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Biochemical responses of *Trioza jambolanae* infected *Syzygium jambos* leaves from Kota District, Rajasthan

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ABSTRACT

Galls are specialized plant structures formed by the alteration of normal plant tissues by the galling organisms. Generally galls provide a food source to the insect for their growth and development. Present study elaborates the biochemical changes in leaf galls of *Syzygium jambos* due to the infection of *Trioza jambolanae.* The amount of total chlorophyll, carbohydrate, protein, phenol, amino acids and proline were compared in healthy and galled leaf extracts. Nutritive tissue, on which the insect feeds, contained high amount of phenol and proline whereas low amount of total chlorophyll, carbohydrate, protein and amino acids compared with healthy leaves. Our results indicate that distribution of biochemical defences in this psyllid gall differs significantly from the leaf tissue from which it is formed. This study suggests that the gall is manipulated by the insect to enhance its food and protective value.

Keywords: Galls, Biochemicals, Nutritive tissue, Psyllids, Defence.

INTRODUCTION

Plant diseases adversely affect the quality of plant by inducing higher level of undesirable constituents in them. Plants may alter their biochemistry to tolerate a particular stress that occurs infrequently, but the metabolic cost is high for such temporary adjustments. Diseases, which are generally detrimental to plant growth, adversely affect metabolism of plant and cause important modifications in them. Such modifications may lead to accumulation or depletion of certain metabolites resulting in an imbalance in the levels of certain metabolites. Galls are the typical plant growths induced by host-specific organisms in which the insect provides the stimulus and the plant initiates the growth response against the galling organism like bacteria, mites and insects.

Syzigium jambos (Family: Myrtaceae) is a plant cultivated in India and propagated by seeds. In Kota district, Rajasthan (India) the gall forming psyllid, *Trioza jambolanae* attacks the leaves of most of the *Syzigium jambos* trees in late April indicating large galls on leaf lamina and at the time of the formation of fruits, this psyllid also attacks on fruits which interfere with the biochemical processes of healthy plant. Changes in normal physiological condition of plant due to disease affect the yield of plant. Therefore the present investigation was carried out to study the effect of *Trioza jambolanae* on the photosynthetic efficiency of *Syzygium jambos* and to evaluate the effect of the psyllid on the quantity of various metabolites like carbohydrate, protein, phenol

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and amino acids Quantitative estimation was made and compared in both healthy and galled leaf tissues.

MATERIAL AND METHODS

All the biochemical experiments were conducted in the laboratory of College. Effect of gall disease caused by *Trioza jambolanae* on *Syzygium jambos* was estimated in terms of biochemical estimations.

Collection of Plant Material

For biochemical studies both Healthy and Galled leaf samples of 15 days, 30 days and 45 days old were collected and surface sterilized with 2% NaOCl (Sodium hypochlorite) for 2 minutes.

Biochemical Estimations

Healthy and gall infected leaves were collected and rinsed in distilled water for 10 min to remove dust and surface debris. They were air-dried by spreading on Whatman (# 1) filter paper for 25-30 min. Each gall sample was trimmed carefully with a sharp razor to avoid any loss of galled tissues. Inhabiting nymphal instars were extracted by slitting the galls partially. Fresh leaf extracts were used for biochemical estimations. Mean values of 3 replicates for every biochemical assay were calculated both for healthy control and galled tissues.

Estimation of total chlorophyll (Arnon, 1949):

One gram of each healthy and insect infected *Syzygium* fresh leaves of 15 days, 30 days and 45 days old were taken crushed and homogenized separately with 80 % acetone and with a pinch of $CaCO_3$ powder in a mortar and pestle. From each clear supernatant was decanted in separate test tubes and residue from both was subjected to grinding repeatedly with acetone, until it became colourless. Acetone extracts of both, the healthy and galled leaves of 15 days, 30 days and 45 days old were centrifuged separately at 3000 rpm for 10 minutes. The supernatants were decanted and the optical density was measured at 663 nm, 652 nm and 645 nm on spectrophotometer. Total chlorophyll contents of both healthy and infected plants were determined by using following formula:-

Total chlorophyll mg/liter = (20.2 X OD 645) + (8.02 X OD 663) X V / a X 1000 X w
Here-
a=length of light path in spectrophotometer (1.0 cm.)
w= weight of plant material
V=volume of extract (supernatant)

Estimation of Total Carbohydrate (Scott, 1960):

100 mg glucose was dissolved in 100 ml distill water for the preparation of standard glucose solution, then 10 ml of the stock solution was diluted in 100 ml distill water for working standard. Standard curve was made by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard ('0' serves as blank). In all the test tubes volume was made 1 ml by adding distilled water. Then 4 ml. of Anthrone reagent was added and heated for 8 minutes in a boiling water bath. It was cooled rapidly and reading was taken at 630 nm when the colour became green to dark green. A standard graph was made by plotting different concentrations of the standard on the X-axis versus absorbance on the Y-axis. From the standard curve the amount of carbohydrate present in the test solutions was calculated by using following formula.
Amount of Carbohydrate present in 100 mg. of the sample= Total carbohydrate mg/100 g =
Mg of glucose X 100/Volume of test sample

For test samples preparation one gram each of healthy and galled plant samples (15, 30, 45 days old) were taken separately. Each sample was extracted into 80% ethanol and after extraction the ethanol was evaporated by taking the beakers on boiling water bath. When all the ethanol is evaporated, 5 ml. of 2.5 N HCl was added to each beaker and again putted on boiling water bath for 1 hour after that it was cooled at room temperature. After cooling it was neutralized with solid Na_2CO_3 until the effervescences ceased. Then volume was made up to 50 ml and centrifuged at 2000 rpm for 10 minutes. Supernatant was collected and from this 1 ml was taken for analysis.

Estimation of total plant protein**-** (by Lowery Method, 1951) :

15, 30 and 45 days old healthy and galled *Syzygium* fresh leaf parts were taken as samples. 1gm each of the samples materials were homogenized with prechilled distil water in mortar and pestle separately and properly. Crushed samples were centrifuged at 5000 rpm for 15 minutes separately. 2 ml. of freshly prepared alkaline $CuSO_4$ reagent was added. Solutions were mixed well and incubated at room temperature for 10 min. After incubation 0.2 ml. of freshly prepared Folin-ciocalteau reagent was added in to the reaction mixtures. The prepared mixtures were incubated at room temperature for 30 minutes. After that optical density was measured at 750 nm by spectrophotometer. Reaction mixture without sample was considered as blank. Amount of protein was calculated from standard curve prepared with albumin.

Estimation of Amino Acid**-** (Moore and Stein, 1948):

For test samples preparation leafy parts of both healthy and infected plants (15, 30 and 45 days old) were taken as sample. 1 gm. of the each sample was crushed in 5 ml. of 80% ethanol. It was then boiled on water bath to extract all the content in solution.

Extract was cooled and centrifuged at 5000 rpm for 10 minutes. 1 ml. of supernatants was taken for further procedure. To the sample a drop of methyl red indicator was added and then neutralized with 0.1N NaOH. After neutralization 1 ml. of freshly prepared ninhydrin reagent was added. Solution was mixed well and placed in a covered conical flask. It was then boiled in a water bath for 20 minutes till purple color became stable. Then OD was taken at 570 nm in spectrophotometer. Amino acid content was calculated from, the standard curve prepared with Glycine.

Estimation of Proline- (Bates et al 1973):

Similarly for the estimations of proline contents in both healthy and galled leafy parts of 15, 30 and 45 days *Syzygium* plant samples. 1 gm. fresh parts of each were crushed separately in 10 ml. 3% sulpho-salysilic acid then centrifuged at 2000 rpm for 10 minutes and clear supernatants were used. To the 2 ml. of the leaf extracts, 2 ml. of glacial acetic acid and 2 ml. of freshly prepared acid ninhydrin were added. Contents were mixed well and heated in boiling water bath at 100°C for 1 hr. Brick red color was developed. After cooling, 4 ml. of toluene was added. Contents were stirred well. A toluene layer gets separated. Its OD was taken at 520 nm in spectrophotometer. All the reaction mixture except the plant sample was taken as blank. Calculation was done using standard curve prepared with D-proline. Calculations were done by using following formula:

µ moles per gram tissue =

µg proline/ml × ml toluene × 5/115.5 gm sample

115.5 is the molecular weight of proline.

Estimation of total phenol (Bray and Thorpe, 1954):

For the estimation of total phenol contents in both healthy and galled (15, 30 and 45 days old) *Syzygium* leaf were taken. 1 gm. leaf samples of each were homogenized separately with 10 ml. of 80% alcohol in a mortar and pestle. Samples were centrifuged at 2000 rpm for 10 minutes. The supernatant were collected and used for total phenol estimation. To the 1 ml. extract of each Folin-ciocalteau reagent (1 ml.) was added followed by 2 ml. of Na_2CO_3. The reaction tubes were shaken vigorously and heated for 1 min. in water bath and cooled under running tap water. The reaction mixture was diluted up to 25 ml. in distil water. Absorbance was recorded at 650 nm in spectrophotometer. Phenol content in the samples was calculated from standard curve prepared with catechol.

Enzyme Activities:

Indole acetic acid oxidase (IAA oxidase) activity**.** (Gordon and Weber, 1951):

The activity of this enzyme was determined by using Salkowski reagent for colorimetric estimation of IAA.

Poly Phenole Oxidase (PPO) Activity **(**Mayer et al 1965):

The activity of the enzyme was expressed as change in optical density at 495 nm/min/g protein.

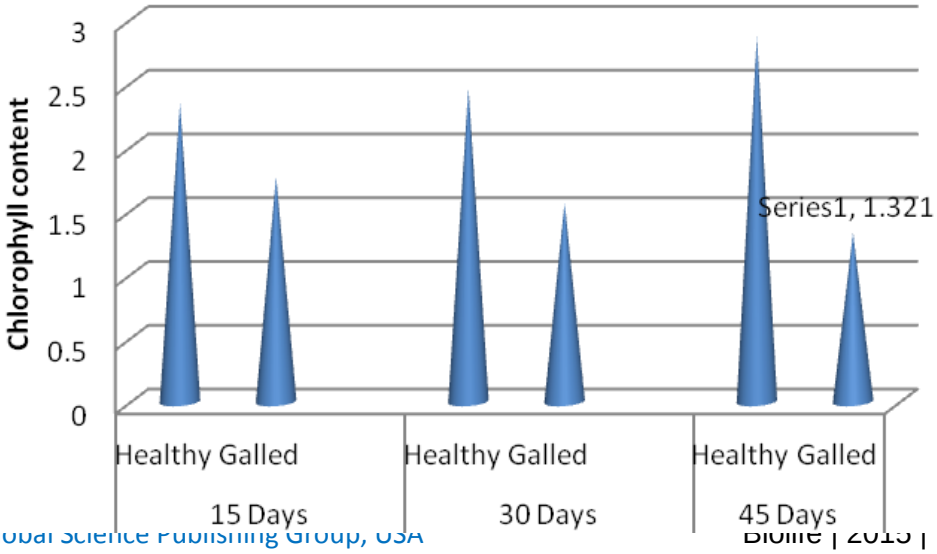
OBSERVATION

Data presented in Table-14 depict comparative quantities of total Chlorophyll, proteins, sugars, amino acids, phenols, and prolines in healthy and *Trioza jambolanae* induced galled leaf tissues at 15, 30 and 45 days of cecidogenesis.

RESULTS & DISCUSSION

The present paper discuss that reduction in photosynthetic activity is the most conspicuous effect

Figure-1. Comparison of TOTAL CHLOROPHYLL content in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages.



of disease. The diseases impose impact upon whole plant physiology including decrease in photosynthesis and increase in respiration, impair growth, interfere in biochemical processes and reduce yield. Plant diseases adversely affect quality of plant by inducing higher level of undesirable constituents in plants (Isawa 1985) it indicates that plant health condition plays a key role in pest management (Zhan Yu Liu *et al* 2007).

Chlorophyll Content:

The data extracted on chlorophyll content indicated that the amount of total chlorophyll was higher in healthy plants over the *Trioza jambolanae* infected plants. In the present study, total chlorophyll contents had been found to be decreasing from 15 to 30 and 45 days old gall tissues, as a result of insect infestation followed by the insect development. The fall in total chlorophyll was from 24.47 percent to 53.89 percent in infected plant over the healthy ones (Figure-1).

In the *Trioza jambolanae* infected *Syzygium* plant more reduction in chlorophyll content may be due to destruction of chloroplast containing leaf and decreased efficiency of chloroplast in carrying Hill reaction. These changes may be partially or completely accounted by reduction in chlorophyll content. These results match with the findings of many workers such as Miles (1968),Uritani (1976) and Purohit et al.(1979) who studied the degradation of chlorophylls compounds in aphid induced galls. Chloroplasts from infected leaves were less efficient in carrying out the Hill reaction than chloroplasts from healthy leaves.

Carbohydrates:

Carbohydrates are synthesized by chloroplasts in the green parts of the plants through the process of photosynthesis. Carbohydrates are the major constituents of nutrition because the entire energy for cellular activity is derived from their breakdown. Carbohydrates are the precursors and the basic molecules for synthesis of phenols, phytoalexins, lignin, callose and frame a skeleton for the synthesis of nucleic acid (Vidyasekaran 2002).

In the present investigation decrease in carbohydrate content of *Syzygium* plant was evident in infected condition over healthy situation. The percent reduction in carbohydrate content of infected *Syzygium* plant over healthy plant ranged from 29.18% to 89.38% at 15, 30 and 45 days of gall and insect development (Figure-2). As Carbohydrates are the precursors for synthesis of different bio molecules. Hence, they play an important role in the defence mechanism of plants. Horsfall and Dimond (1957) assigned a major role for sugars in disease resistance.

Oxidation of various metabolites mainly carbohydrates was also observed by Allen (1942) with increasing infection intensity. In the present study sugar levels were also found to be decreasing with the advent of disease development and this decline was directly proportional to the infection intensity.

Protein:

In the present investigation, amount of protein content was higher in healthy leaves than in the galled leaves of *Syzygium* at all the growth stages. The decrease in protein content ranged from 48.90% to 57.53% in infected plant over healthy plant.

The protein biosynthesis of the host is widely assumed to be significant feature of pathogenesis. Ananthakrishnan (2001) suggested that various plant

Figure-2. Comparison of CARBOHYDRATE content in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages.

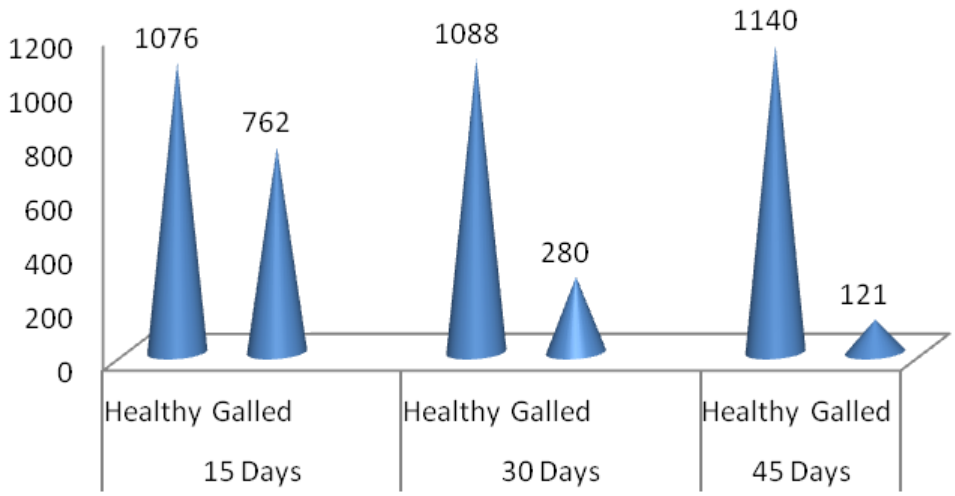


Figure-3. Comparison of PROTEIN content in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages

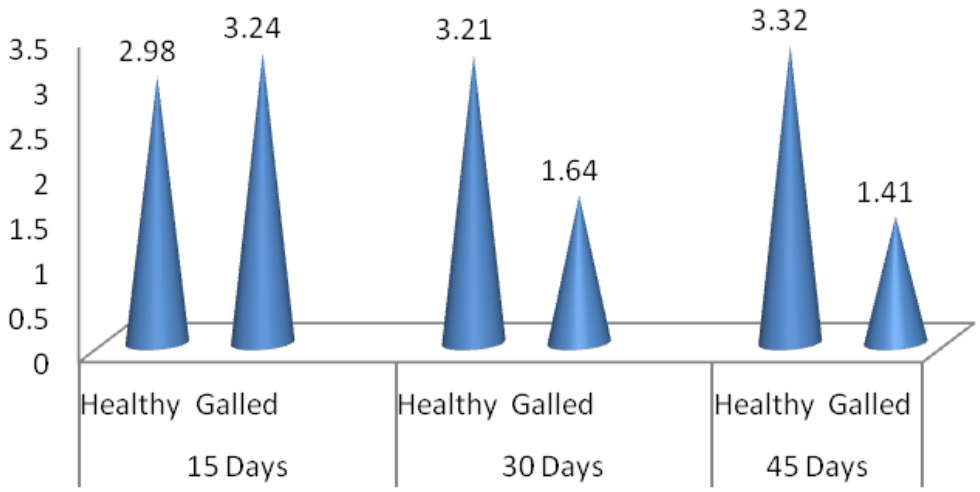
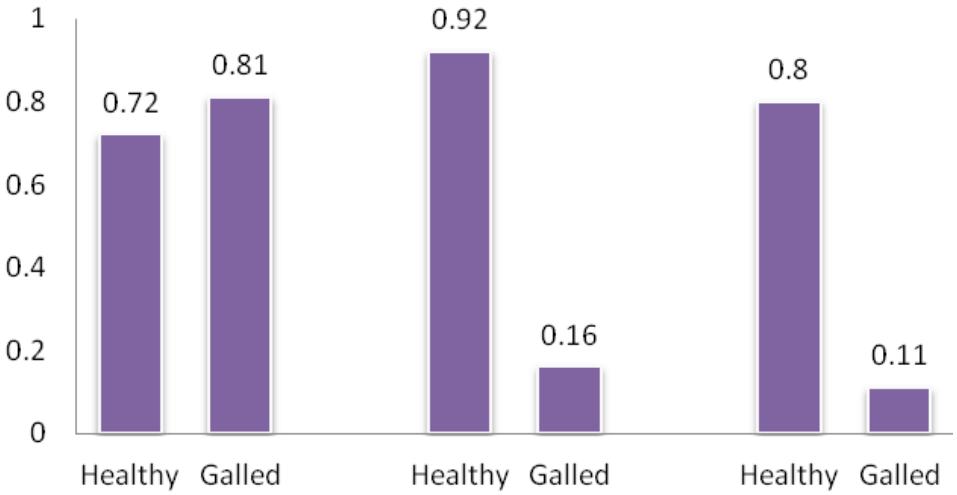


Figure-4. Comparison of AMINO ACID content in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages



proteins are synthesized by infected plant for defence. So when the plants are attacked by the insects the plant cells generate signals and one of these signals is the inhibition of the expression of certain polypeptides that may be useful in providing the basis for new crop protection strategies. It is believed that synthesis of diverse plant proteins is important for defence. In the present study initially there was 8.72% increase in Protein content in 15 days old young galls as compared to healthy, after that remarkable decline in protein content in 30 and 45 days old galled tissues was observed (Figure-3). These results are in accordance with the findings of Anders (1958) who found that the, saliva of aphid contains proteolytic enzymes like protease and peptidase which degrade proteins into amino acids. In the present study same may be the cause of decrease in Protein content at the time of the maturity of the galls for providing nutrition to the insect.

Amino Acid:

Amino acids are of great biological importance since they play an important role as growth promoting factors. Initially there was 12.5 percent increase in the amount of amino acids in galled tissues as compared to healthy leaf tissues, which showed marked decline of 82.60 to 86.41 % at the maturity of the galls and development of the *Trioza jambolanae* insect (Figure-4).

The increase in the quantity of amino acid initially in the gall tissues may be due to break down of proteins into utilizable units by the protease enzyme secreted by the salivary glands of the insect. Similar findings were also given by Miles and Lloyd (1967) and Miles (1968). The infection caused a breakdown of plant proteins and release of amino acid to react with endogenic phenolic acid and produce IAA thus causing hypertrophy. During the later stages of

cecidogenesis deficiency of amino acids may be due to their utilization by the developing insect. Similar possibilities were given by Anders (1958).

Proline:

Proline is an amino acid, which is though not involved in the synthesis of protein but reflects its importance at the time of stress. It acts as a cytoplasmic osmoticum and plays an important role in osmoregulation when the plants are subjected to stress condition (Moftah and Michel, 1987). While the Proline metabolism has been widely studied in response to abiotic stresses (Hare and Cress, 1997; Verbruggen and Hermans, 2008; Szabados and Savoure, 2010), few investigations have characterized it under conditions of pathogen attack.

Diseases in plants are considered as an important biotic stress. The results of present investigation revealed that there was an increase in proline content in the galled leaves of *Syzygium* as compared to ungalled healthy leaves at all the growth stages and this trend was followed up to maturity of the gall. Percent increase in the proline content in diseased *Syzygium* plant over healthy was reported to be ranging between 172 % to 307.22 % (Figure-5). The increased level of proline in galled leaves might confirm the idea that galling has stressful effect on the infested leaves of *Syzygium*. Gibon *et al* (2000) also suggested Proline accumulation in response to stress. They also suggested that proline plays a role in stress adaptation within the cell.

Phenol:

Among all the biochemical components of different hosts, phenol stands out as the most important component in imparting resistance to several plant diseases. Higher concentration causes an instant lethal action by a general tanning effect, while low concentration causes gradual effect on the

Figure-5. Comparison of PROLINE content in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages

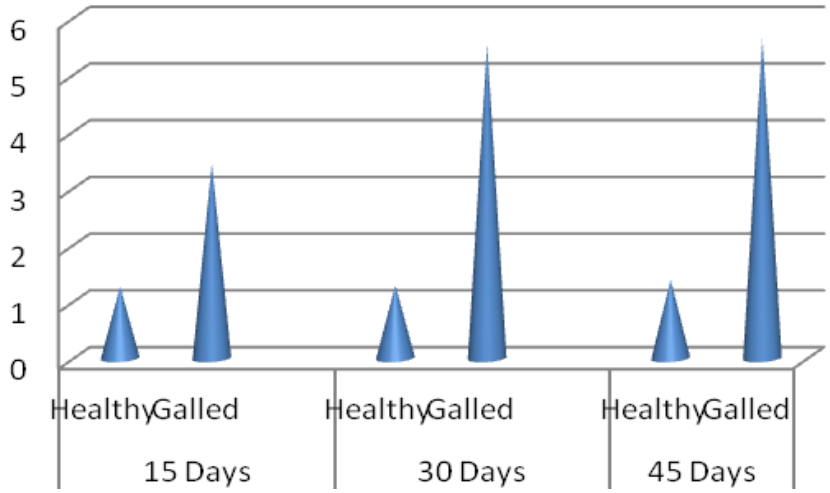


Figure-6. Comparision of PHENOL content in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages

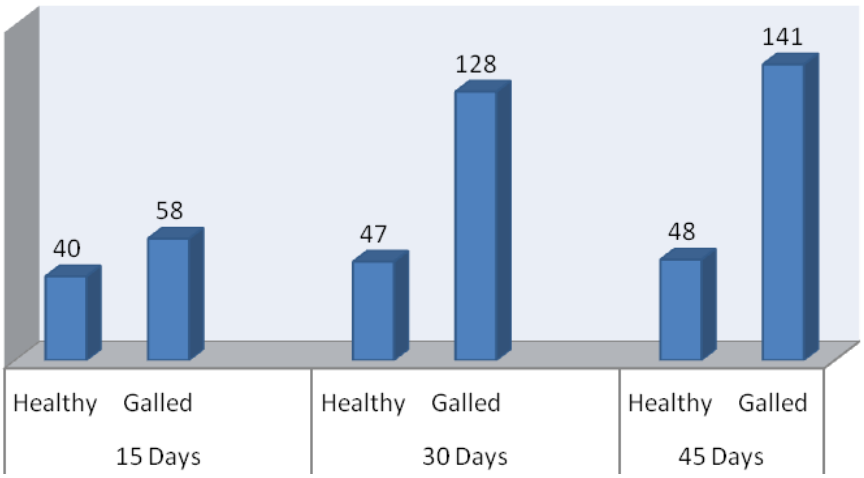


Figure-7. Comparison of IAA OXIDASE ACTIVITY in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages

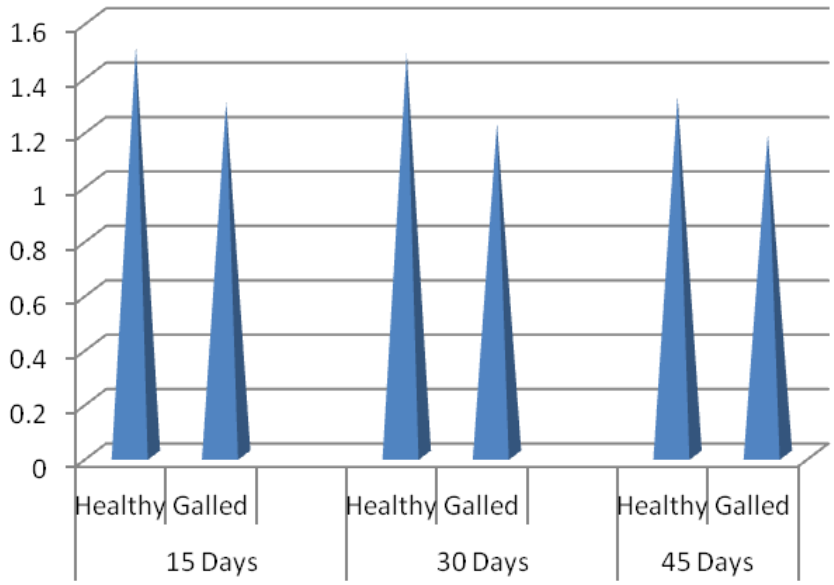
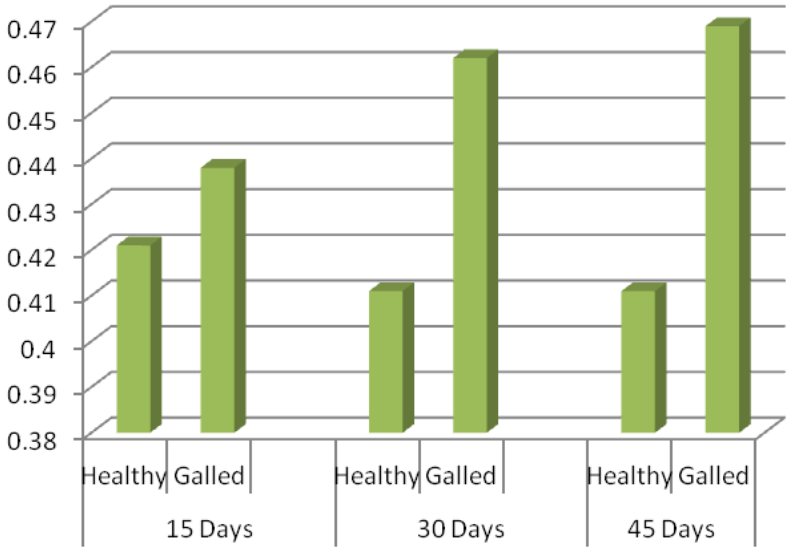


Figure-8. Comparison of PPOxidase ACTIVITY in the leaves of Healthy and Galled *Syzygium jambos* at different growth stages



cellular constituents of the parasite (Dasgupta, 1988).

In the present study the quantity of phenol in diseased plant was significantly higher than that of healthy plant at all the 3 developmental stages. The increase in phenolic content was ranging from 45 percent to 193.75 percent in the infected plants as compared to the healthy plants at all the growth stages (Figure-6). The increase in total phenol contents was probably because of the activity of peroxidase and polyphenol oxidase enzymes. The increase in phenols in the diseased tissues revealed that they play an important role in inducing resistance against further invasion of the pathogen as they are toxic to pathogens. Many experiments have indicated that there is a correlation between the degree of resistance and the phenol level in healthy plants (Mehrotra, 1980).

A marked accumulation is shown in mature galls which help in gall formation. Accumulation of phenols at increased level might be the tendency of the host to isolate the pathogen at original site of infection (Legrand, 1983; Ride, 1983).

IAA oxidase and PPO activity:

As shown in Figure-7 & 8, there is a marked difference in IAA oxidase and PPO activity between the ungalled, and galled tissue. The IAA oxidase activity decreased to 13.83% while PPO activity

decreased up to 4.03 percent to 14.11 percent in infected plant over the healthy one, with mean 10.18 percent decrease. This may be one of the reasons for the increased level of auxin concentration in the same sample.

CONCLUSION

Changes occurred in the *Syzygium* plant due to *Trioza jambolanae,* interfered in the growth progression of plant and accounted for many alterations in the biochemical alterations in the physiological and biochemical status of the infected plant. Amount of polysaccharide, starch, lipid and phenols were more near the gall cavity. Comparative studies were made on Chlorophyll contents, Carbohydrate, Protein, Amino acids, Proline and Phenol in galled plant tissues as compared to healthy plants. There was quantitative decrease in Chlorophyll, Carbohydrate, Protein, and Amino acids whereas the amount of Proline and Phenol were higher in infected tissues. The IAA oxidase and PP oxidase activity decreases in infected tissues over the healthy ones. It gives an idea about the nature and defence mechanism of the host plant against *T. jambolanae*.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. **Allen P J (1942).** Changes in the metabolism of wheat leaves induced by infection with powdery mildew. Am.y. Bot., 29, 425.
2. **Ananthakrishnan T N (2001).** Phytochemical defence profiles in insect-plant interactions. *In: Insects and Plant Defence.* Pp: 1-21. Dynamics Sci. Pub., USA.
3. **Anders F (1958).** Amino sauren als gallener stofeeder *Reblaus* (Vi tens). *Experientia* **14:**62-68.
4. **Arnon D I (1949).** Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris. Plant Physiol.* 24: 1-15.
5. **Bates L-S, Waldren R P and Teare I D (1973)**. Rapid determination of free proline for water stress studies, Plant and soil, 39, 205-208.
6. **Bray H G and Thorpe W V (1954).** Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. **1:**27-52.
7. **Dasgupta M K (1988).** Principles of Plant Pathology, Published by Allied Publishers Private Limited, pp. 470-500.
8. **Gibon Y, R Sulpice and F Larher (2000).** Proline accumulation in conola leaf discs subjected to osmotic stress is related to loss of chlorophylls and to the decrease of mitochondrial activity. *Physiol. Plant.,* 110: 469-76.
9. **Hare PD, Cress WA (1997).** Metabolic implications of stress- induced proline accumulation in plants. Plant Growth Regulation 21, 79–102.
10. **Horsfall J and Dimond A (1957).** Interactions of tissue sugar, growth substances and disease susceptibility. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, vol. 64, no. p. 415-421.
11. **Isawa K (1985).** Deterioration in the chemical composition and nutritive value of forage-crops caused by foliar diseases. VIII. Chemical composition and nutritive value of grass and legume leaves artificially killed by physical or chemical treatments. Bull. Natl. Grassland Res. Ins. 31: 73-80.
12. **Legrand M (1983).** Phenylpropanoid metabolism and its regulation in disease. Pages 367-384. *In:* Biochemical Plant Pathology. J. A. Callow, ed. John Wiley & Sons, Chichester, UK.
13. **Lowry O R, Rosebrough R J, Farr A L and Randall R J (1951).** Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* **193:**265-275.
14. **Mehrotra R S and Agarwal A (2003)**. Plant Pathology 2nd edi. Tata Mc Graw Hill Publishing com. Ltd.
15. **Miles P W (1968).** Insect secretions in plants. *Ann. Rev. Phytopath.* **6:**136-164.
16. **Miles P W and Lloyd J (1967).** Synthesis of a plant hormone by the salivary apparatus of plant sucking bugs. *Nature* (London). **203:** 801-802.
17. **Moftah A E and Michel B E (1987)**. The effect of sodium chloride on solute potential and protein accumulation in soybean leaves. *Plant Physiol,* 83, 238-240.
18. **Moore S and Stein W H (1948)**. Photometric ninhydrin method for use in chromatography of amino acids. Journal of Biological Chemistry. 176: 367-386.
19. **Purohit S D, Ramawat K G and Arya H C (1979).** Phenolics, peroxidise and phenolase as related to gall formation in some arid zone plants. *Curr. Sci.* 148: 714-716.
20. **Ride J P (1983).** Structural barriers in defence. Pages 215-236 in: Biochemical Plant Pathology. J. A. Callow, ed. John Wiley & Sons, Chichester, UK.
21. **Scott F G (1960).** Effect of super optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus* L. *Plant Physiol.* 35: 653-668.
22. **Szabados L, Savouré A (2010).** Proline: a multifunctional amino acid. Trends Plant Sci 15: 89–97.
23. **Uritani I (1976).** Protein metabolism. In: *Encyclopedia of Plant Physiol.* (New Series). R. HEIT-Fuss and P. H. WILLIAMS (Eds.). Springer-Verlag, Heidelberg and New Y or k. **4:**509-521.
24. **Verbruggen N, Hermans C (2008)**. Proline accumulation in plants: a review.Amino Acids **35**: 753–759.
25. **Vidhyasekaran P (2002).** Bacterial disease resistance in plants: molecular biology and biotechnological applications. Binghamton, NY: Haworth Press.
26. **Zhan-Yu-Liu, Huang J, Shi J, Tao R, Zhou W and Zhang L (2007).** Characterizing and estimating rice brown spot disease severity using stepwise regression, principal component regression and partial least-square regression. *J. Zhejang Univ Sci B,* 8(10): 738-744.

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