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# *LOCALIZATION OF METABOLITES AND ENZYMES IN LEAF GALL OF TERMINALIA ARJUNA*

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The purpose of the present investigation was to study the possible alteration in metabolic activity due to insect attack on *Terminalia arjuna*. Histochemical localization of metabolites and enzymes was studied in leaf gall of *T. arjuna* (Linn.) induced by *Trioza flectcheri* (Homoptera) . These studies revealed higher activity of various metabolites in gall tissue, especially near the nutritive zone. A functional elaboration in the cells closer to the feeding site of the cecidozoan during cecidogenesis was evident. Also, a different response of metabolites and enzymes at cellular level of the host proved advantageous to the insect toward gall formation.

**Keywords :** Enzymes; Histochemical; Leaf gall; Metabolites, *Terminalia arjuna*.

## Introduction

Arjuna (*Terminalia arjuna)* is a large tree, the bark is used in certain herbal combinations as a powerful, soothing tonic for the heart. It is good for both the physical heart as a muscle, as well as for the emotions associated with the heart. Arjuna is used for loneliness, sadness and frustration. It strengthens the emotions to decrease excessive response to stress and trauma. It helps in strengthening the body's natural rejuvenative processes, hastening the replacement of dead or weak cells with fresh, vital ones. In proper combinations, Arjuna helps to stabilize an erratic heart beat.

The bark of *T. arjuna* is astringent and is used in fevers, fractures and contusions; it is also taken as cardiac tonic. Clinical evaluation of this botanical medicine indicates that it can be of benefit in the treatment of coronary artery disease, heart failure, and possibly hypercholesterolemia. It has also been found to be antibacterial and antimutagenic[1,2]. *Terminalia's* active constituents include tannins, triterpenoid, saponine (arjunic acid, arjunolic acid, arjungenin, arjunglycosides), flavonoides (arjunone, arjunolone, luteolin), gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCS), phytosterols, calcium, magnesium, zinc and copper[2].

The possible alteration in metabolic activity caused by insect attack was studied by histochemical localization of metabolites and enzymes in leaf galls of *T. arjuna* caused by *Trioza fletcheri* of the order Homoptera. Galls are generally epiphyllous, globose, indehiscent and generally peristant pouch cum covering galls. The ostiole is narrow.

## Materials and Methods

The normal leaf and leaf galls of *Terminalia arjuna* were collected from western Uttar Pradesh (Dist. Mathura), Eastern Rajasthan (Dist. Bharatpur) and adjoining areas, and their morphology was studied. Fresh hand cut sections of leaf were used for histochemical analysis. The metabolites, starch and cellulose[3], carbohydrates[4,5], proteins[6], lipids[7], lignin[3] and tannins[8], and enzymes *viz.*, polyphenol oxidase[9], peroxidase[10] and acid phosphatase[11] were localized and documented. Their qualitative increase or decrease in localization was assessed in terms of intensity of stainin. The degree of distribution of the stain in various tissues was recorded as low (+), moderate (++) and high (+++).

## Observations and Discussion

Results obtained for localization of metabolites and enzymes in normal and leaf gall tissues are presented in Table 1 and Fig. 1-3.

*Starch* - In normal leaf, starch granules were observed in the mesophyll region. The concentration of starch was more in palisade tissue as compared to spongy parenchyma. Nutritive zone of leaf gall tissue showed high intensity of starch grains (Fig. 1 C and D). Presence of starch near the gall cavity suggests that the insect may be utilizing starch as food material as such. Starch present in nutritive zone suggests a possible diffusion of soluble saccharides produced by starch hydrolysis[12].

*Cellulose*-Cellulose was observed more in the outer surface of epidermis and it was less in mesophyll region of normal leaf. In gall tissue of the leaf, the cellulose was present in high quantity in nutritive zone and palisade parenchyma present (Fig. 1 E and F). Cell walls of nutritive zone, palisade parenchyma, inner lining of mesophyll and vascular region showed more contents of cellulose in gall tissues as compared to the normal tissues. High amount of cellulose in gall tissue has been reported in *Prosopis cineraria* rachis gall[13].

*Total insoluble polysaccharides*-Almost all the tissues of normal leaf showed the presence of insoluble polysaccharide in the cell walls. A very strong reaction to the stain was observed in the leaf gall mesophyll cells and nutritive zone (Fig. 1G and H).

*Lipids*-Palisade cells of mesophyll of normal leaf showed strong reaction to the stain and epidermis showed less quantity of lipids. Nutritive zone of leaf gall tissue showed very strong reaction to the stain. Almost all the tissues of gall showed high quantity of lipids (Fig. 2 A and B). Normal

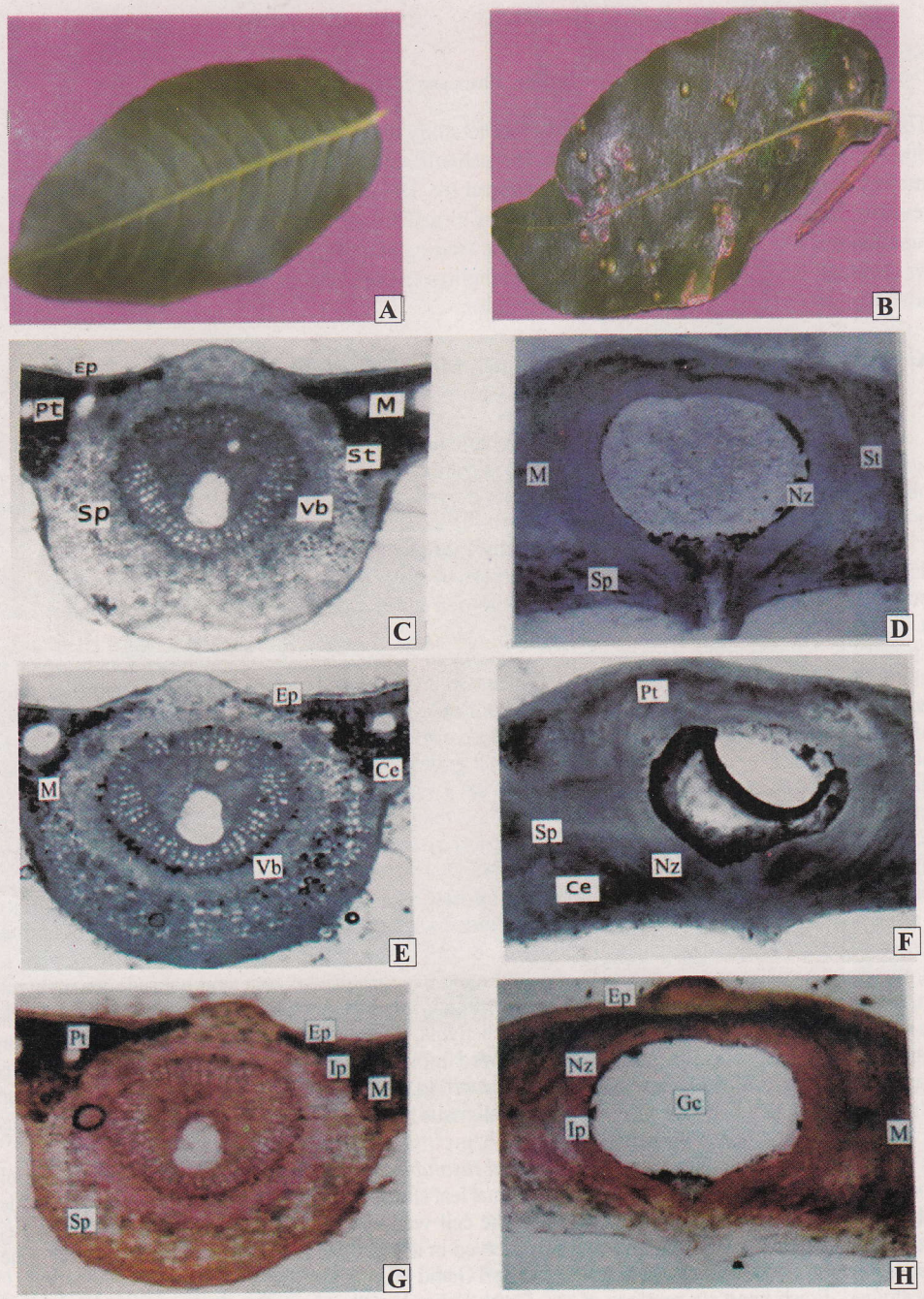


**Fig. 1**. Localization of various metabolites in normal and leaf gall of *Terminalia arjuna.,* A, C, E, G-normal leaf, B,D,F,H- leaf gall, C and D- localization of starch, E and F- localization of cellulose, G and H- localization of total insoluble polysaccharides.
(Ep= Epidermis, Pt= Palisade tissue, Sp=Songy parenchyma, St=Starch, Vb=Vascular bundle, M=Mesophyl)
C x=400, D x=370, E x=370, F x=150, G x = 370, H x=370

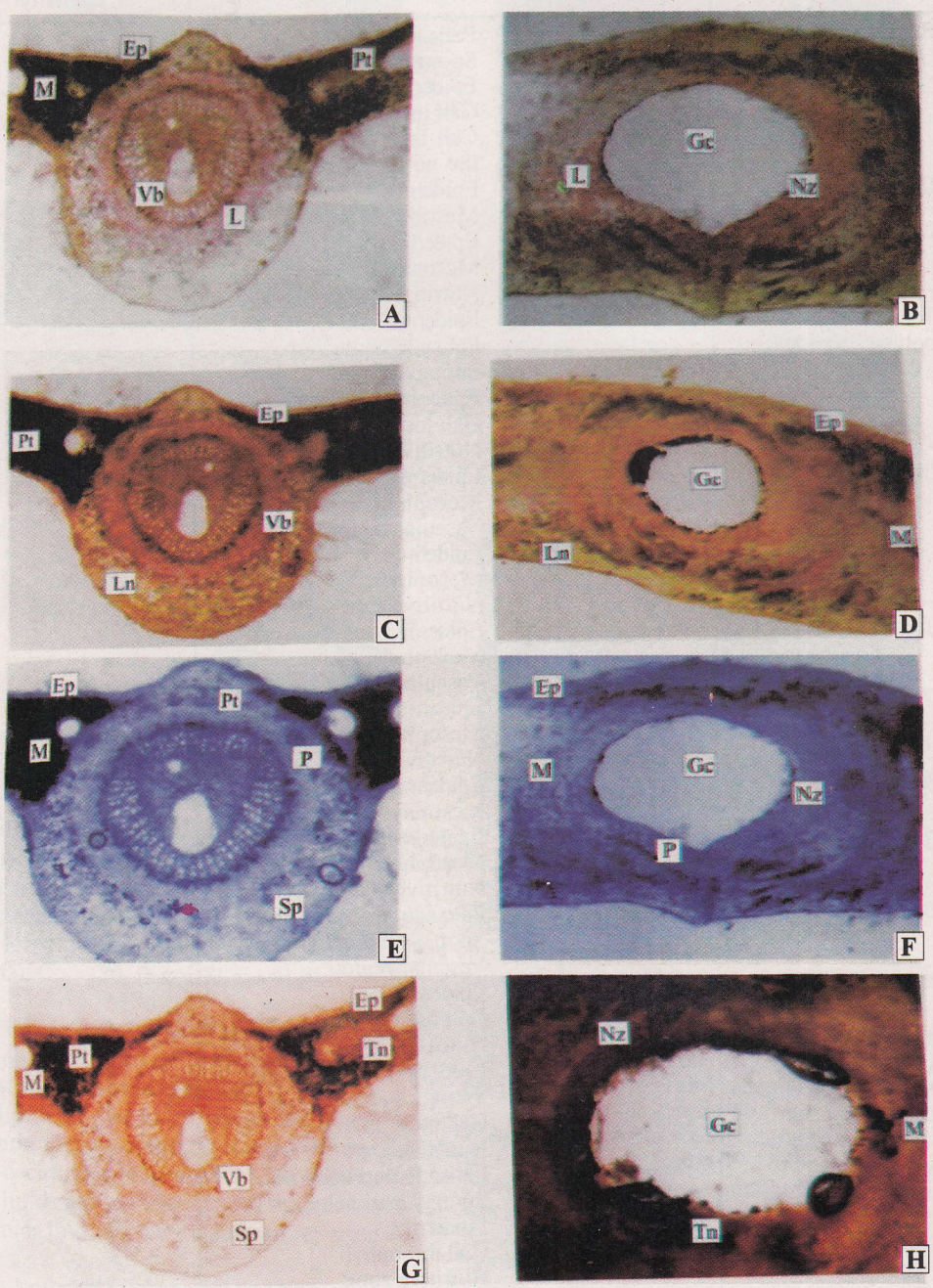


**Fig. 2**. Localization of various metabolites in normal and leaf gall of *Terminalia arjuna*. A, C, E, G normal leaf, B,D,F,H leaf gall, A and B-localization of Lipids, C and D -localization of Lignin,
E and F -localization of Proteins, G and H -localization of Tannins.
(Ep= Epidermis, L=Lipid, M=Mesophyll, Pt=Palisade tissue, Vb=Vascular bundle, Gc=Gall cavity, Nz=Nutritive zone, Sp=Spongy tissue, P=Protein, Tn=Tannins)
A x=370, B x=200, C x=370, D x=370, E x=370, F x=200, G x=370, H x=130

**Table 1.** Histochemical localization of metabolites and enzymes in normal and leaf gall tissues of *Terminalia arjuna*.

| S.No. | Metabolite | Normal/Gall | Regions localized | Intensity |
|---|---|---|---|---|
| 1. | Starch | Normal | Palisade parenchyma | ++ |
| | | | Spongy parenchyma | + |
| | | Gall | Epidermis | + |
| | | | Gall mesophyll | + |
| | | | Cell layer just above the nutritive zone | +++ |
| 2. | Cellulose | Normal | Epidermis | + |
| | | | Mesophyll | + |
| | | Gall | Epidermis | + |
| | | | Mesophyll | ++ |
| | | | Nutritive zone | +++ |
| 3. | Total insoluble polysaccharides | Normal | Epidermis | + |
| | | | Palisade parenchyma | + |
| | | | Spongy parenchyma | + |
| | | Gall | Epidermis | + |
| | | | Gall mesophyll | +++ |
| | | | Nutritive zone | +++ |
| 4. | Lipids | Normal | Epidermis | + |
| | | | Mesophyll | +++ |
| | | | Vascular region | + |
| | | Gall | Epidermis | + |
| | | | Mesophyll | +++ |
| | | | Nutritive zone | +++ |
| 5. | Lignin | Normal | Epidermis | + |
| | | | Mesophyll | + |
| | | | Vascular region | + |
| | | Gall | Epidermis | + |
| | | | Mesophyll | + |
| | | | Nutritive zone | ++ |
| 6. | Protein | Normal | Epidermis | + |
| | | | Mesophyll | +++ |
| | | Gall | Epidermis | + |
| | | | Mesophyll | + |
| | | | Nutritive zone | +++ |
| 7. | Tannin | Normal | Palisade parenchyma | +++ |
| | | | Spongy parenchyma | + |
| | | | Vascular region | + |
| | | Gall | Epidermis | + |
| | | | Gall mesophyll | + |
| | | | Nutritive zone | +++ |
| 8. | Acid Phosphatase | Normal | Epidermis and Palisade | +++ |
| | | | Spongy parenchyma | + |
| | | Gall | Gall mesophyll | ++ |
| | | | Nutritive zone | +++ |
| 9. | Peroxidase | Normal | Palisade parenchyma | + |
| | | | Spongy parenchyma | + |
| | | Gall | Epidermis | + |
| | | | Gall mesophyll | + |
| | | | Nutritive zone | +++ |
| 10. | Polyphenol | Normal | Palisade parenchyma | + |
| | | | Spongy parenchyma | + |
| | | | Vascular region | + |
| | | Gall | Epidermis | + |
| | | | Gall mesophyll | +++ |
| | | | Nutritive zone | +++ |

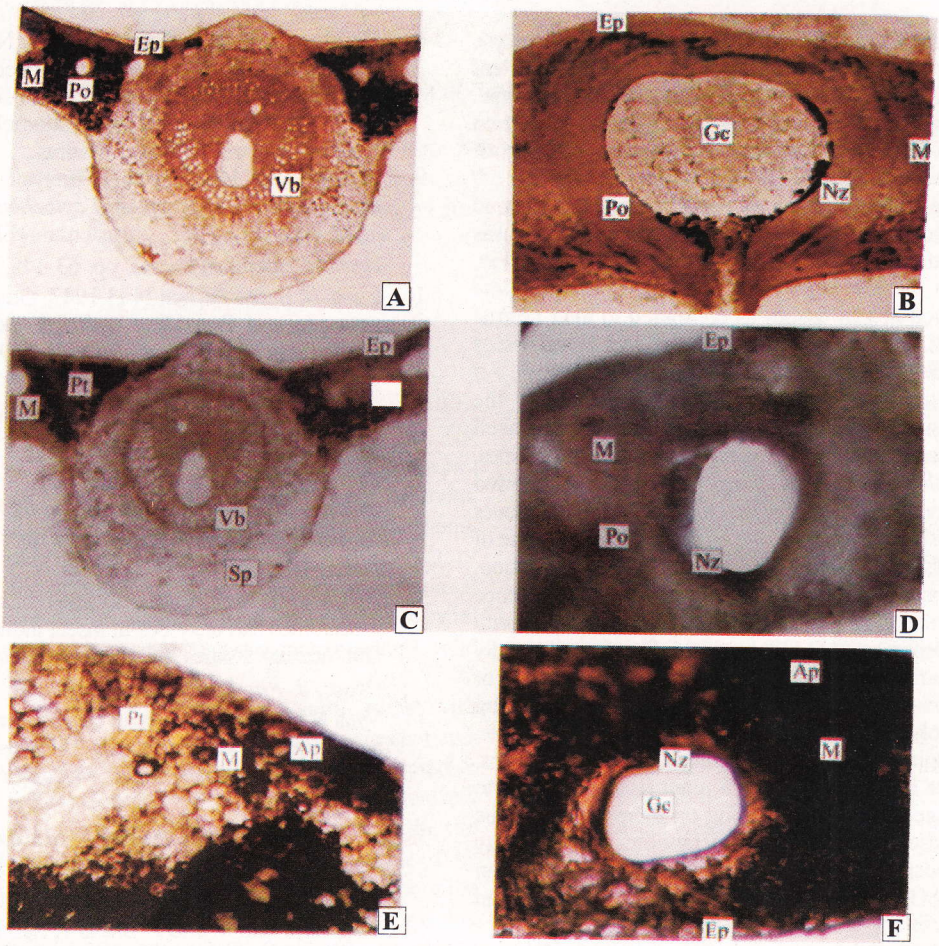


**Fig. 3**. Localization of various enzymes in normal and leaf gall of *Terminalia arjuna.* A, C, E normal leaf, B, D, F leaf gall
A and B showing activity of Polyphenol oxidase; C and D showing activity of peroxidase;
E and F showing activity of Acid phosphatase.
(Ap= Acid phosphatase, Ep= Epidermis, M=Mesophyll, Po=Polyphenol oxidase, Vb=Vascular bundle,
Gc=Gall cavity, Nz=Nutritive zone, Sp=Spongy parenchyma, Pt=Palisade tissue, Po=Peroxidase)
A x=370, B x=200, C x=130, D x=200, E x=130, F x=130

tissues showed feable staining and all the gall tissues showed higher concentration of total carbohydrate of insoluble polysaccharides in and around the gall cavity. Abundance of lipids in the nutritive region could be related to the continuous wounding as a result of feeding activity of the cecidozoa which alters the metabolic pathway. These lipids are, in turn utilized by the insects for survival. Similar findings have also been reported by several workers[14,17].

*Lignin*-Lignin was moderate in normal leaf tissues. Very less quantity of lignin was observed in epidermis. It was localized intensely in nutritive zone and mesophyll region of leaf gall tissues and it was feable in epidermal cells (Fig. 2 C and D). In general, parenchymatous cells showed less amount of lignin because infection with any pathogenic agent might have delayed the process of lignification in cortical and pericycle region[18].

*Proteins* -The palisade tissue in mesophyll of normal leaf region showed high intensity of proteins and moderate in spongy parenchyma and vascular cells. Nutritive zone of leaf gall region showed very high intensity of proteins. Mesophyll cells showed moderate amount of proteins in leaf gall (Fig. 2 E and F). In increased incidence of proteins, the maximum amount was noticed in nutritive tissues which helps the cecidozoan in its growth and develoment. Wounding is known to accelerate protein synthesis[19].

*Tannins* - Mesophyll region of normal leaf showed high intensity of tannins. Epidermis showed feable amount of tannins. Very strong reaction to the stain was observed in leaf gall nutritive region and feable reaction was observed in epidermal cells and spongy parenchyma (Fig. 2 G and H). An increased amount of tannins in gall tissues could be attributed to the higher incidence of polyphenol oxidase and peroxidase activity. Similar observation was also made

by Gopinathan[20] in thrips induced leaf gall of *Mimusops*.

*Polyphenol oxidase* - Mesophyll region of normal leaf showed positive reaction with polyphenol oxidase enzyme. Epidermis showed feable staining. High intensity of reaction was observed in nutritive zone and mesophyll region of leaf gall tissue (Fig. 3A and B).

*Peroxidase* - Almost all the tissues of normal leaf showed positive reaction of enzyme. Intensity of enzyme action was more in leaf gall nutritive zone and mesophyll region (Fig. 3 C and D). Increase in polyphenol oxidase and peroxidase was observed in gall tissues as compared to their normal counterparts. Higher activity of these enzymes in gall tissues has been observed in several insect induced galls[17,20]. An increased activity of polyphenol oxidase leads to the stimulation of auxin activity in plants which is well substantiated by maximum hyperplasy seen in gall tissues.

*Acid Phosphatase* - Mesophyll region of normal leaf showed high activity of acid phosphatase enzyme. Almost all tissues showed positive reaction of the enzyme. Nutritive zone of leaf gall tissues showed high intensity of enzyme action. Epidermis and mesophyll cells showed moderate presence of enzyme action (Fig. 3 E and F). A predominant activity of acid phosphatase was observed in gall tissues especially near the nutritive zone. This enzyme might be directly or indirectly linked with energy transfer mechanism by bringing about hydrolysis of some suitable substances, for example, sugar phosphate for making available phosphate groups. An increase in rate of growth of plant tissues is likely to be advantageous to insect feeding on them primarily due to an increased mobilisation of metabolites. High activity of acid phosphatase in nutritive regions has also been reported by Gopinathan and Suresh[21] and Gopinathan and Ananthakrishnan[22].

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