International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 14-08-2024, Accepted: 13-09-2024, Published: 28-09-2024
Volume 9, Issue 9, 2024, Page No. 74-77

Estimation of bioactive metabolites in *Mitragyna parvifolia* leaves

Dr. Om Prakash Meena[1], Dr. B M Meena[2]
[1] Associate Professor, Department of Botany, Govt. College Rajgarh, Alwar, Rajasthan, India
[2] Associate Professor, Department of Zology, SRP Govt. College Bandikui, Dausa, Rajasthan, India

Abstract
Histochemical studies have shown that galll -inducing agent can alter the cell and tissue metabolism on the host plant part. Morphologically changes in the host tisues are ultimately linked with physiological changes. Histrochemistry enables the localization of various metabolides present in the tissue.
To understand the morphological changes in term of physiology certain histochemical studies have been carried out and attempt has been made to interrupt the biochemical data in term of cells,tissue and tissue system. Fresh hand cut sections were used to study the changes brought about in the distribution of various metabolites and enzymes of the normal and galled tissue.The metabolites studies during the present investigations were starch,protein,tannins and lignin.

Keywords: Histochemistry, metabolites,galls

Introduction
Plant tissue are extremely complex in both morphology and physiology. Infection of any kind causes changes in the host morphology and physiology. Many biochemical changes take place at the infection site in the plant during the establishment of host-parasite relationships. Qualitative histochemical studies on the localisation of various metabolites provide an inside into the biochemical phenomenon at the cellular level. Cecidogenesis is a phenomenon that modifies both the plant anatomy and its metabolic activities. Histo chemical analysis are done to observe such physiological changes.such observations prove to be useful in providing data related to the dispersal and concentration of different metabolites and enzymes at the cellular level.These help in understanding the physiology of parasites and the relationship between the host and parasite.The kadamba is one of the most common vegetation in tropical area. For shade, tree are planted across avenues, along roadside and in communites. A fully kadamba tree can reached height of 45 m.It is a massive tree with a large crown and a straight cylindrical bole. Fresh hand cut sections of gall and normal leaf were used for the localisation of starch, cellulose total carbohydrates of insoluble polysaccharides, lipids protein,tannin, lignin and the activity of enzyme like acid phosphate peroxides and polyphenol oxides. These were immediately stained and photomicrographed.

Material and methods
Survey and collection
Normal leaves and leaves with gall form from the tree were taken from kadam Dongri and surrounding area of Jaipur for this investigation. The sample collection was performed between July and November. Galls were divided into three categories young,mature and old, and were fixed in F.A.A and 70% alcohol. In the same way, they are normal counterparts were corrected. For numerous experimental studies, freshly fixed and dried materials of normal and galled issue were used.

Starch
Starch was restrained by the method of IKI reaction *i.e.* Iodine-potassium iodide reaction after Johansen, 1940 [11].

Preparation of IKI Solution
2.0 g of potassium iodide was liquidated in 100 ml of distilled water and then
0.2 g of iodine was dissolved in it.

Procedure
Hand-cut fresh sections were laid in the IKI solution for a few minutes and in the same solution, they were mounted and observed.

Proteins
Localization of proteins was done by the method of Amido-black (Weime, 1959) [14].

Preparation of Amido-black dye
The preparation of Amido-black dye was done by the addition of 0.5 g of an amido-black solution containing 5.0 g of mercuric chloride and 5.0 ml of glacial acetic acid dissolved into 100 ml of distilled water. The tinge was utilized after filtration.

Procedure
Hand-cut fresh sections were tinged by amido-black stain for 2-3 mins and eventually drenched in 2.0% acetic acid solution for 5 minutes and then transferred into distilled water. The sections were fixed in glycerine and photomicrographed.

Lipids
Total lipids content were localized by the method described by Chiffelle and Putt (1951) [3].

Preparation of Sudan-III dye
In 100 mL of ethylene glycol, 0.7 g of Sudan III dye was dissolved. The solution was heated to 100-1100 degrees Celsius and thoroughly agitated. The dye was filtered and kept in a brown bottle using Whatman filter paper No. 2.

Procedure
Fresh hand-cut portions were immersed in ethylene glycol for 3-5 minutes and shaken occasionally. After that, the slices were dyed for 5-7 minutes with Sudan-III dye. These

pieces were placed in ethylene glycol and distilled water and agitated for another 2-3 minutes. The samples were fixed in glycerine and photomicrographed after a thorough washing in distilled water for 3-5 minutes

Lignin
The phloroglucinol HCl test was used to locate lignin (Johansen, 1940; Siegel, 1953) [11, 12].

Preparation of Dye
1.0 g of phloroglucinol was liquidated in 100 ml of 95% ethyl alcohol.

Procedure
Hand-cut fresh sections were laid in phloroglucinol solution. One drop of 20% HCl was added to the above tinge. The sections were then taken off and fixed in glycerine, observed and photomicrographed.

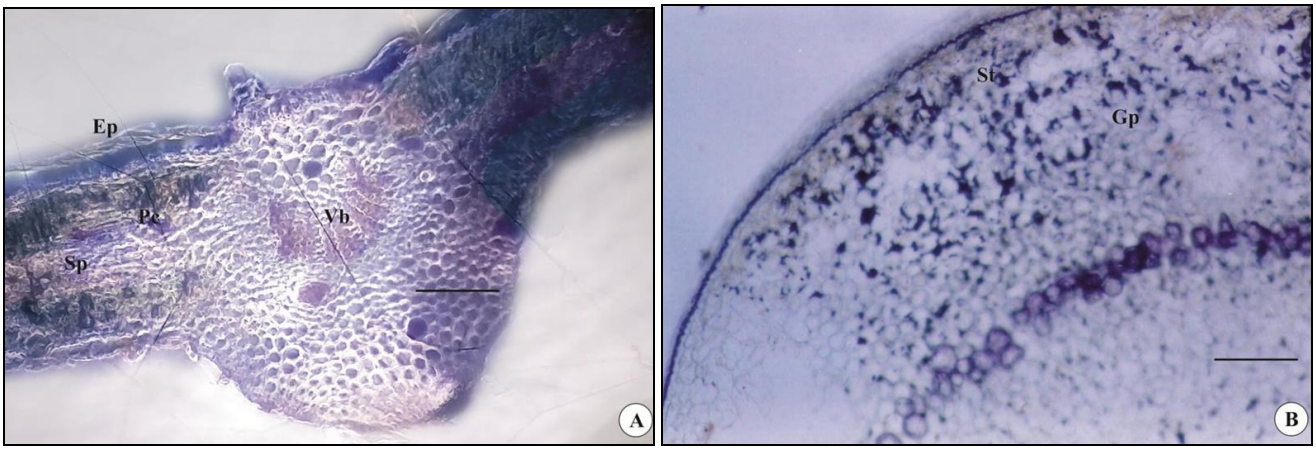
Starch
Results are depicted in Table 1 and Plate 1, Figs. A-B.
The most important carbohydrate reserve in plants, Starch, are localized as blue to black granules in cells.
It was observed in very high intensity in leaf gall parenchyma especially in the regions between the mechanical zone and epidermis although the intensity was low in the gall epidermis. In normal leaf, starch was observed at very high intensity in palisade tissues while the intensity was lower in the epidermis, spongy parenchyma and vascular bundles [Plate 1, Figs. A-B].

Proteins
Results are depicted in Table 1 and Plate 2, Figs. A-B.
Proteins were stained blue in colour. In all the four galls studied, the protein was found to be present throughout the gall tissues except sclerenchyma and the intensity was high in tissues of galls as compared to respective normal counterparts leaves [Plate 2, Figs. A-B].
The leaf gall parenchyma and palisade tissue of normal leaf showed a very high abundance of protein. It was observed in very low intensity in the gall epidermis and in the epidermis of the normal leaf. Spongy parenchyma showed a moderate amount of protein [Plate 2, Figs. A-B].

Lipid
Results are depicted in Table 1 and Plate 3, Figs. A-B.
Lipids are globules that range in colour from yellow to pink. The intensity of lipids was found higher in gall tissues than in normal tissues. Lipids were found in
high and moderate concentrations in the leaf gall epidermis and gall parenchyma, respectively. Lipid concentrations were moderate in the epidermis and palisade cells, but lower in the spongy parenchyma of normal leaves [Plate 3, Figs. A-B].

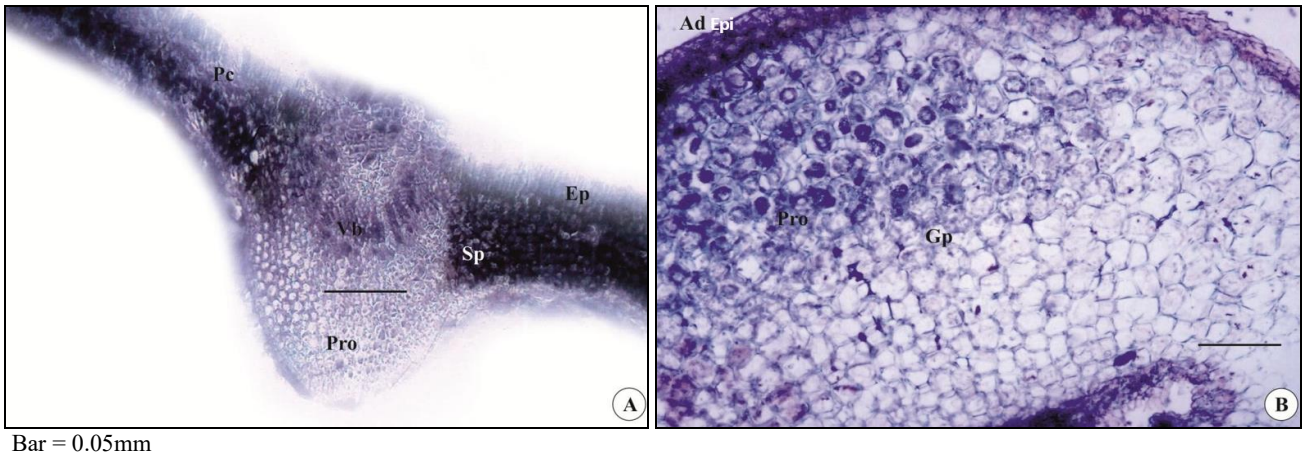
Lignin
Results are depicted in Table 1 and Plate 4, Figs. A-B.
Lignin was stained pinkish-brown to purple in colour in the tissues. It was totally absent in the parenchymatous regions of the normal and galled tissues. It was localized intensely in the mechanical zone of leaf gall and vascular region of normal leaf [Plate 13, Figs. A-B].

Plate 1



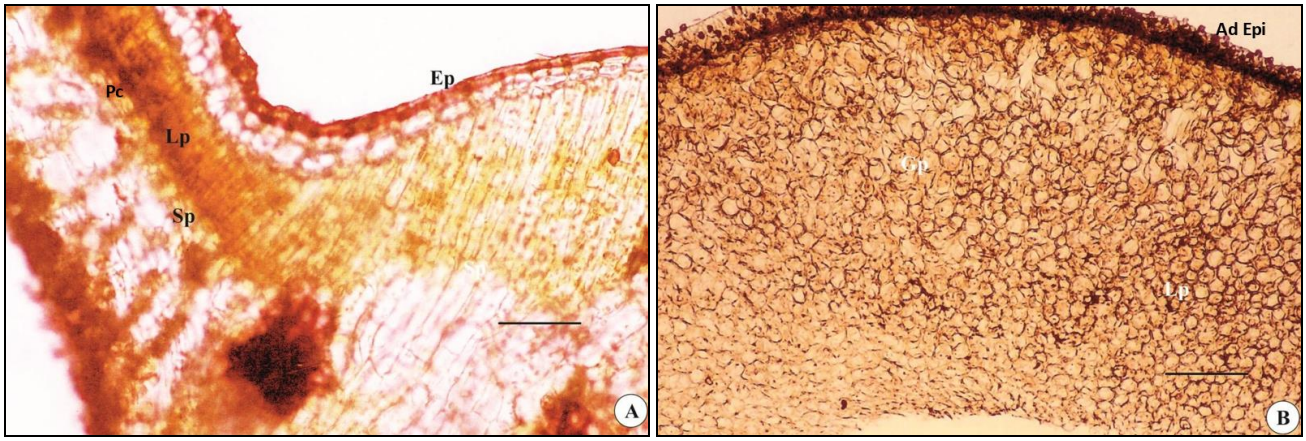
Bar = 0.05mm

Plate 2



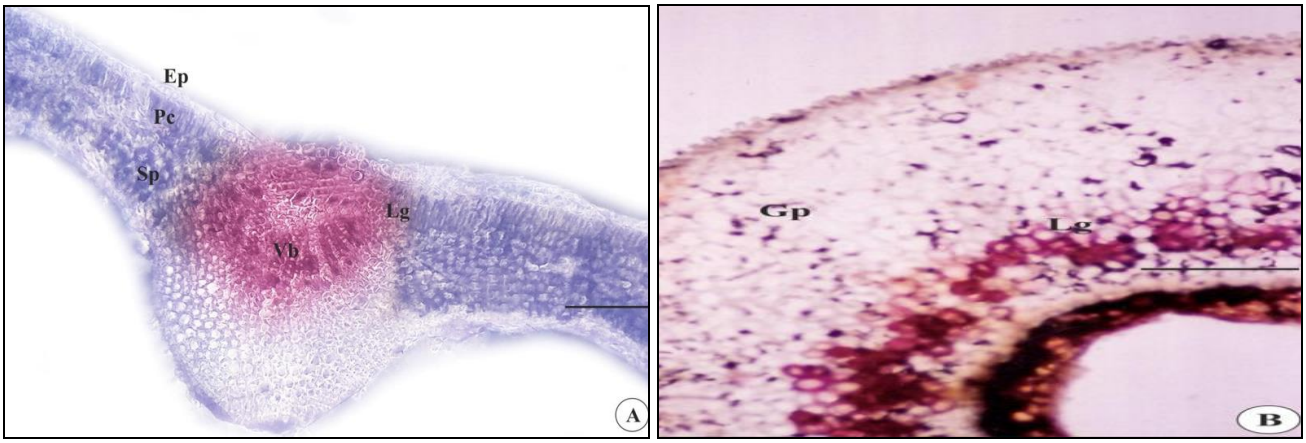
Bar = 0.05mm

Plate 3



Bar = 0.05mm

Plate 4



Bar = 0.05mm

Table 1

Metabolites	Normal or Gall	Regions Showing localization					
		Epidermis	Palisade	Spongy Parenchyma	Vascular Bundle	Gall Parenchyma	MT
Starch	N	+	+	+	++	X	X
	G	+	x	x	X	+++	+
Cellulose	N	++	-	++	++	X	X
	G	++	x	++	X	++++	++
Carbohydrates	N	+++	++++	++	++	X	X
	G	++	x	x	++	++	++
Proteins	N	+	+	+	+	X	X
	G	++	++++	x	X	++	-
Lipids	N	++	++	++	+	X	X
	G	+	x	x	X	+++	++
Lignin	N	-	-	-	++	X	X
	G	-	x	x	X	-	++++
Tannins	N	++	+	+	+	X	X
	G	+	x	X	X	+++	++++
Acid phosphatase	N	-	+	+++	-	X	X
	G	+	x	X	X	+++	+++
Peroxidase	N	+	++++	++	+	X	X
	G	++	x	X	X	+++	+++
Polyphenol oxidase	N	++	+++	+	+	X	X
	G	+++	x	x	x	+++	+++

N = Normal
G = Gall
- = Nil
+ = Low intensity
++ = Moderate intensity
+++ = High intensity
++++ = Very High Intensity
x = Metabolites Absent
mz = Mechanical Zone

Refrences

1. Arora DK, Patni V. Localization of metabolites and enzymes in insect induced rachis gall and normal tissues of *Prosopis cineraria* (Linn.) Druce. J. Phytol. Res.,2001:14(2):179-181.
2. Arya S. Studies on nematode induced root galls of cowpea (*Vigna unguiculata*) *in vivo* and *in vitro*. Ph. D. Thesis, Univ. of Rajasthan, Jaipur, India, 1989.
3. Chiffelle TL, Putt FA. Propylene and ethylene glycol as solvents for Sudan IV and black B. Stain Test.,1951:26:51-56.
4. Debnath M, Sharma SL, Sharma S, Kant U. Differential metabolic change in midge induced leaf gall of *Mangifera indica*. J. Ind. Boi. Soc.,2002:81:293-299.
5. Gopinathan K. Morphological patterns and histochemical profile in *Mimusops-Arrhenothrips* gall system. Proc. Indian Acad. Sci.,1987:97(3):203-214.
6. Gortner RA, Gortner WA. Outline of Biochemistry III Ed. John Wiley and Sons, Inc., New York Chapman & Hall Limited, London, 1949.
7. Isaias R, Ferreira B, Alvarenga D, Barbosa L, Salminen JP, Steinbauer M. Functional compartmentalization of nutrients and phenolics in the tissue of galls induced by *Leptocybe invasa* (Hemiptera: Eulophidae) on *Eucalypyus camaldulensis* (Myrtaceae): Nutrients and phenolics in *Leptocybe invasa* galls. Austral Entomolgy, 2018, 10-17.
8. Kahl G. Metabolism in plant storage tissue slices. Bot. Rev.,1974:40:263-314.
9. Kumar A. Physiology and host-parasite interaction of insect induced galls of *Prosopis cineraria in vivo* and *in vitro*. Ph D.Thesis, University of Rajasthan, Jaipur, 1996.
10. Mathur M. Studies on insect induced galls of certain economically important tree species. Ph.D. Thesis, University of Rajasthan, Jaipur, 2002.
11. Johansen DA. Plant microtechnique. McGraw-Hill Book Co inc., Newyork, 1940, 523.
12. Siegel SN. Biosynthesis of lignins. Physiol. Plantarum.,1953:6:134-139.
13. Vyas K. Studies on some important galls of leguminous plants induced by insects and mite. Ph.D. thesis, University of Rajasthan, Jaipur, India, 1989.
14. Weime. Studies on agar electrophoresis Arcia nitgraphens, NY Brussels and Elsevier Amsterdam, 1959, 1965.