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Studies on the foliar biochemical changes in the Gall (*Trioza fletcheri minor*) infested tasar food plants *Terminalia arjuna* and *Terminalia tomentosa*

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Abstract
Food plants of tropical tasar silkworm infested by many pests causing huge foliage loss of about 15 % and upto 90% in heavy infestation. Amongst, *Trioza fletcheri minor* is most important insect pest commonly causing leaf gall on the primary tasar food plants. In the present study, the impact of gall infestation on the vital biochemical composition of the leaf in two primary food plants such as *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan) was analysed and compared. Leaves samples were collected and categorized into gall infested leaf and uninfested (healthy) leaf. Further, biochemical changes were recorded consequent to severity of infestation. Lower level of moisture in the infested leaf was observed on contrary, there was an increase in the protein concentration upto 26% compared to the ungalled leaves. Similarly, higher sugars and phenols were recorded as consequence of gall infestation. Significant increase of proline in the infested leaf can be used as an indicator of stress.

Keywords: leaf gall infestation, *Trioza fletcheri minor*, *Terminalia arjuna*, *Terminalia tomentosa*, biochemical profile

1. Introduction
Infestation of Gall by the insect *Trioza fletcheri minor* in the tropical tasar silkworm food plants such as *Terminalia arjuna* (Arjun) and *T. tomentosa* (Asan) is very common and causing significant damage to the foliage this in turn impact on the total tasar cocoon production [1]. Unlike a majority of gall-inducing psylloids that are generally host and site specific [2], *T. fletcheri minor* is known to form the leaf galls in many species of genus *Terminalia* [3]. The gall insect activates a perturbation in growth mechanisms and alters the differentiation processes in the host plant, modifying the physio-chemical activity in turn the plant architecture to its advantage [4]. The gall inducers generally show a high level of specificity to their host plants and to specific plant organs [5, 6]. The phytophagous insects are known to extract nutrients from the phloem, xylem or non-conducting plant cell [7]. Growth of gall tissues is associated with the changes in the levels of their cellular contents such as sugars, proteins and other secondary chemicals [8]. Several studies have analysed the mechanisms of manipulation and alteration of host plant traits by galling insects [9, 10].Quality of leaf plays a major role among the various factors influencing silkworm growth. Moisture content of the leaves has a direct effect on growth and development of silkworm by favouring the ingestion, digestion and assimilation of leaf nutrients. Leaves containing more water, total sugar and soluble carbohydrate and less mineral are best relished by silkworms [11, 12]. Nutritive requirement of silkworm larvae vary with the maturity of leaves fed. Young age silkworms required leaves of high moisture content as it is easy to digest and late age silkworms required mature leaves with less moisture content as late age silkworms have the strength to digest mature leaves. On the other hand too much mature leaves do not contain sufficient biochemical contents and moisture content is not suitable to feed silkworms [13]. Keeping in view, in the present study the impact of gall infestation on the biochemical constituents in the leaves of primary food plants of tasar silkworm *T. arjuna* (Arjun) and *T. tomentosa* (Asan) were analyzed and compared.

2. Materials and Methods
2.1. Collection of Leaf sample: Leaf samples for the study was collected from the *T. arjuna* and *T. tomentosa* plants maintained at CTR&TI, field laboratory during June-August 2014. Simple visual scoring method was adopted for observation of gall infestation in tasar host plants. Leaves samples were categorized into gall infested leaf and ungalled (healthy) leaf

further based on the number of galls on the abaxial surface of the leaves separated into 5 gall, 15 gall and 28 gall in both the host plant leaves. A control ungalled leaves were also kept for the comparison.

2.2. Analysis of leaf moisture content: Leaf moisture content was determined on fresh weight basis [14]. For each maturity, 25 leaves /replicate were harvested separately from a longest shoot and leaves were wiped with a muslin cloth to remove dust particles and fresh weight was recorded immediately. Then leaves were kept in normal environmental conditions (26±1°C temperature; 70±5% relative humidity) for 6 hours. Then leaves were dried in hot air oven at 80 °C for 48hours till constant weight was attained and dry weight was recorded. Average leaf moisture content of leaves was calculated by using following formula and expressed in percentage (%).

$$\text{Leaf moisture content (\%)} = \frac{\text{Fresh weight of leaves - Dry weight of leaves}}{\text{Fresh weight of leaves}} \times 100$$

2.3. Quantitative analysis of total chlorophyll: Total chlorophyll content was estimated following the method mentioned by [15] in fresh leaves infested with gall and ungalled leaves. One gram of fresh leaf was homogenized with 10 ml of acetone. Centrifuged the homogenate at 3000 rpm for 10 min. Supernatant was collected in a separate tube and repeated the tissue extraction with acetone until the extract was free from pigment. Optical density was measured at 645 and 663 nm and the total chlorophyll was calculated.

2.4. Biochemical analysis of the leaf: Important biochemicals such as proteins, total sugars, phenols and proline in the leaves were analysed using appropriate standard protocol as follows.

Total Proteins were measured following the method of [16] using Folin-phenol reagent. The leaf protein was extracted using 80% ethanol in dry leaf powder and further precipitated using 10% trichloro acetic acid (TCA) as mentioned by [12]. Quantitative estimation was done using copper reagent and total protein concentration was calculated by preparing standard curve with bovine serum albumin (BSA).

Total Sugars was extracted initially with the hot ethanol in the dry leaf powder and quantitative estimation was carried out using methodology [15]. The OD was measured at 625 nm then calculated the total sugars comparing the glucose standard.

Total Phenols - The phenolic content in the leaf sample was extracted using 80% ethanol as extracting solvent [17]. Estimated using reaction mixture contains 0.1ml of extracted samples and its volume was made up to 3ml with double distilled water in a clean test tube. 0.5ml of FCR was added. Then after 2 min, 1ml of 20% Na_2CO_3 (sodium carbonate) was added and mixed thoroughly. Then the mixture was kept in a boiling water bath for about 5min and cooled in running tap water. The absorbance was taken against blank at 650nm. The total phenolic content was expressed in mg/g using a standard curve prepared from catechol (mg/ml).

Proline- Leaf proline was extracted in the fresh leaf homogenate with 3% Sulpho salicylic acid and the extract obtained was used for quantitative estimation by reaction with ninhydrin in acidic conditions (pH 1.0) to form the chromophore (red color) and optical density was measured at 520 nm. The proline content was calculated comparing with the standard proline solution [18].

2.5. Statistical analysis: The data collected was subjected to statistical analysis and their mean values were calculated. One-way ANOVA was used to test the significance of differences between the mean values of independent observations. Comparisons were performed with DMRT using SPSS 10.

3. Results

The gall infestation by T. *fletcheri minor* observed in the *T. arjuna* and *T. tomentosa* has impacted significantly on the biochemical as well as on moisture and chlorophyll content of the leaves. The observations made in the gall infested and ungalled leaves are represented as below.

3.1. Moisture content of the leaf: The moisture content recorded in the leaf reduced corresponding to the severity of infestation. Leaf with higher number of leaf gall (28 gall) recorded 72.46±2.30% in *T. tomentosa* and 65.57±0.96% in *T. arjuna* when compared to the ungalled leaves 75.53±2.30% and 69.75±1.09%. Since there was change in the moisture content in the infested leaf compared to control ungalled leaf, but no significant reduction was observed (Table. 1).

3.2. Total Chlorophyll: The leaves with gall infestation showed a decrease in the chlorophyll content. There is a significant variation in the chlorophyll content of the differentially infested leaves. Higher the number of gall converse the content of chlorophyll was observed in the leaves of both the host plants. The leaves with 28 gall recorded 51 mg/g tissue of chlorophyll compared to the ungalled leaves 64.63 mg/g in *T. tomentosa.* Similarly, it was 49.16 mg/g in 28 gall and 69.75 mg/g in the ungalled in case of *T. arjuna* (Table. 1).

3.3. Total Protein: Higher protein content was recorded in the infested leaf and significant change compared to control. The protein content was observed 148±1.74 mg/g in ungalled leaves of *T. tomentosa* correspondingly it was 134±1.12 mg/g in *T. arjuna* in normal leaf. The protein content showed an initial increase 163±1.8 mg/g and 147.23±1.10 mg/ml in 5 gall, 186.5±1.73, 157.1±1.15 in 15 gall and it was 147.53±1.73, 128.4±1.15 mg/ml in 28 gall of *T. tomentosa* and *T. arjuna* respectively (Table. 1).

3.4. Total sugars: The level of total sugars in the gall infested leaves showed variation with the number of galls. A steady increase of sugar content is noticed in the galled leaves initially and reduced in the higher infestation. Comparatively a significant difference was observed in ungalled and the gall infested leaves (Table. 1).

3.5. Total Phenols: Higher phenol was recorded in the gall infested leaf, increase in the phenol content observed corresponding to the severity of the gall infestation. The ungalled leaf phenol recorded 28.2±1.8 mg/g and 29.62±1.06 mg/g in *T. tomentosa* and *T. arjuna* respectively (Table. 1).

Table 1: Biochemical analysis in the leaves of *T. arjuna* and *T. tomentosa* on Gall infestation

	Ungalled/no. of galls	Moisture (%)	cholophyll (mg/g)	Protein (mg/g)	Sugars (mg/g)	Phenol (mg/g)
T.tomentosa	Un galled	75.53±2.30	64.63±2.02	148.20±1.74	188.53±1.73	28.20±1.87
	05 gall	74.77±2.37	64.28±1.12	163.7±1.81	205.60±1.90	36.82±2.39
	15 gall	74.05±2.31	58.06±1.73	186.50±1.73	215.46±1.73	38.24±1.76
	28 gall	72.46±2.30	51.51±1.76	147.53±3.0	195.13±2.09	42.26±1.64
F value		0.316 NS	13.35 **	108.0**	39.9**	9.29**
r value		-0.323 NS	-0.907**	-0.026 NS	0.123 NS	0.769**
T.arjuna	Un galled	69.75±1.09	60.71±1.07	134.33±1.12	210.50±1.15	29.62±1.06
	05 gall	67.63±1.16	59.04±1.48	147.23±1.91	228.30±1.17	33.35±1.18
	15 gall	67.61±1.32	55.69±1.09	157.10±1.15	236.20±1.13	35.56±1.27
	28 gall	65.57±0.96	49.16±1.12	128.40±1.15	205.46±1.18	40.52±1.24
F value		2.26NS	17.89**	128.6**	155.9**	14.5**
r value		-0.617*	-0.929**	-0.264 NS	-0.260 NS	0.900**

3.6. Proline: Significant elevated level of proline was recorded in the gall infested Asan and Arjun leaves. The increase was more than the two folds compared to the proline content in the ungalled leaves. The ungalled leaves of Asan recorded 08±1µg/ml and 10.3±1.9 µg/ml in Arjun plant and gall infested leaves showed higher proline the leaf with 5 gall recorded 12±2 µg/ml, 20±2 µg/ml in 15 gall and 35±2 µg/ml in leaf with 28 gall of Asan plant. Similar trend was also observed in the Arjun plants (Fig. 1).

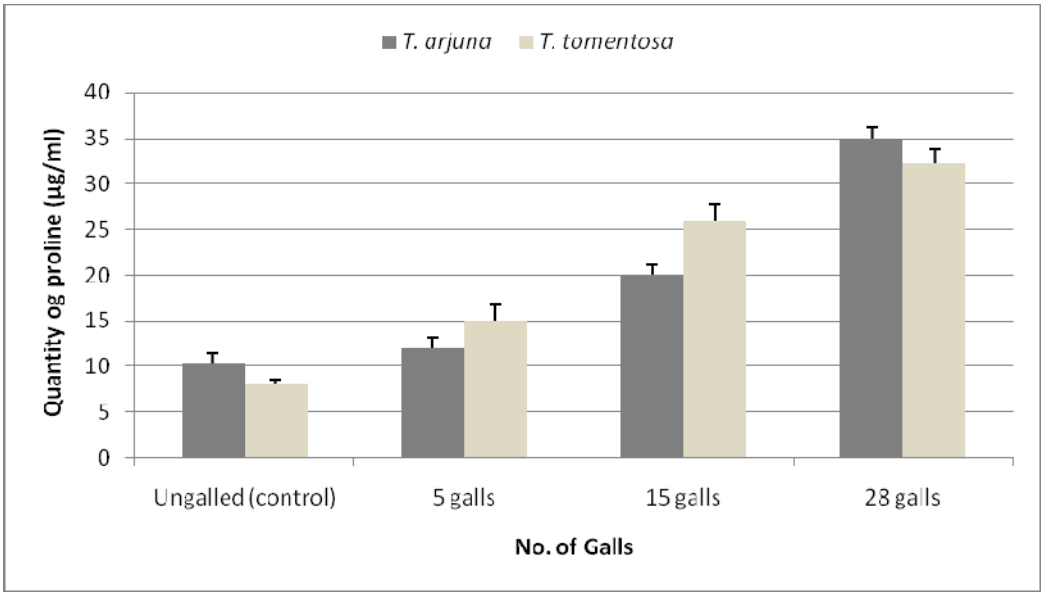

Fig 1: Analysis of Proline level in the leaves of *T. arjuna* and *T. tomentosa* on Gall infestation

Plate: 1-3: Gall infested leaves of *T.arjuna T.tomentosa* and Adult of *Trioza fletcheri minor*

4. Discussion

The gall-inducing psylloids *Trioza fletcheri* menaced in many important agriculture as well as forest based plants causing extensive damage to foliage and in addition to plant tissue by forming gall. Generally a gall insect disturbs the normal physiological function of the host plant and alters the cell differentiation [19]. It initiates increased production of normal plant growth hormones [20]. These plant hormones causes localized plant growth that can result in increase in cell size (hypertrophy) and/or cell number (hyperplasia) which leads to modify the phenotype of their host plants. Besides, the direct manipulation of the plant morphology and physiology in the immediate environment of the gall [21]. Gall phenotypes that provide clues to their adaptive significance are their chemical composition involving nutritive and defensive element. Gall forms may be able to manipulate host nitrogen levels to their own advantage and secondary metabolites, notably phenolic compounds play an essential role in gall formation [22].

The results in the present study showed variations in important cellular content of the host plant due to gall formation. Moisture content observed reduced in the gall infested leaf. The decrease of moisture was corresponding to the severity of the infestation or number of galls. This may be due to the fact that, the gall insects are known to extract moisture content and nutrients from the leaf tissue of host plant cell [7] for the formation of gall on the leaf tissue. Growth of gall tissues is associated with the changes in the levels of host plant cellular contents such as sugars, proteins and other secondary chemicals [8]. Hence the increased infestation leads to more loss of moisture in the leaf. Chlorophyll content of gall tissues showed a decrease as growth progressed. This loss of chlorophyll is responsible for the decolourisation of the area of the leaf where egg was laid on leaves [23]. The low chlorophyll content in galled tissues was due to the loss of palisade tissues, disappearance of chloroplast and modifications of spongy mesophyll.

Remarkable changes in the some of the biomolecules were observed on gall infestation in the leaves of tasar host plants. The higher protein concentration observed in the galled tissue corroborates the observations of earlier reports [24, 25]. The formation of gall requires mechanical and chemical stimuli. The fluid which probably contains enzymes and other cecidogenic substances released/injected into the plant by the insect at the time of egg laying triggers gall induction. The action of the stimulus leads to the formation of new tissues, which cover the nymph in order to isolate the invader, the gall forming insects. Synthesis of diverse plant proteins are believed to be of important in defence is also known [26]. The increased secretion of defensive proteins by host plants that block the action of proteolytic enzymes from herbivores contributes higher proteins in the leaves on infestation. Similar observations were reported in many plant defence mechanism. These proteins, known as proteinase inhibitors, rapidly seem to accumulate throughout plants that are being fed upon by insects and even accumulate in undamaged areas of plants that are far from the initial feeding site [27]. Further there is a reduced total protein in the highly infested leaf which might be due to the lower protein requirement and host plant would reach the saturated level in secretion of proteins [28]. The level of total sugars in gall infested increased in initial stages due to the tissue accumulates starch which is generally less in the ungalled tissue. The stimulus of gall forming insects redirect growth and differentiation of cells which act as a sink of nutritive substances from the host plants by normal flow of resources and/ or by the active mobilization of neighbouring parts of the gall [29]. The elevated levels of phenols during development of psylloid galls are very common [30]. The plants synthesize many secondary chemical and phenols based on its genotype and as a defensive purpose against the invading pest and pathogens [31]. Ortho-dihydroxy phenols oxidize in to quinines which very possibly react with tryptophan released by the hydrolysis of host-plant proteins to stimulate the synthesis and secretion of IAA which is essential for gall formation [32]. This aspect supports the observation of present study wherein increased level of phenol in the gall infested leaves. An increase of proline content in the galled leaves of Arjun and Asan occurs compared to the ungalled leaves. Induction of proline in galled tissues indicates that this has been produced due to the stress. Proline is produced as a defence mechanism to protect from invaders (biotic stress) or stress factors (abiotic stress) and is believed to be an adaptive response to the altered conditions. Increase in proline content was observed in galled leaves of *Populus* [33]. Proline accumulation is known to be a response to stress condition in plants [34]. It is also reported that galls are formed, secondary chemicals get isolated from the nutritive tissue and are concentrated in the peripheral tissues at proportions ten times higher than that of non-galled leaves [35, 22].

5. Conclusion

It can be concluded that when plants are attacked by insects they generate certain signal chemicals and one of these signal molecules is the initiator of expression of certain polypeptides that may be useful in providing the basis for new crop protection strategies. As observed in the present study, the increased secretion of proteins is needed to be studied in detail for better clarity through characterization and the significant elevation of proline level in the gall infested leaves which can be used as marker for the biotic stress. As such it is the high time to take up some strategic planning or new crop protection strategies to control or suppress the *Trioza fletcheri* pest population at minimum level by integrated pest management practices.

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