but lesions were more prominent on the upper side.

Fig. 36 Diseased leaves of Anogeissus latifolia Wall.

Fig.37 Diseased leaves of Madhuca indica Gmel.

FIG.36

FIG. 37

The distribution of proanthocyanidins, iridoids, alkaloids, saponins and tannins in the healthy and infected leaves of A.latifolia and M.indica is presented in Table XIV. Alkaloids were absent in both the healthy and infected leaves of A.latifolia and M.indica. Saponins and iridoids were present in healthy and infected leaves of M.indica, while they were absent in A.latifolia. Tannins and proanthocyanidins were present in healthy and infected leaves of A.latifolia, but absent in the leaves of M.indica.

The post-infectional phenols present in healthy and infected leaves of A.latifolia and M.indica are presented in Table XV. The flavonols, 3'4'-diOMe quercetin and 7,3'4'-triOMe quercetin were present in both healthy and infected leaves of A.latifolia. In addition the infected leaves contained a glycoflavone also. The concentration of the flavonoids in the infected leaves were much higher in the infected leaves. The phenolic acids, vanillic and ferulic acids were also present in both the healthy and infected leaves of A.latifolia.

The infected leaves of M.indica contained myricetin in place of 4'-OMe myricetin of the healthy leaves. p-Hydroxybenzoic acid and vanillic acids were present in both healthy and infected leaves of M.indica.

The diffusates from the leaves of A.latifolia and M.indica treated with spores of the pathogenic fungus and a non-pathogen F.solani showed no qualitative difference from that of the control. However in M.indica when leaves were subjected to the facilitated diffusion technique after treatment with spores of the pathogen, the extract from control leaves showed the presence of 4'-OMe myricetin while the extract from treated leaves contained myricetin.

DISCUSSION

The chemical constitution in both the plants were altered as a result of infection. The presence of a glycoflavone in the infected leaves of A.latifolia is quite interesting. This glycoside possesses more biological activity than the aglycones and therefore the production of a flavone-C-glycoside (glycoflavone) in the infected leaves of A.latifolia suggests that the plant would have resorted to the production of a more toxic compound as a immediate response to microbial attack. Since many parasites have been known to have β-glucosidase (Davis et al., 1953; Siebs, 1955; Oku, 1959; Pridham, 1960), it is supposed that hydrolysis of phenolic glycosides releasing aglycone may play a role in disease resistance (Holowezak et al., 1962). This supposition is based on the fact that phenolic glucosides are generally more stable than their split products to auto-oxidation (Hattori and Shiroya, 1955; Shiroya et al.,1955). Therefore the production of C-glycoside which is immune to β-glycosidase mediated hydrolysis is in fact a step towards stopping the β-glucosidase activity and the production of labile aglycone. The reports on the antifungal activity of a phenolic glycoside from *Anagallis arvensis* (Nene and Thaplayal, 1965) and inhibition of *Aspergillus luchuensis* by a glycoside (Jung and Hubbes, 1965) corroborated the above view on the response of the plant.

The production of myricetin in place of the methoxylated myricetin in infected leaves of *M.indica* clearly shows the process of demethylation which is also seen in *Cassia fistula* and *Morinda tomentosa* (see Chapter IV). The *in vitro* studies with leaves of *M.indica* on treatment with the pathogenic fungus also indicate the same results. The process of demethylation can be considered as the best immediate possible response offering maximum resistance to the invader in curbing the spread of the disease.

Table XIV : Distribution of proanthocyanidins, iridoids alkaloids, saponins and tannins in healthy and infected leaves *Anogeissus latifolia* and *Madhuca indica*.

Name of the plant	1	2	3	4	5
Anogeissus latifolia					
Healthy leaves	+	•	•	•	+
Infected leaves	+	•	•	•	+
Madhuca indica					
Healthy leaves	•	+	•	+	•
Infected leaves	•	+	•	+	•

Table XV: Distribution of pre-infectional and post-infectional phenols in *Anogeissus latifolia* and *Madhuca indica*

Name of the plant	Pre-infectional compounds	Post-infectional compounds
Anogeissus latifolia	3'4'-diOMe quercetin	3'4'-diOMe quercetin
	7,3'4' triOMe quercetin	7,3'4' tri OMe quercetin
	Vanillic acid	Vanillic acid
	Ferulic acid	Ferullic acid
		Glycoflavone
Madhuca indica	4-OMe myricetin	Myricetin
	p-hydroxybenzoic acid	p-hydroxybenzoic acid
	Vanillic acid	Vanillic acid