



QUANTITATIVE ESTIMATION OF SOME METABOLITES AND ENZYMES IN INSECT INDUCED LEAF GALLS OF *FICUS RELIGIOSA*

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ABSTRACT

This Paper reports the quantitative estimation of some metabolites and enzymes in insect induced leaf galls of *Ficus religiosa*. The parameters assayed were total soluble sugar, reducing sugar, starch a-amylase activity and invertase activity compared to normal tissues. Galls showed significantly higher content of total soluble sugar, reducing sugar, starch a-amylase activity and invertase enzymes activity and lower content of reducing sugar.

KEY WORDS: Total soluble sugar, Reducing sugar, Starch a-amylase activity, Invertase activity, *Ficus religiosa*.

INTRODUCTION

Ficus religiosa is found scattered in forests, where it propagates as an epiphyte on other trees specially widely found in uplands and plane area. The wood of fig trees is often soft and the latex precludes its use for many purposes. It was used to make mummy caskets in ancient Egypt. The species are mostly planted near Buddhist temples as it is referred to as sacred in India. Hindus associate the tree with fertility in women. It is also an important host to many insects. The present investigation is concerned with the leaf galls of *Ficus religiosa* induced by the Depteran, *Pipaldiplosis-pipaldiplosis*. Higher concentration of carbohydrates around gall cavity has been observed by several workers in *Prosopis* rachis gall (Arora and Patni, 2001), *Ficus* leaf gall (Singh, 2006).

MATERIALS AND METHODS

Normal and heavily galled *Ficus religiosa* leaf of equal size were collected from Sodala region or University campus of Rajasthan Jaipur and their biochemical study was done.

(1) Estimation of total soluble sugar

The amount of total soluble sugars was estimated by phenol sulphuric acid reagent method (Dubois *et al.*, 1951). 500 mg each of fresh normal and galled plant material was homogenized with 10.0 ml of 80 percent ethanol. Each sample was centrifuged at 2000 rpm for 20 aminutes. The supernatants were collected separately to 1.0 ml of alcoholic extract, 1.0 ml of 5% phenol was added and mixed. Then 5.0 ml of 96% sulphuric acid was added rapidly. Each tube was gently agitated during addition of sulphuric acid and then allowed to stand in water

bath at 26-30°C for 20 minutes. The OD of the characteristic yellow orange colour thus developed was measured at 490 nm in a spectrophotometer after setting for 100% transmission against the blank, standard curve was prepared by using known concentrations of glucose. The quantity of total sugar was expressed as mg/g fresh weight of tissue.

(2) Estimation of starch

Estimation of starch was carried out by the method of McCready *et al.* (1950). The residual mass obtained after the extraction of total soluble sugars of normal and gall plant material was suspended in 5.0 ml of distilled water and subsequently 6.5 ml of 52% perchloric acid was added to the residue after stirring of the mixture, the contents were centrifuged for 20 minutes at 2000 rpm. The supernatant was decanted and collected and the whole procedure was repeated thrice. Supernatant of each step were then poured and the total volume was made up to 100.0 ml with distilled water. The mixture was then filtered through Whatman filter paper (No.42). 1.0 ml aliquot of this filtrate was analyzed for starch content following the same procedure as that of total soluble sugars. 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 tissue.

(3) Estimation of reducing sugar

Estimation of reducing sugar was done by the method of Miller (1972). 500 mg plant material was treated with 10.0 ml of 80% ethyl alcohol. Sample was centrifuged at 2000 rpm for 20 minutes. 1.0 ml of extract was collected separately in test tube. To this 1.0 ml DNSA (3,5-dinitro salicylic acid) reagent was added. The mixture was heated for 5 minutes in boiling water bath. After the colour had developed, 1.0 ml of 40% sodium-Rochelle salt was added when the contents of the tubes were still warm. The tubes were cooled under running tap water. Absorbance was measured by spectrophotometer at 515 nm against the standard prepared from glucose. The quantity of reducing sugars was expressed as mg/g fresh weight of tissue.

(4) Estimation of Alpha amylase activity

Alpha amylase activity was determined by measuring the production of maltose and other reducing sugars from amylopectin of amylase using 3, 5-dinitrosalicylic acid (DNSA) colorimetric procedure of Bernfeld (1955). 200 mg of fresh weight of each tissue sample of normal and gall tissues were crushed in 4.0 ml of 0.02 M phosphate buffer (pH 6.9). The homogenate was centrifuged at 2500 rpm for 20 minutes. The supernatant was used to determine the enzyme activity. The reaction mixture consisted of 1.0 ml of enzyme extract and 1.0 ml of substrate solution (1.0 gm soluble starch dissolved in 100 ml of 0.02 M phosphate buffer, pH 6.9 containing 0.0067 M NaCl). The reaction mixture was then incubated at 30°C for 45 minutes and subsequently the reaction was stopped by adding 1.0 ml of DNSA reagent. The tubes were kept in boiling water bath for 15 minutes and then cooled by keeping under tap water immediately. 20 ml of distilled water was added to the cooled mixture. A yellow colour developed due to the unhydrolyzed starch. Optical density of this mixture was read at 560 nm against a zero min. blank. The activity was expressed in terms of mg starch hydrolysed per hour per g fresh weight of tissue.

(5) Estimation of Invertase activity

A modified method of Harris and Jaffcoat (1974) was used for the estimation of invertase activity. 500 mg each of normal and galled plant material was crushed in 5.0 ml of 0.2 M acetate buffer (pH 4.8). In a test tube 0.4 ml of 0.4 M sucrose was added. To the reaction mixture consisted of 0.4 M enzyme extract was added to make the volume 1.0 ml Control was prepared by adding sucrose solution to the test tube in which enzyme was inactivated by boiling for 5 minutes. After incubation at 30°C for 30 minutes, 1.0 ml of 3,5- DNSA reagent was added. Tubes were put in boiling water bath for 10 minutes and then contents were diluted to 10.0 ml by adding distilled water. Optical density was measured at 560 nm. Activity was expressed as mg sucrose hydrolyzed / hour/ g fresh weight of the tissue.

RESULTS

The results are presented in Fig. A-E. Total soluble sugar contents were more in leaf gall of *Ficus religiosa* (young, mature and old) as compared to normal counter parts. Mature gall tissues showed slightly higher amount of total soluble sugar contents than young and old galls (Fig. 1). Reducing sugar content was more in normal plant tissue as compared to galls. In gall, maximum reducing sugar was recorded in mature leaf galls of *Ficus religiosa* than young and old galls. It decreased slowly as the galls old (Fig. 2). Total starch contents were recorded more in young leaf galls as compared to their normal counter parts. It decreased in mature and old galls of *Ficus religiosa* (Fig. 3). Alpha amylase activity decreased in all the gall tissues as compared to their normal counter parts. In the gall tissues, maximum alpha amylase activity was found in young gall which decreased gradually with the age of the galls (Fig. 4). Invertase activity was recorded more in gall tissues as compared to their normal counter parts. Maximum activity was recorded in mature leaf gall of *Ficus religiosa* (Fig. 5).

DISCUSSION

The quantity of total soluble sugar was considerably high in gall tissue as compared to normal tissue. According to Mehrotra and Agarwal (2003), sugar has large numbers of stereo-isomer, because they contain several asymmetric carbon atoms (Lindhrost and Thisbe, 2003). Galls have often been described as physiological sinks. Increase in sugar contents in galls might be due to accumulation of these substances. The accumulation may involve the translocation of soluble sugars from the neighbouring healthy tissues to physiological sink. This view is supported by the findings of Shaw and Samborski (1956). High sugar contents in young and mature galls may be due to increased metabolic activity under stress which may in turn be responsible for additional synthesis of sugar. Similarly carbohydrates may also accumulate by depletion of starch due to the activated alpha-amylase activity and other enzymes, Garg and Mandhar (1975), Shekhawat (1980) and Purohit (1980) also reported increased activity of alpha amylase along with increased sugar contents.

Figure 1
Estimation of total soluble sugar

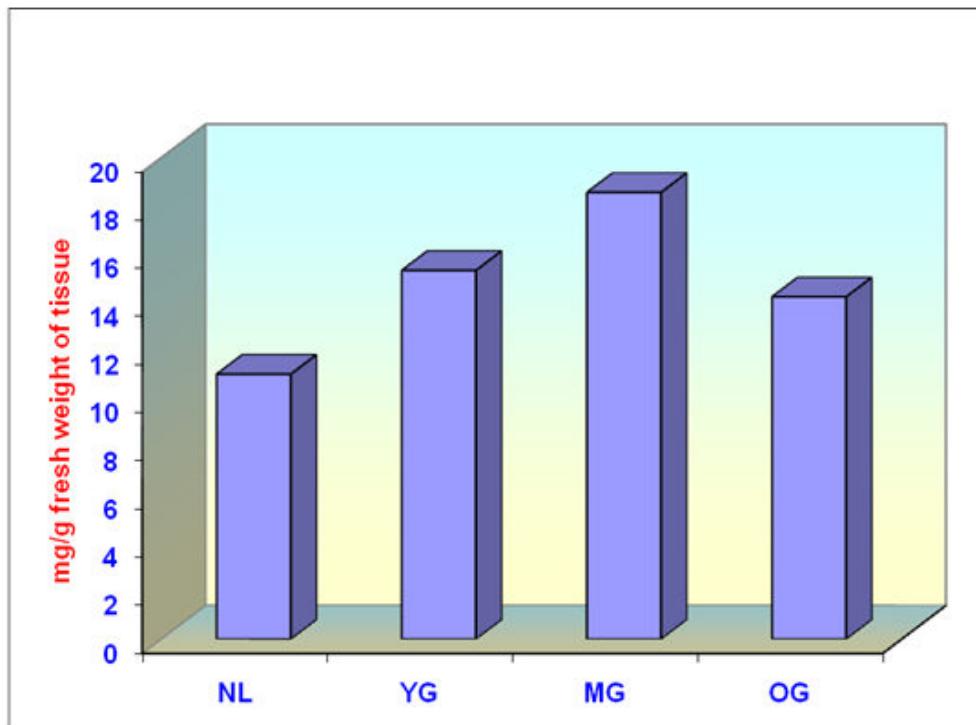


Figure 2
Estimation of reducing sugar

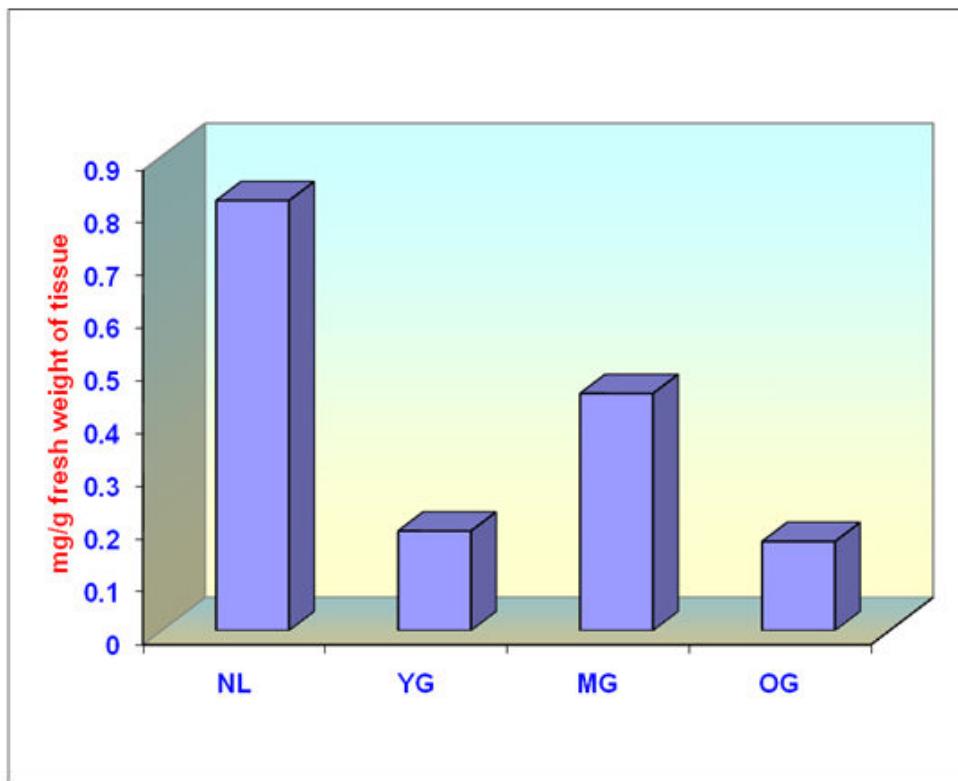


Figure 3
Estimation of total starch

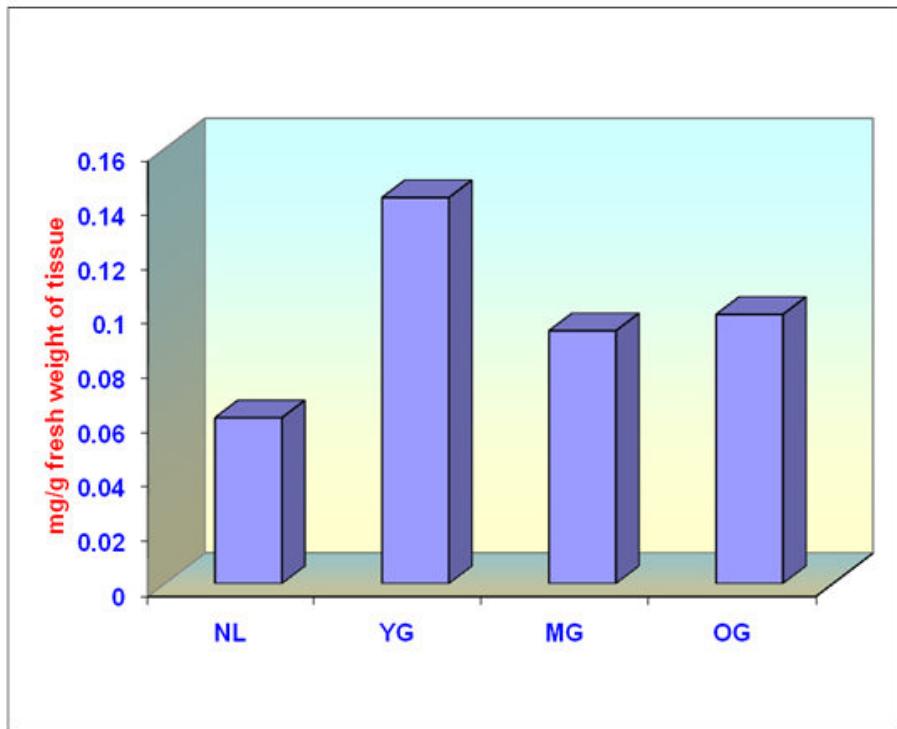


Figure 4
Estimation of alpha-amylase activity

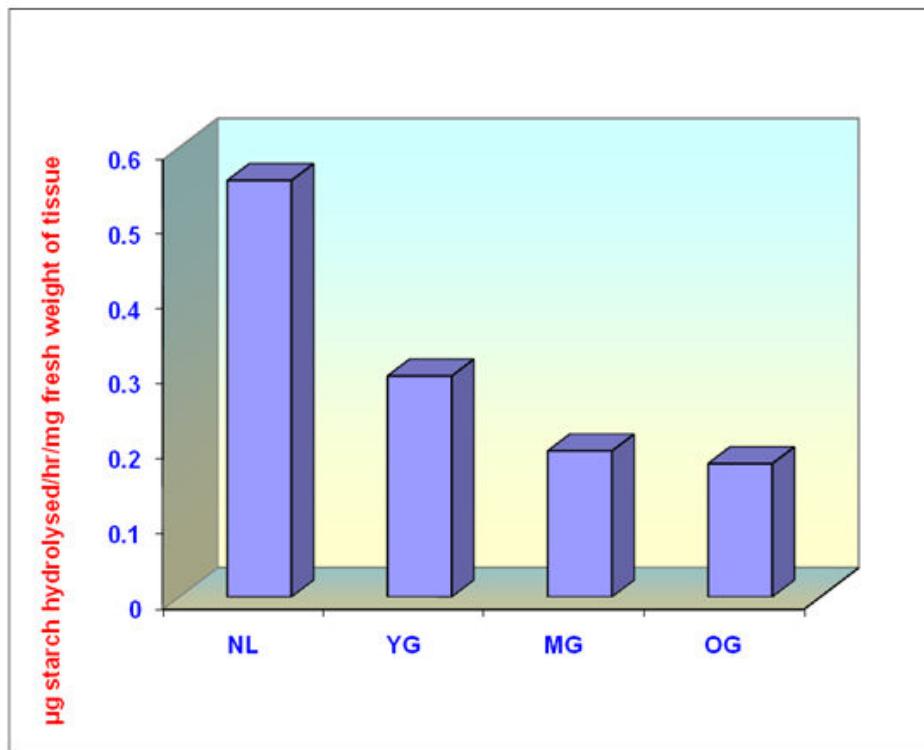
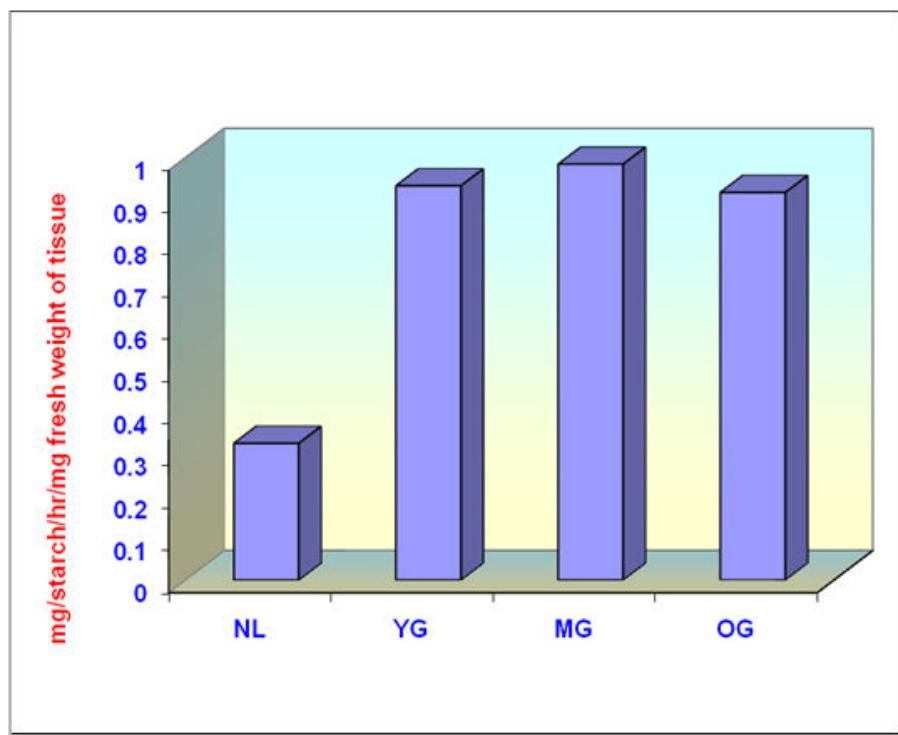


Figure 5
Estimation of invertase activity



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ABBREVIATIONS

NL = Normal Leaf, YG = Young Gall, MG = Mature Gall, OG = Old Gall

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