



Biochemical Changes in the leaf galls of *Cinnamomum Verum Presl.* (Syn. *Cinnamomum zeylanicum*) induced by unknown *Eriophyes sp.*

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ABSTRACT

The genus *Cinnamomum* is a member of a tropical evergreen tree of the Lauraceae family. In its wild state, it grows up to 7m (56 ft). *Cinnamomum verum Presl.* (Syn. *Cinnamomum zeylanicum*) is of commercial value and is extensively used in culinary as a spice in food and the ayurvedic system of medicine. One of the major diseases, causing severe losses in yield, thus affecting the economy in India is the leaf gall disease of Cinnamon. To understand the host-pathogen interactions, it becomes obligatory to estimate the proteins, carbohydrates, enzymes, etc. present in a particular host plant quantitatively, to draw meaningful conclusions on host-pathogen interaction. The pathogen is always associated with the infection caused to a healthy plant. Eventually, biochemical changes take place in the diseased tissue. The pathogenic organism releases cell secretion, which comprises of various cell metabolites which alter the metabolism of the diseased tissue. In the present investigation, changes in the biochemical profile of healthy and diseased leaf of Cinnamon has been attempted, and the results have been discussed in the light of pathogenicity, induced by unknown *Eriophyes sp.*



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INTRODUCTION

Mani (1973) reported Four types of galls on *Cinnamomum verum Presl.* (Syn. *Cinnamomum zeylanicum*). However, only some preliminary work has been done on the morbid anatomy of leaf galls of *Cinnamomum verum Presl.* (Syn. *Cinnamomum zeylanicum*) and no work, to the best of our knowledge, has been reported on the biochemical aspects of the leaf galls *Cinnamomum verum Presl.* (Syn. *Cinnamomum*

zeylanicum). Changes in the healthy metabolism of host following infection is a widespread phenomenon in a plant. Any symptom due to infection, brought by a pathological organism in plants is associated with biochemical changes in the tissues. Several workers have studied the biochemical changes in various plants after infection with the pathological organism (Parashar and Lodha, 2007; Meena et al., 2014). When a disease is developed in a plant, the pathogen induces cell response by releasing its secretion, which comprises of auxin, enzymes and toxins to alter the host cell metabolism. Thus equilibrium in metabolism is established between the host and the parasite in a localised phase of infection (Sharma, 2004). Daly (1976) reported that the imbalance in photosynthesis, respiration and translocation due to foliar disease, reduces productivity even in uninfected parts of the plants.

The altered metabolism in plant galls induced by insects and mites is best understood by estimating the number of various metabolites present in the normal as well as the gall tissues. Hence the present investigation is undertaken to study the changes

in the biochemical profile of leaf galls and regular counterparts of *Cinnamomum verum Presl.* (*Syn. Cinnamomum zeylanicum*) *in vivo*.

Leaf galls of *Cinnamomum verum Presl.* (*Syn. Cinnamomum zeylanicum*) induced by unknown *Eriophyes sp.* were selected for the present study. Healthy and Infested shoots were collected from the experimental field and studied. The knowledge of the carbohydrate contents of both normal and gall tissues will aid in understanding the metabolism of the diseased and healthy tissues. Hence total soluble sugar contents, reducing sugar contents, starch contents and activity of enzymes viz. alpha-amylase and invertase of leaf galls and normal counterparts *Cinnamomum verum Presl.* (*Syn. Cinnamomum zeylanicum*) were analysed.

MATERIALS AND METHODS

Total soluble sugar contents were studied by the phenol sulphuric acid reagent method of [Dubois et al. \(1958\)](#). 500 mg each of standard and gall tissues were homogenised with 10.0 ml of 80 per cent ethanol, centrifuged at 2000 rpm for 20 minutes, and the Supernatants were used for the estimation of total Soluble Sugar, expressed as mg/g fresh weight of tissue.

The dinitrosalicylic acid (DNSA) method of [Miller \(1959\)](#) was used for the estimation of Reducing Sugars. To sample the extracts, of each healthy and diseased material, 1.0ml DNSA reagent was added, heated in boiling water bath for 5 minutes, 1.0 ml of 40% sodium-potassium tartrate was added, while it was still warm, cooled the tubes in running tap water, and the absorbance was measured at 575 nm against the blank. The quantity of reducing sugars was expressed as mg/g fresh weight of tissue.

The method of [McCready et al. \(1950\)](#) was used for the estimation of Starch. 500 mg each of standard and gall tissues were homogenised with 10.0 ml of 80% ethanol, centrifuged at 2000 rpm for 20 minutes, the supernatant was discarded, and the residue was suspended in 5.0 ml of distilled water. Subsequently, 6.5 ml of 52% perchloric acid was added. After stirring the content for 15 minutes, the mixture was centrifuged for 20 minutes at 2000 rpm, and The supernatant was collected. The whole procedure was repeated thrice. Supernatants of each step were then pooled, and the total volume was made up to 100.0 ml with distilled water. The filtrate of 1.0 ml aliquot was analysed for starch content. Quantity of starch was calculated in terms of glucose equivalent, and factor 0.9 was used to convert the value of glucose to starch. Quantity of starch was expressed in terms of mg/g fresh weight of tis-

sue.

[Bernfeld \(1955\)](#) method was used for the determination of Alpha-amylase. The enzymes were extracted from normal and gall tissues, 200 mg each, homogenised in 4.0 ml of 0.02 M phosphate buffer (pH 6.9). The homogenate was centrifuged at 2500 rpm for 20 minutes, and the supernatant was used to determine the enzyme activity. 1.0 ml each of enzyme extract and Substrate Solution (1% soluble starch in 0.02M Phosphate buffer containing 0.0067M NaCl at pH 6.9) was heated at 300 degree Celsius for 45 minutes. 1.0 ml of DNSA reagent was added to stop the reaction, and the tubes were boiled in boiling water bath for 15 minutes, cooled by adding 20 ml of distilled water to develop the yellow colour due to unhydrolysed starch, measured at 560nm. The Alpha-amylase activity was expressed in terms of mg starch hydrolysed per hour per g fresh tissue.

Invertase activity of normal and gall tissues was estimated by the method of [Harris and Jeffcoat \(1974\)](#). The enzyme was extracted from normal and gall tissues, 500 g each, homogenised in 5.0 ml of 0.2 M acetate buffer (pH 4.8), centrifuged at 2000rpm for 20 minutes and the supernatant was used as enzyme extract. Enzyme activity was measured by adding in a test tube 0.4 ml of 0.4 M sucrose and 0.4 ml of enzyme extract and 1.0 ml of DNSA reagent incubated at 300 degree Celsius for 30 minutes, cooled in the water bath for 10 minutes. Then contents were diluted to 10.0 ml by adding distilled water. Control tubes contained 0.4 M Sucrose only. Optical density was measured at 560 nm. The activity was expressed as mg sucrose hydrolysed / hour/ g fresh tissue.

RESULTS

Leaf galls induced by unknown *Eriophyes Sp* resulted in an increased level of Total Soluble Sugars, Reducing Sugars, alpha-amylase and Invertase activities in *Cinnamomum verum Presl.* (*Syn. Cinnamomum Zey lanicum*) compared to its normal leaf tissues. In comparison, Total Starch content was more in normal leaves than the induced gall tissues of *Cinnamomum verum Presl.* (*Syn. Cinnamomum Zeylanicum*).

DISCUSSION

Many investigators reported that stimulus of metabolic products secreted by gall-forming organisms leads to abnormal growth in plants ([Dropkin, 1955](#); [Braun et al., 1958](#)). Carbohydrates play a vital role in various metabolic processes of plants

and are indispensable for tissues grown in culture. These are formed by storage of energy trapped from sunlight in the process of photosynthesis. Secondly, carbohydrates are the main constituents of the supporting tissues of the plants.

A large number of plants of economic importance are affected by *Eriophyes sp.* Feeding punctures caused by unknown *Eriophyes sp.* are partially callosified by the host, permitting the repair of cell wall integrity following punctures. Mani (1964) and Channabasavanna and Nangia (1984) have referred that each host plant responds differently to feeding activity by unknown *Eriophyes sp.* mites. According to them, the damaged tissues contain a large number of free amino acids, indicating a disturbed protein synthesis. Auxin and Phenolic contents are also generally higher in the galls than of the normal leaf tissues (Balasubramanian and Purushothaman, 1972; Purohit *et al.*, 1979).

Maretski *et al.* (1974) reported that the growth and metabolic events of the cells and tissues are proportional to the quality and quantity of carbohydrates along with plant growth substances. Changes in Carbohydrate metabolism induced by the disease may be due to the disturbance of several regulatory systems that regulate the steady metabolic state of a healthy plant cell (Kosuge and Kimpel, 1981). The subject has been reviewed by many workers (Goldsworthy, 1964; Street, 1969; Maretski *et al.*, 1974). Srivastava (1992) reported an increase in carbohydrate content in stem galls of *Coriandrum sativum* induced by *Protomyces* as compared to the normal stem.

Increase in sugar contents in leaf gall of *Achyranthes Aspera* has been reported by Shekhawat *et al.* (1978). Tandon and Arya (1979) reported that insect induced *Zizyphus* stem gall tissues contained more reducing sugars and showed increased alpha-amylase activity as compared to normal tissues. Karanawat (1988) reported a decrease in total sugar contents in insect induced galls of *Syzygium jambolanum*. Kumar (1996) reported higher reducing sugar and starch content and lower alpha-amylase activity in leaf gall tissue of *Prosopis cineraria*. Mathur (2002) reported higher reducing sugar and alpha-amylase activity in flower gall of *Caligonum polygon sides* as compared to normal tissue.

For a proper understanding of insect and plant interaction, it becomes necessary to study the changes in the biochemical profile of gall and normal tissues. In the present investigation, biochemical studies of leaf gall induced by unknown *Eriophyes sp.* in *Cinnamomum verum Presl.* (*Syn.Cinnamomum*

zeylanicum) compared to its normal counterparts were conducted *in vivo*. Carbohydrate contents and hydrolysing enzymes were used as the parameters to study the biochemical changes in the leaf galls. The results showed increased Total soluble sugar contents reducing sugar, alpha-amylase activity and invertase activity in gall tissues as compared to normal tissue. These studies revealed that the pathogen caused a derangement in the metabolism of the host tissues as reported in the leaf galls of *Achyranthus Aspera* (Shekhawat *et al.*, 1978), *Zizyphus* stem gall tissues (Tandon and Arya, 1979), flower gall of *Caligonum polygon sides* (Mathur, 2002). It is concluded that the biochemical changes, particularly the carbohydrates, play a vital role in induced leaf galls in *Cinnamomum verum Presl.* (*Syn.Cinnamomum zeylanicum*). While feeding on plant tissues, the insect and mites inject and secrete a chemical substance into the plant tissue that causes the plant to grow abnormally and produce galls. They remain in the gall as inquiline until the completion and maturity of their life stages and emerges from the exit cavities. The biochemical analysis revealed the changes happened in the plant tissue variably due to the secretion and limit the production of new tissues. In such foliar diseases, each of the critical processes governing carbon flow such as photosynthesis, respiration and translocation can be affected. Normal and gall tissues of *Cinnamomum verum Presl.* (*Syn. Cinnamomum zeylanicum*) were therefore isolated to study the extent of transformation.

CONCLUSION

It is concluded that the biochemical changes in the healthy plant of *Cinnamomum verum Presl.* (*Syn.Cinnamomum zeylanicum*), the carbohydrates, especially the total soluble sugars, reducing sugar, alpha-amylase and invertase activities are compared to the diseased tissue, play a vital role in the induced leaf galls of *Cinnamomum verum Presl.* (*Syn. Cinnamomum zeylanicum*) by unknown *Eriophyes sp.*

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Conflict of interest

No conflict of interest received from any group with the findings.

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