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PHENOLICS AND OXIDATIVE ENZYMES IN LEAF GALLS OF *QUERCUS LEUCOTRICHOPHORA*

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Quercus leucotrichophora A. Camus (Bluejack oak) is found distributed throughout Himalayan region in India. Epiphyllous, pustule like galls on the tree are caused by *Eriophyes* mite. Hypophyllous, fruit like galls are induced by cecidomyiid (Dipteran). Biochemical studies including different phenolics and oxidative enzymes of both the galls and normal leaf have been carried out. Most of the phenolics, involved in defense mechanism have been estimated higher in the gall tissues. Isozyme analysis of peroxidase was analysed by PAGE (Poly Acrylamide Gel Electrophoresis). Two bands were detected in normal and gall tissues, banding intensity was more in galls. Intense activity and banding pattern of peroxidase and polyphenol oxidase activity could be correlated to accumulation of phenolics and condensed tannins in galls.

Keywords: Camus; Cecidomyiid; *Eriophyes*; Galls; Oak; *Quercus leucotrichophora*.

Introduction

Specific interaction between animals and plants are very common in nature. Among these interactions, those of gall inducing insects and their host plants are believed to be the most intimate[1]. Gall causing parasites release growth-regulating chemicals as they feed, causing adjacent plant tissues to form a gall. Entomogenous galls are pathological structures which have originated from neoformed tissues, as a mechanical and/or chemical insect stimuli[2]. Several different groups of insects and one family of mites have developed the ability to induce plant galls. Many different types of galls have been reported on oak plants. Oak trees are distributed throughout the Mediterranean region, Europe, Asia and North America. In India, *Quercus leucotrichophora* is conspicuous in the Himalayan region (1300-3000 masl). The tree is important for its timber, aesthetic and medicinal values.

Epiphyllous galls caused by *Eriophyes* mite and hypophyllous mango like cecidomyiid galls on the tree have been studied. Cecidomyiidae (gall midges) is the most abundant group which induce galls in India[3]. Galls on oak are the potential source of dyestuff, gallic acid, secondary metabolites, tannins, pigments etc. The levels of nutrients and secondary metabolites in gall tissues are usually markedly different from those of normal leaf. Morphological, histochemical and biochemical studies of both the leaf galls of *Q. leucotrichophora* have been carried out[4]. The present investigation deals with the study of some biochemical changes in the leaf galls of *Q. leucotrichophora* infested by *Eriophyes* mite and cecidomyiid.

Material and Methods

Normal leaves and both type of leaf galls of *Quercus leucotrichophora* were collected from Mussoorie, Dehradun and Nainital. Biochemical changes viz. phenolics and oxidative enzymes in gall and normal leaf were determined by various methods as described. Quantitative analysis of total phenols was made by the method of Bray and Thorpe[5], O-dihydroxyphenol by Johnson and Schall[6], peroxidase activity by Worthington enzyme Manual[7], and polyphenol oxidase activity by the method of Palmer[8].

Determination of condensed tannins was carried out by Butanol-HCL-iron method of Porter *et al.*[9]. Phenyl Ammonia Lyase (PAL) activity was assayed by the method of Higuchi[10].

Isozymes of peroxidase were analysed by the method of Davis[11]. Polyacrylamide gel (10%) was prepared and poured between two glass plates, comb was inserted between them. Samples were prepared by homogenizing fresh plant material in phosphate buffer (pH 7.0). After centrifugation, supernatant thus obtained was mixed with sucrose grains, a few drops of 0.01% bromophenol blue (BPB). The samples were loaded in the wells. Electrophoresis was started by supplying a current of 100

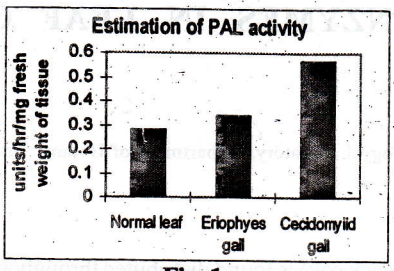
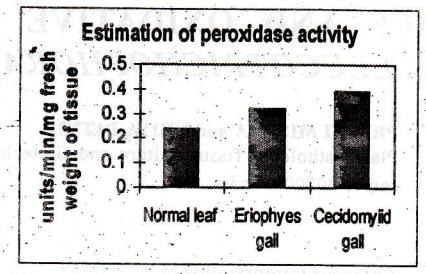


Fig. 1.



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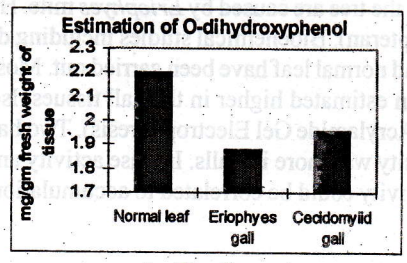

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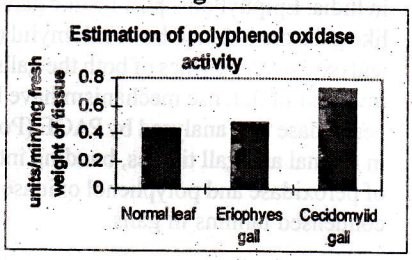

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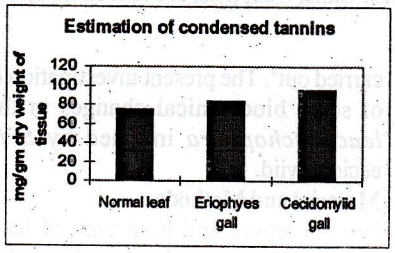
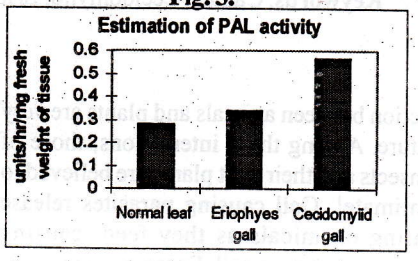


Fig. 3.



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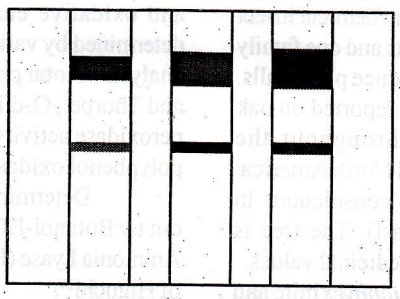

NL:- Normal leaf
EG:- Eriophyes Gall
DG:- Dipteran Gall

Fig. 7. Zymogram showing intensities of bands

V till the tracking dye (BPB) reached almost the lower end of the gel. After running, the gel was removed from the glass plates and transferred to an appropriate staining solution (acetate buffer and 0.1% O-dianisidine). Bands were photographed and Rm value was calculated by making zymogram.

Results and Discussion

Leaves of *Q. leucotrichohpora* (bluejack oak) were isobilateral, amphistomatic, dull green, white wooly beneath with serrate margins. Thick cuticle was present on lower and upper epidermis. Galls caused by *Eriophyes* mite were hemispherical, pustule like, glabrous above and below with brown erineum. Cecidomyiid galls showed great anatomical complexity. Mesophyll of the gall was differentiated into parenchymatous, chlorenchymatous and sclerenchymatous compact layers. The gall was two chambered where larva developed. Feeding action of larva inflicts wound and secretion of salivary chemicals, alters

Table 1.

Sample	Band	Rm Value	Intensity
NL	Band 1	0.208	Intense
	Band 2	0.458	Faint
EG	Band 1	0.229	Intense
	Band 2	0.458	Intense
DG	Band 1	0.229	Very intense
	Band 2	0.458	moderate

the subcellular chemistry in host tissues; both of these when they occur concurrently, stress the host plant cells. As a neutralizing response to the stress, the plant translocates different metabolites to the site where the insect feeds. Continued feeding activity by the insect induces the plant to produce different type of defensive metabolites[12].

Phenolics- The quantity of total phenols was significantly higher in both the galls as compared to normal leaf (Fig. 1). The increase in levels of phenols may be attributed to defense mechanism. According to Rana *et al.*[13], the resistance to disease caused by aphids is due to the presence of high amount of phenols. Motta *et al.*[14] assayed higher contents of soluble phenols, tannins, lignins, lipids etc. in galls and suggested that these substances represent the main energy source for the insect. This increase may be due to the action of enzymes such as polyphenol oxidase, peroxidase or pre-existing phenolic compounds or through the release of bound phenolic compounds[15].

O-dihydroxyphenols have been estimated low in the galls (Fig. 2). It is known that polyphenol oxidase helps in conversion of O-dihyroxyphenols to tannins (higher phenols). Several studies reported that total tannin content and/or total phenolics were negatively correlated with growth rates of feeding herbivores[16]. Condensed tannins have been estimated highest in the cecidomyiid galls, than in pustule like galls (Fig. 3). Some different galls have also exhibited elevated condensed tannin concentration than the leaves[17].

Enzymes- Activity of oxidative enzymes viz. peroxidase, polyphenol oxidase and Phenyl Ammonia Lyase (PAL) were found to be higher in galls (Figs. 4 to 6). Galls of the cynipid wasp on chestnut oak had significantly greater peroxidase and polyphenol oxidase activities[18].

Peroxidases play an important role in plant metabolism and physiology and are involved in the responses of plants to infectious and abiotic stress stimuli. Increased peroxidase activity could be due to increased phenol concentration, where phenols are factors of peroxidases and hence influence resistance in the host. Polyphenol oxidases are involved in total phenols and tannins biosynthesis. High polyphenol oxidase and PAL activities have been detected in *Prosopis cineraria* galls by Ramani and Kant[19].

Isozyme analysis of peroxidase - An isozyme is one of the multiple forms of an enzyme separable by electrophoretic procedure and having similar or identical catalytic activities. These forms vary in contents with the species, the tissue, the stage of growth and the application of growth substances.

Two bands/isoperoxidases of each sample were recorded. Band first of sample NL (normal leaf) showed same Rm value as sample EG (*Eriophyes* gall) and DG (Dipteran gall) but with less intensity. Band second of NL was very faint. Band first and band second of EG sample was very intense. Band first of DG was highly intense than the previous two. Band second of DG was not equally intense as band second of sample EG (Fig. 7).

Activity of specific peroxidase isoforms was also detected differentially in galls on red oak[20]. It can be concluded that the isoperoxidase activity is markedly high in the galls.

Overall higher accumulation of phenolics and condensed tannins in both the galls could be attributed to high activity of polyphenol oxidase and peroxidase. This could be confirmed by the high intensity of banding pattern of isoperoxidases due to gall formation.

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