Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 46 Issue 4 July 2025 pp. 572-582
DOI: http://doi.org/10.22438/jeb/46/4/MRN-5391

J. Environ. Biol., 2025
© Triveni Enterprises, Lucknow (India)
Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Effect of *Pauropsylla tuberculata* herbivory on morpho-anatomical and biochemical responses in *Alstonia scholaris*

J. Singh[1], N. Kaur[2], A.K. Gill[3]* and K.S. Sangha[1]

[1] *Department of Entomology, Punjab Agricultural University, Ludhiana-141 001, India*
[2] *Department of Botany, Punjab Agricultural University, Ludhiana-141 001, India*
[3] *Department of Forestry & NR, Punjab Agricultural University, Ludhiana-141 001, India*

Received: 22 May 2024 **Revised:** 26 August 2024 **Accepted:** 10 October 2024

***Corresponding Author Email:** arshpau@pau.edu ***ORCiD:** http://orcid.org/0009-0006-3855-4342

Abstract

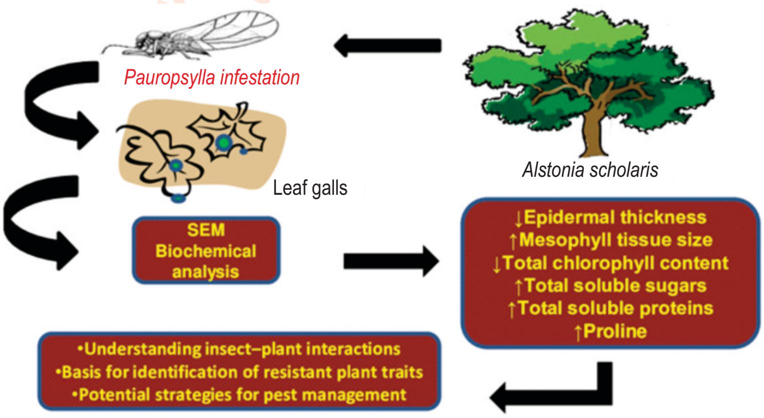
Aim: To study the alterations in growth mechanisms, cellular differentiation and biochemical processes in *Alstonia scholaris* (L.) R. Br. post attack of Hemipteran bug, *Pauropsylla tuberculata* Crawford (Psyllidae: Hemiptera).

Methodology: The studies were conducted in the Department of Forestry & Natural Resources, Punjab Agricultural University where healthy and psyllid galled leaves at different developmental stages were collected from five trees. Data on the morphological, anatomical and biochemical parameters of *P. tuberculata* infested *Alstonia* leaves and pods were recorded.

Results: The perusal of data suggested that the diameter of gall chamber of *P. tuberculata* on *Alstonia* leaves ranged from 0.1 to 1 cm. The upper and lower epidermal thickness decreased in infested leaves. In mesophyll cells, the thickness of palisade tissues, spongy tissues and vascular bundles increased in infested leaves as compared to healthy leaves. The total soluble sugars content in infested leaves (2.90 mg g^{-1} f.wt.) and pods (3.90 mg g^{-1} f.wt.) were significantly higher than healthy leaves (1.61 mg g^{-1} f.wt.) and pods (1.61 mg g^{-1} f.wt.), respectively. Also, the total soluble protein and proline content were significantly higher in the infested leaves and pods than healthy leaves and pods. The total chlorophyll content in healthy leaves (2.25 mg g^{-1} f.wt.) and healthy pods (2.19 mg g^{-1} f.wt.) was significantly higher than total chlorophyll in infested leaves (0.26 mg g^{-1} f.wt.) and infested pods (0.27 mg g^{-1} f.wt.), respectively.

Interpretation: *P. tuberculata* herbivory resulted in morpho-anatomical and biochemical changes in *A. scholaris* as a plant response against biotic stress.

Key words: *Alstonia*, Gall insect, Herbivory, *Pauropsylla tuberculata*



Triveni Enterprises (Educational Services Set-up)

How to cite: Singh, J., N. Kaur, A.K. Gill and K.S. Sangha: Effect of *Pauropsylla tuberculata* herbivory on morpho-anatomical and biochemical responses in *Alstonia scholaris*. *J. Environ. Biol.*, **46,** 572-582 (2025).

Introduction

The genus *Alstonia* are evergreen trees and shrubs belongs to family Apocynaceae contains 40–60 species, native to America, Africa, Polynesia, Australia and tropical and subtropical regions of South-east Asia (Zhao *et al.,* 2020). *A. scholaris* contains alkaloids (Yu *et al.,* 2021) and triterpenoids (Hu *et al.,* 2021) and possess anti-microbial (Zhao *et al.,* 2021), anti-bacterial (Wang *et al.,* 2016), anti-asthmatic (Shang *et al.,* 2010), anti-urolithiasis properties (Zehra and Sanaye, 2021) and anti-cancerous property (Datta and Dhiman, 2016). Several insect pests infest *A. scholaris* (L.) R. Br. including lactid borer, leaf skeletonizer and leaf gall former (Tripathi *et al.,* 2018). The superfamily Psylloidea of Order Hemiptera are sap-sucking, exclusively plant-feeding found both as free-living, lerp-forming, and gall-inducing taxa. Generally the psyllid, *Pauropsylla tuberculata* Crawford induces leaf galls on all plant parts of *A. scholaris* (Biswas *et al.,* 2014) including stem, leaves, inflorescence, fruits etc., which adversely affects its aesthetic and economic value (Dhiman *et al.,* 2012; Chander, 2014).

The gall inducing psyllids are host specific, phytophagous both in nymphal as well as adult stages (Sharma and Raman, 2022). In psyllid induced galls, the number of nymphs per chamber can be one or more than one. Also gall distribution on plant parts varies as isolated or aggregated (Albert *et al.,* 2011). Psyllid feeding disseminate plant diseases and drain away the vital food and water which is detrimental to plants (Jain and Dhiman, 2014). Galls on the leaves of *A. scholaris* were first noticed in 2005 in Chandigarh (Chander, 2014). In 2010, the galls on *A. scholaris* leaves appeared in an epidemic form in Punjab, Haryana, Chandigarh and Delhi. Photosynthetic activity was impaired in galled leaves as leaves were distorted, stunted growth and tree dries up. Inflorescence and follicle fruit galls affected reproduction of plant (Jain and Dhiman, 2014). Losses in seedling production was more because they become bushy, fail to attain expected height and cannot be used for transplantation making total failure of the plantation programme (Tripathy *et al.,* 2018). As insect attack modifies the plant architecture to its advantage by altering the growth mechanisms and cellular differentiation processes in the host leaf (Raman, 2007).

The insect induced morpho-anatomical and biochemical aspects of galls still remain unexplored, which can be utilized as stress markers for biotic conditions. Recently, studies on biology of *Pauropsylla tuberculata* on *Alstonia scholaris* (Sawarkar and Mahajan, 2024; Pasayat and Tripathy, 2020), seasonal incidence (Singh and Sangha, 2018; Muhammad *et al.,* 2023; Rajput and Saini, 2025) and studies on tissue ultrastructure and phytochemistry (Pandey and Kate, 2024) have been studied, however limited work has been done on the morpho-anatomical and biochemical changes occuring in the foliage and pods of *A. scholaris* due to the galls induced by psyllid, *P. tuberculata* in North Indian conditions. Based on the present studies future defence tactics against the psyllid population can be developed using the investigated characteristics.

Materials and Methods

Sampling: Five infested and uninfested trees of *A. scholaris* growing around Punjab Agricultural University and Guru Angad Dev Veterinary and Animal Sciences University were selected to collect mature galled and ungalled leaves. Identification of trees was confirmed by Dr. G. P. S. Dhillon, Principal Tree Breeder, Department of Forestry & Natural Resources, Punjab Agricultural University, Ludhiana. Sampling sites included Roadside sites (Location A: Agronomy Department; Location B: Biotechnology Department and Location C: Floriculture Department) and Field sites (Location D: Gate number four and Location E: GADVASU campus). From each tree,10 galled leaves were collected randomly at maturity stage. Based on gall diameter, the youngest developmental stage was detected, which appeared as a small bulged spot. The different developmental stages of galls and mature ungalled leaves were taken for laboratory analyses of morphological and anatomical features. To observe various stages of psyllid development, the galls present on the leaves were dissected daily.

Morphological analysis: Under dissecting microscope, the galls were observed for morphological features in the laboratory and photographs of different developmental stages of leaf galls were taken. The insect and its nymphal stages from dissected galls were collected in bottles.

Anatomical analyses: For studying the structure of healthy and infested leaves, the samples were fixed on the spot in FAA (Sass, 1958), gradually dehydrated using ethyl alcohol series which consisted of 10, 30, 50, 70, 90 and 95% alcohol and two changes in absolute alcohol for 2 hr at each step. The samples were then subjected to ethyl alcohol and TBA (tertiary-butyl alcohol) series (3:1, 1:1, and 1:3). Finally the material was kept in pure TBA with two changes of 6 hr each. Infiltration of plant material was undertaken using paraffin-TBA mixture prepared by mixing equal amount of molten paraffin and TBA. Thereafter, the material was embedded in pure paraffin wax on the lid of coupling jar. For microtomy, small rectangular blocks of embedded material were mounted on wooden block. Samples were sectioned (10 µm) on a rotary microtome and the slides were prepared using formalin solution followed by oven drying at 40º C, followed by dewaxing using xylene: alcohol (3:1, 1:1 and 1:3) and alcohol (absolute, 95, 70, 50, 30 and 10% series). The slides were stained with erythrosine and crystal violet (Jensen, 1962).

Biochemical analyses: Fresh samples from five infested and uninfested trees of *A. scholaris* were processed for biochemical analysis. Collected samples were grouped as ungalled and galled leaves. Standard procedures were followed for the analysis of total soluble sugars (Dubois *et al.,* 1956), total soluble proteins (Lowry *et al.,* 1951), total chlorophyll content (Hiscox and Israelstem, 1979) and proline (Bates *et al.,* 1973).

Statistical analyses: For anatomical changes, the mean± SE of data of different parameters was calculated and was tested for significance with t- test. For biochemical analyses, the data were statistically analyzed using Factorial Completely Randomized Design for determining significance of differences at various levels of treatment means and the means of combinations (interactions) among various levels of different treatments.

Results and Discussion

Galls were formed on both the upper and lower surfaces of the leaves (Fig. 1), however, they were abundant on the lower surface. Gall formation was initiated when *P. tuberculata* oviposited on the leaves. Slight decolorization was visible on the oviposition sites which enlarged to form a small outgrowth placed in a depression on the upper side of leaf. Initially, the growth of gall was towards the lower side and its growth towards the upper side occurred later. Majority of galls were observed on the lateral veins. Initially, the gall tissue enclosed a nymph within, which grew over the insect to enclose it. The maximum galls were found lateral to the second order veins. In case of severe infestation of leaves, the lamina was completely reduced into cellular mass. Both leaves were equally infested irrespective of their maturity. With an increased infestation, in terms of number of galls, the appearance of leaf became crumbled and deformed.

A depression was formed during the later stage of gall development and the formed chamber by means of small opening was open to the lower side (Fig. 2). The mature pouch galls were either single chambered (unilocular) or 3-4 chambered (multilocular). The multilocular appearance of galls was due to coalesced adjacent galls. At maturity, the gall was similar in colour with the host leaf and apically yellow in colour. It was monothalamus, subcylindrical with succulent wall. The chamber was subcuneatein shape with distal pointed edges. The gall opening was subcircular and apical. The galls remained persistent on the leaf even after the adult psyllid escaped. The number of galls varied from 25 to numerous on each leaf. Different stages of galls did not differ much from each other, except in size, and the diameter of gall chamber range from 0.1 cm to 1 cm. At senescent stage, small orifices due to the emergence of psyllid adults were present on the gall surface, and the cells close to the chamber and exit canals appeared as a yellowish brown rim forming a protective layer.

Galls formed by *P. tuberculata* on *A. scholaris* represent cecidozoa enclosed in a covering pouch. Morphologically, different galls produced by different insects on same plant have been reported (Scareli-Santos and Varanda, 2003; Albert *et al.,* 2011; Sharma and Raman, 2022. In *A. scholaris,* only single type of galls were observed on the leaves regardless of leaf age as an indication of host species reaction against inducing insects.

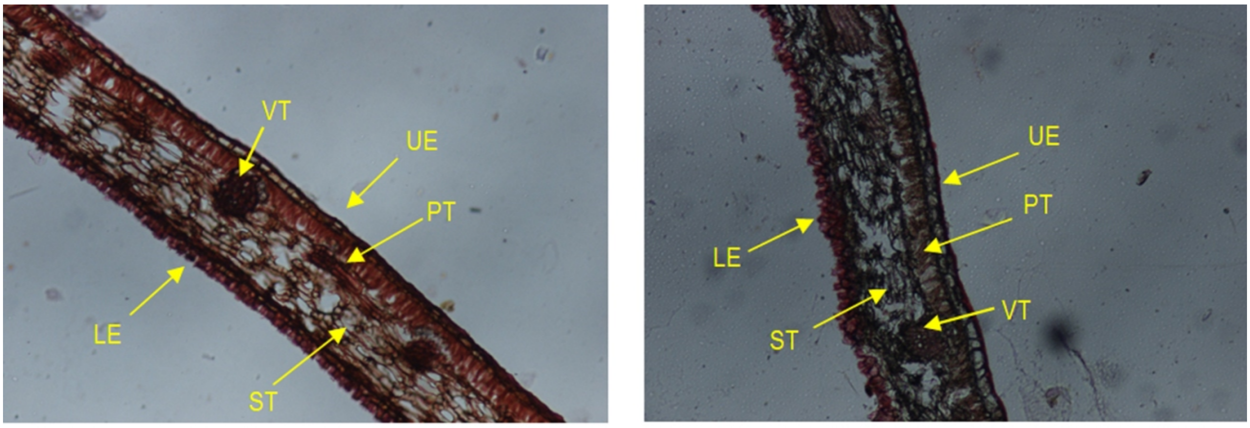
Data pertaining to anatomical changes in the galled leaves caused by *P. tuberculata* is presented in Table 1. Overall, the damage due to gall formation resulted in increase or decrease of various anatomical parameters *viz.,* thicknesses of upper epidermis, lower epidermis, palisade tissue, spongy tissue and vascular bundle tissues (Fig. 3,4). There were significant differences observed in thicknesses of upper epidermis, palisade tissue, spongy tissue and vascular bundle tissues except thickness of lower epidermis in healthy and infected leaves (df=6; 95% confidence level). The upper epidermal layer thickness was more in healthy leaves (18.70 µm) than infected leaves (18.18 µm). The percentage decrease in the upper epidermal layer thickness over healthy was 3.25 per cent. The lower epidermal layer thickness was more in healthy leaves (15.07 µm) than infected leaves (12.62 µm). The percentage decrease in the lower epidermal layer thickness over healthy was 19.18 per cent. In mesophyll cells, the thickness of palisade tissues was low in healthy leaves (41.19 µm) than infected leaves (47.21 µm). The percentage increase in the thickness of palisade tissues over healthy was 14.62 per cent. The thickness of spongy tissues was lesser in healthy leaves (138.37 µm) than infected leaves (163.13 µm). The percent increase in thickness of spongy tissues over healthy was 17.89 per cent. During gall development, the increase in gall size resulted due to division of cells of spongy parenchyma. The thickness of vascular bundles was lesser in healthy leaves (84.03 µm) as compared to infected leaves (119.87 µm). The infected galls when compared with healthy leaves showed further increase in the thickness of vascular bundles and mesophyll.

In *Ficus microcarpa,* the number of mesophyll cell layers and their thickness increased due to cell division in the homogeneous parenchyma cortex, induced by *Gynakothrips ficorum* (Souza *et al.,* 2000). Similarly, in *Copaifera langsdorffii*, an undescribed species of Cecidomyiidae induced similar changes through cell division in the parenchyma cortex (Oliveira Isaias, 2010). The epidermis covering the nymphal chamber showed reduced thickness (Dias *et al.,* 2011). Cells of the epidermal and spongy cells multiply to hypertrophied (Albert *et al.,* 2011). Hyperplasia and hypertrophy leads to increased area of parenchyma cells during gall formation (Oliveira and Isaias, 2010; Moura *et al.,* 2009). Souza *et al.* (2000) reported the development inhibition in vascular system and protoxylem cells collapse during cell hypertrophy in young galls.

Overall, the damage caused due to gall formation on leaves and pods resulted in increase or decrease of various biochemical parameters *viz.,* total chlorophyll, total soluble protein, total soluble sugars and total proline. Insects forming galls stimulate the growth and differentiation of cells, which act as a sink for nutritive substances from the host plants. This occurs either through the normal flow of resources or by actively mobilizing neighbouring parts of the gall (Hartley, 1998). There were significant differences recorded with respect to chlorophyll a, chlorophyll b and total chlorophyll contents among healthy and infested leaves, among various locations and combination of both factors. The healthy leaves showed significantly higher chlorophyll a (1.34 mg g^{-1} f.wt.), chlorophyll b (0.5 mg g^{-1} f.wt.) and total chlorophyll (2.25 mg g^{-1} f.wt.) contents as compared to gall infected leaves (Table 2). Among various locations, the maximum total chlorophyll content in leaf samples (1.58 mg g^{-1} f.wt.) was recorded at location D, followed by (1.39 mg g^{-1} f.wt.) location C, while the minimum chlorophyll content was (0.22 mg g^{-1} f.wt.) was recorded in leaf samples collected from location B.

Fig. 1: Morphological changes in *Alstonia* leaves (i) Galls on upper surface of leaf; (ii) Galls on lower surface of leaf; (iii) Galls on lateral vein (upper surface) and (iv) Galls on lateral vein (lower surface).

Fig. 2: Morphological changes in *Alstonia leaves* (i) Galls crumbled and completely deformed; (ii) Galls become scattered and isolated; (iii) Galls on the lower surface showing depression in the centre and (iv) Yellowish brown periderm formed at the rim of opening.



Healthy leaf

Initial enlargement of PT, ST and VT

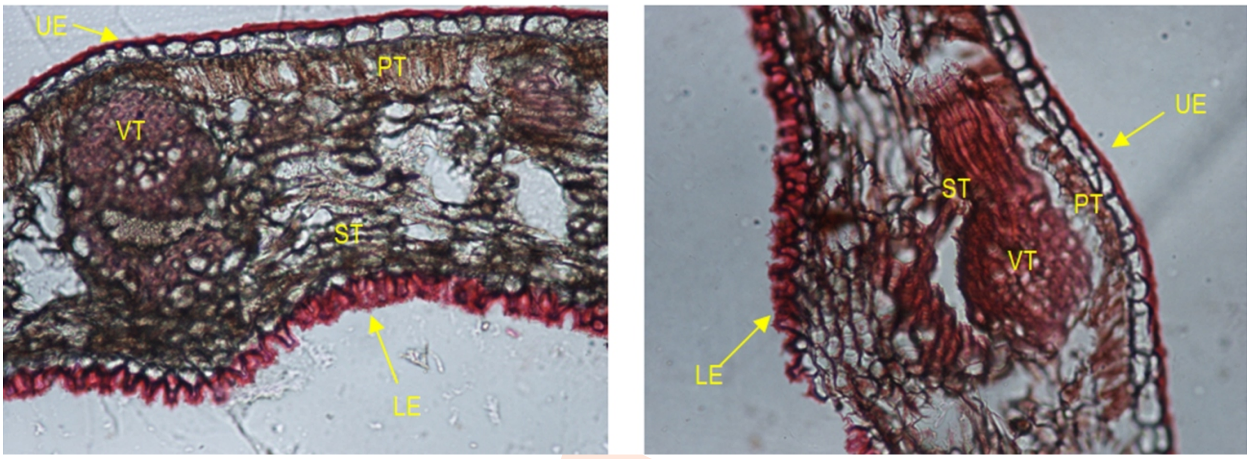


Fig. 3: Anatomical changes in gall and healthy leaves. LE: Lower epidermis; UE: Upper epidermis; ST: Spongy tissues; VT: Vascular tissues and PT: Palisade tissues.

Among various combinations of plant condition and locations, the maximum total chlorophyll (2.83 mg g^{-1} f.wt.) was recorded in healthy leaves at location D, which was at par with total chlorophyll (2.58 mg g^{-1} f.wt.) in healthy leaves at location C. However, the maximum chlorophyll a (1.54 mg g^{-1} f.wt.) was recorded in healthy leaves at location C, which was at par with healthy leaves at location D (1.53 mg g^{-1} f.wt.). Among various combinations, the maximum chlorophyll b contents (1.28 mg g^{-1} f.wt.) was recorded in healthy leaves at location D, followed by chlorophyll b (1.01 mg g^{-1} f.wt.) in healthy leaves at location C. The chlorophyll a (1.34 mg g^{-1} f.wt.), chlorophyll b (0.88 mg g^{-1} f.wt.) and total chlorophyll (2.19 mg g^{-1} f.wt.) contents were significantly higher in healthy pods as compared to gall infected pods (0.21 mg g^{-1} f.wt., 0.06 mg g^{-1} f.wt. and 0.27 mg g^{-1} f.wt.) (Table 3). The maximum total chlorophyll (1.44 mg g^{-1} f.wt.), chlorophyll a (0.86 mg g^{-1} f.wt.) and chlorophyll b (0.58 mg g^{-1} f.wt.) contents was recorded at location D. Among various combinations of plant condition and locations, the maximum total chlorophyll content (2.57 mg g^{-1} f.wt.) was recorded in healthy pods collected from location D, which was at par with total chlorophyll (2.42 mg g^{-1} f.wt.) in healthy pods collected from location C. Similarly, the maximum chlorophyll a (1.47 mg g^{-1} f.wt.) and chlorophyll b (1.1 mgg g^{-1} fresh weight) contents was recorded in healthy leaves at location D. In *Ficus* leaves with progressive growth, the chlorophyll content of gall tissues decreased and chlorophyll less, caused due to egg deposition, was responsible for decolorization of leaf area (Moghe, 1980). The loss of palisade tissues, disappearance of chloroplasts, and modifications in spongy mesophyll resulted in a low chlorophyll content in galled tissues. The increased gall growth results in decreased chlorophyll content of galled tissues (Albert *et al.,* 2011). The results are in agreement with the findings of Mandal *et al.* (2014), who observed a decrease in chlorophyll content as gall development advanced. Similar findings have been recorded earlier (Carneiro *et al.,* 2014; Yang *et al.,* 2007; Samsone *et al.,* 2011, 2012; Gailite *et al.,* 2005; Ajoykumar and Subitha, 2019), where galled plants generally exhibit lower chlorophyll content. Based on these observations, it can be inferred that the total chlorophyll content serves as a biochemical marker for foliar gall resistance in *Alstonia scholaris.* Similar decrease in chlorophyll content in gall

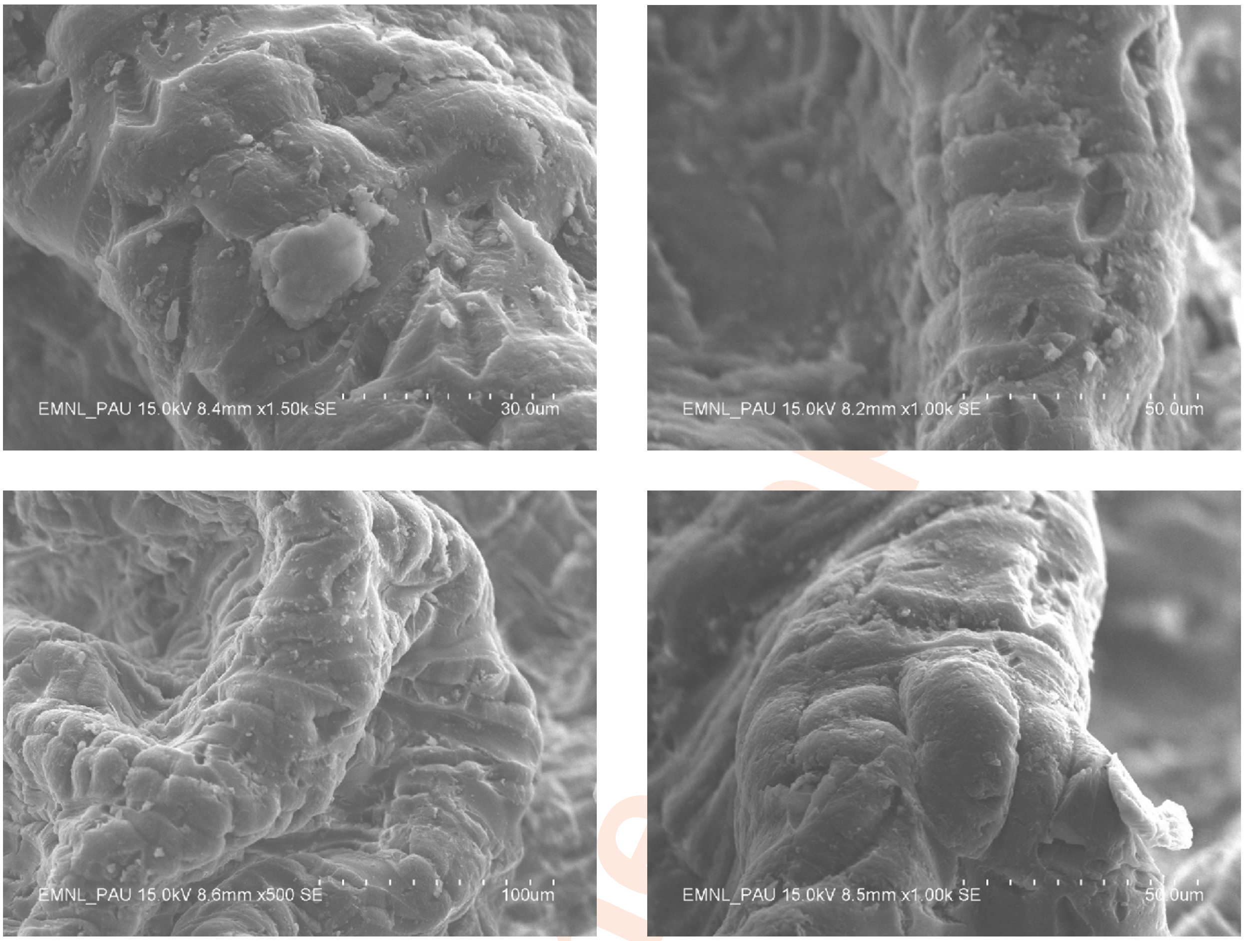


Fig. 4: Stereo Electron Microscope images of *A. scholaris* galls.

infected leaves of *T. arjuna* and *T. infestation* have been reported by Mukherjee *et al.* (2016).

The chlorophyll a, chlorophyll b and total chlorophyll content was recorded to be higher in healthy leaves as compared to gall infected leaves of *Eucalyptus oblique* (Khattab and Khattab, 2005). Gall formation is linked to reduced photosynthetic capacity (Moura *et al.,* 2008) and results in new functions of mesophyll cells, serving both as a feeding site for the insect and a protective barrier (Rohfritsch, 1992; Moura *et al.,* 2008). Plants infested with galls have shown reduced growth, productivity (Gonzales *et al.,* 2005) and ornamental value. The total soluble protein content was recorded to be lesser in healthy (1.61 mg g^{-1} f.wt.) as compared to gall infected leaves (2.90 mg g^{-1} f.wt.) (Table 4). Among various locations, the maximum total soluble protein was recorded at location C (2.47 mg g^{-1} f.wt.), which was at par with total soluble proteins in leaves at location A, D, E. Among various combinations of plant condition and locations, the maximum total soluble protein was recorded in infected leaves (3.10 mg g^{-1} f.wt.) collected from location C, although all combinations differed non-significantly. The total soluble protein content in pod galls showed similar trend as observed in leaf galls and recorded to be lesser in healthy (1.61 mg g^{-1} f.wt.) as compared to gall infected pods (3.07 mg g^{-1} f.wt.) (Table 4). Among various locations, the maximum total soluble proteins was recorded in location D (2.50 mg g^{-1} f. wt.), which was at par with total soluble proteins in pods at all other locations, except total soluble proteins at location E (2.14 mg g^{-1} f.wt.). Among various combinations of plant condition and locations, the maximum total soluble protein was recorded in infected leaves (3.33 mg g^{-1} f.wt.) collected from location D, although all combinations differed non-significantly. The total protein content in normal leaves of uninfested plants and infested trees was more or less similar.

The formation of galls require both mechanical and chemical stimuli. The fluid released or injected into the plant by the insect during egg laying likely contains enzymes and other cecidogenic substances that trigger gall induction. This leads to the formation of new tissues covering the invading insect in order to isolate the invader. The synthesis of diverse plant proteins is

Table 1: Anatomical changes in leaf tissues due to galls formation caused by *P. tuberculata*

Parameters	**Healthy leaves (µm)***	**Infected leaves (µm)***	**Per cent increase/ decrease over healthy**
Thickness of Upper epidermis	18.70±1.71	18.18±2.71	(-)3.25
Thickness of Lower epidermis	15.07±2.24	12.62±0.72	(-)19.18
Palisade tissues thickness	41.19±19.31	47.21±9.98	(+)14.62
Spongy tissues thickness	138.37±26.21	163.13±69.59	(+)17.89
Vascular tissues thickness	84.03±7.96	119.87±12.22	(+)42.65

*Values are mean of four replications, S.E., t calculated value (0.05) ('t' test); Thickness of Upper epidermis = 0.705; t-table value = 0.397 (df = 6); Thickness of Lower epidermis = 0.043; t-table value = 2.55 (df = 6); Palisade tissues thickness = 0.523; t-table value = -0.678 (df = 6); Spongy tissues thickness=0.446; t-table value = -0.816 (df = 6) and Vascular tissues thickness=0.001; t-table value = -6.020 (df = 6)

Table 2: Changes in chlorophyll content of healthy and gall infected leaves*

Locations	**Chlorophyll a (mg g^{-1} f.wt.)**		**Mean**	**Chlorophyll b (mg g^{-1} f.wt.)**		**Mean**	**Total Chlorophyll (mg g^{-1} f.wt.)**		**Mean**
	Healthy	**Infected**		**Healthy**	**Infected**		**Healthy**	**Infected**	
Location A=Agronomy Department	1.06	0.22	**0.64**	0.89	0.04	**0.47**	1.96	0.27	**1.12**
Location B= Biotechnology Department	1.33	0.19	**0.77**	0.36	0.08	**0.22**	1.7	0.27	**0.22**
Location C= Floriculture Department	1.54	0.16	**0.84**	1.01	0.04	**0.54**	2.58	0.18	**1.39**
Location D= Gate number four	1.53	0.23	**0.88**	1.28	0.05	**0.69**	2.83	0.29	**1.58**
Location E= GADVASU campus	1.25	0.22	**0.74**	0.8	0.04	**0.47**	2.07	0.26	**1.18**
Mean	**1.34**	**0.2**	**0.77**	**0.5**	**0.2**	**0.35**	**2.25**	**0.26**	**1.26**
LSD(p=0.05) for:									
Plant condition (A)=	0.055			0.071			0.084		
Locations(B)=	0.087			0.11			0.13		
A×B=	0.12			0.16			0.19		

*Mean of five replications

Table 3: Changes in chlorophyll content of healthy and gall infected pods*

Locations	**Chlorophyll a (mg g^{-1} f.wt.)**		**Mean**	**Chlorophyll b (mg g^{-1} f.wt.)**		**Mean**	**Total Chlorophyll (mg g^{-1} f.wt.)**		**Mean**
	Healthy	**Infected**		**Healthy**	**Infected**		**Healthy**	**Infected**	
Location A=Agronomy Department	1.19	0.22	**0.71**	1.02	0.06	**0.54**	2.22	0.28	**1.25**
Location B= Biotechnology Department	1.25	0.2	**0.73**	0.38	0.08	**0.23**	1.64	0.27	**0.96**
Location C= Floriculture Department	1.4	0.21	**0.81**	0.99	0.02	**0.51**	2.42	0.23	**1.33**
Location D= Gate number four	1.47	0.25	**0.86**	1.1	0.05	**0.58**	2.57	0.3	**1.44**
Location E= GADVASU campus	1.38	0.19	**0.79**	0.90	0.07	**0.48**	2.11	0.26	**1.17**
Mean	**1.34**	**0.214**	**0.77**	**0.88**	**0.06**	**0.47**	**2.19**	**0.27**	**1.23**
LSD(p=0.05) for:									
Plant condition (A)=	0.029	0.070	0.066						
Locations(B)=	0.046	0.11	0.104						
A×B=	0.064	0.16	0.148						

*Mean of five replications

believed to be important in the plant's defense mechanism (Reinbothe *et al.,* 1994). Upon infestation, the host plants secrete elevated levels of defensive proteins, inhibiting the action of proteolytic enzymes from herbivores and leading to increased protein content in the leaves. These proteolytic enzymes, referred to as proteinase inhibitors, accumulate rapidly throughout plants under insect attack and may even accumulate in undamaged plant areas (Anantha Krishnan, 1998). The protein content in the unburst galled tissue exhibited an almost two-fold increase. Similar increase in total soluble protein content in gall infected leaves of *T. arjuna* and *T. tomentosa* have earlier been reported by Mukherjee *et al.* (2016). The galled leaves recorded 186.50 mg g^{-1}

Table 4: Total soluble protein content of healthy and gall infected leaves and pods

Locations	Total Soluble Proteins in leaves (mg g^{-1} f. wt.)*		Mean	Total Soluble Proteins in pods (mg g^{-1} f. wt.)*		Mean
	Healthy	Infected		Healthy	Infected	
Location A=Agronomy Department	1.55	3.01	**2.28**	1.52	3.27	**2.40**
Location B= Biotechnology Department	1.51	2.49	**2.00**	1.69	2.81	**2.25**
Location C= Floriculture Department	1.84	3.10	**2.47**	1.75	3.10	**2.43**
Location D= Gate number four	1.63	3.05	**2.34**	1.66	3.33	**2.50**
Location E= GADVASU campus	1.53	2.84	**2.19**	1.45	2.83	**2.14**
Mean	**1.61**	**2.90**	**2.26**	**1.61**	**3.90**	**2.76**
LSD(p=0.05) for:						
Plant condition (A)=	0.19	0.16				
Locations(B)=	0.31	0.25				
A×B=	NS	NS				

*Mean of five replications

Table 5: Total Soluble Sugars of healthy and gall infected leaves and pods

Locations	Total Soluble Sugars in leaves (mg g^{-1} f.wt.)*		Mean	Total Soluble Sugars in pods (mg g^{-1} f.wt.)*		Mean
	Healthy	Infected		Healthy	Infected	
Location A=Agronomy Department	9.26	9.97	**9.62**	8.47	8.87	**8.67**
Location B= Biotechnology Department	8.59	9.83	**9.21**	8.10	8.46	**8.28**
Location C= Floriculture Department	5.19	10.80	**8.00**	5.45	10.02	**7.74**
Location D= Gate number four	6.02	8.54	**7.28**	6.82	8.77	**7.80**
Location E= GADVASU campus	6.87	9.64	**8.26**	7.18	8.96	**8.07**
Mean	**7.19**	**9.76**	**8.48**	**7.20**	**9.02**	**8.11**
LSD(p=0.05) for:						
Plant condition (A)=	0.48	0.38				
Locations(B)=	0.76	0.60				
A×B=	1.07	0.84				

*Mean of five replications

tissue of total soluble proteins content as compared to the ungalled leaves 148.20 mg g^{-1} in *T. tomentosa.*

The total sugar soluble content was recorded to be lesser in healthy leaves (7.19 mg g^{-1} f.wt.) as compared to gall infected leaves (9.76 mg g^{-1} f.wt.) (Table 5). Among various locations, significant differences were observed and maximum total soluble sugar (9.62 mg g^{-1} f.wt.) was recorded in healthy leaves at location A, which was at par (9.21 mg g^{-1} f.wt.) with location B. Among all the combinations, the maximum total soluble sugar (9.26 mg g^{-1} f.wt.) was recorded in healthy leaves at location A (Agronomy Department), however, the maximum total soluble sugar (10.80 mg g^{-1} f.wt.) was recorded in gall infected leaves at location C. The total soluble sugars content was recorded to be lesser in healthy leaves (7.20 mg g^{-1} f.wt.) as compared to gall infected pods (9.02 mg g^{-1} f.wt.) (Table 5). The maximum total soluble sugars content (8.67 mg g^{-1} f.wt.) was recorded at location A. Among various combinations, the maximum total soluble sugars content (10.02 mg g^{-1} f.wt.) was recorded in infected pods at location C. The accumulation of sugar in the gall chamber indicates that these cells may function as nutritive tissues. The gall maker's stimulus redirects the growth and differentiation of cells, causing them to act as a sink for nutritive substances from the host plant. The results are in line with Manna (2020) who reported a steady increase of sugar content in the galled fruits induced by *Pseudophacopteran* sp., a psylloid herbivores than normal fruits on *Alstonia scholaris*. Mukherjee *et al.* (2016) recorded 195.13 mg g^{-1} tissue of total sugars content in galled leaves as compared to the ungalled leaves 188.53 mg g^{-1} in *T. tomentosa*. Similarly, it was 205.46 mg g^{-1} in gall infected leaves and 210.50 mg g^{-1} in ungalled leaves in *T. arjuna*. Ajoykumar and Subitha (2019) also recorded enhanced sugars in the leaf galls caused by *Trioza pallida* as compared to the normal leaves of *Mallotus philippensis.*

The total proline content was recorded lesser in healthy leaves (0.0295 µg g^{-1} f.wt.) as compared to gall infected leaves (0.0107 µg g^{-1} f. wt.) (Table 6). The maximum total proline (0.0228 µg g^{-1} f.wt.) was recorded at location C although differences among various locations were non-significant. Among various combinations, the maximum total proline content (0.0150 µg g^{-1}

Table 6: Total Proline content of healthy and gall infected leaves and pods

Locations	Total proline content in leaves (µg g^{-1} f.wt.)*		Mean	Total Proline content in pods (µg g^{-1} f.wt.)*		Mean
	Healthy	Infected		Healthy	Infected	
Location A=Agronomy Department	0.0062	0.0509	**0.0285**	0.0092	0.0273	**0.0182**
Location B= Biotechnology Department	0.0104	0.0212	**0.0159**	0.0153	0.0206	**0.0199**
Location C= Floriculture Department	0.0150	0.0304	**0.0228**	0.0286	0.0381	**0.0333**
Location D= Gate number four	0.0083	0.0189	**0.0136**	0.0237	0.0174	**0.0205**
Location E= GADVASU campus	0.0139	0.0260	**0.0200**	0.0126	0.0227	**0.0177**
Mean	**0.0107**	**0.0295**	**0.0201**	**0.0179**	**0.0252**	**0.0215**
LSD(p=0.05)for:						
Plant condition (A)=	0.0132	0.0062				
Locations(B)=	NS	0.0099				
A×B=	NS	NS				

*Mean of five replications

f.wt.) was recorded at location C in healthy leaves, although differences among combinations were non-significant. The total proline content was recorded to be lesser in healthy pods (0.0252 µg g^{-1} f.wt.) as compared to gall infected pods (0.0179 µg g^{-1} f.wt.) (Table 6). The maximum total proline content in pods (0.0333 µg g^{-1} f.wt.) was recorded at location C. Among various combinations, the maximum total proline content (0.0381 µg g^{-1} f.wt.) was recorded at location C in infected pods although differences among combinations were non-significant. Proline is produced as an adaptive response to adverse conditions, acting as a defense mechanism against both biotic stress (such as pathogens or herbivores) and abiotic stress (like drought, salinity, or extreme temperatures). It helps plants cope with these stress factors by stabilizing proteins and membranes, protecting cells from oxidative damage, and maintaining cellular hydration. Proline also serves as an osmoprotectant and an energy source, while potentially functioning as a signaling molecule in stress response pathways.

The accumulation of proline is recognized as a response to stress conditions in plants (Bates *et al.,* 1973), while proline content in normal leaf was not detectable. A normal leaf from an infected branch of an infected tree showed a very low concentration, however, a significant increase in proline content was observed in the galled tissue, indicating a stressed condition. The results are in line with the findings of Rasool *et al.* (2019) who reported high proline contents in *P. tuberculata* induced galled leaves as compared to healthy leaves in *A. scholaris.* Proline content in galled leaves caused by *T. pallida* in *M. philippensis* when compared with normal leaves increased approximately 4-fold in young leaves, 3-fold in mature leaves, and 9-fold in senescing leaves (Ajoykumar and Subitha, 2019). Increase in total proline content in gall infected leaves of *T. arjuna* and *T. tomentosa* have earlier been reported by Mukherjee *et al.* (2016). In *T. tomentosa*, the galled leaves recorded a total proline content of 26 µg ml^{-1}, whereas the ungalled leaves showed 8 µg ml^{-1}. Proline is considered an adaptive response to altered conditions, serving as a defense mechanism against invaders (biotic stress) or stress factors (abiotic stress). The galled leaves of *Populus* showed increase in proline content (El-Akkad, 2004).

The study on *P. tuberculata* infestation in *Alstonia* leaves and pods is highly relevant for understanding plant stress responses to pest attacks. It highlights the physiological and biochemical changes in the plant, such as cell differentiation, alterations in mesophyll structure, soluble sugars, proteins, proline, and chlorophyll content, ultimately resulting in gall formation. These insights can inform pest management strategies, as changes in these biomarkers could serve as early indicators of infestation. Additionally, the study provides ecological relevance by showing how pest outbreaks can affect plant health and ecosystem dynamics, especially when the affected plant is crucial part of local flora. The findings also have broader agricultural implications, offering valuable data on plant defense mechanisms that can be applied to improve crop resilience and pest control practices. Ultimately, the research contributes to the development of more effective pest management strategies and a deeper understanding of plant-pest interactions.

Acknowledgments

The authors express gratitude to Punjab Agricultural University, Ludhiana, Punjab, India, for their generous provision of materials and laboratory support, which facilitated the successful execution of the experiment.

Authors' contribution: J. Singh: Carried out research work; **K.S. Sangha:** Formulated the research work, supervised field research, provided field facilities and editing of the manuscript; **N. Kaur:** Provided laboratory facilities; **A.K. Gill:** Corresponding author and drafted the manuscript.

Funding: Not applicable.

Research content: The manuscript's content is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: Not applicable.

Consent to publish: All authors give their consent to publish the article in *Journal of Environmental Biology.*

References

Ajoykumar, K.N. and P.P. Subitha: Physiochemical changes associated with foliar gall formation by a psyllid insect T*rioza pallida* Haupt. in *Mallotus philippensis* Muell. *Arg. Int. J. Res. Advent. Technol.*, **7**, 721-728 (2019).

Albert, S., A. Pandhiar, D. Gandhi and P. Nityanand: Morphological, anatomical and biochemical studies on the foliar galls of *Alstonia scholaris* (Apocynaceae). *Rev. Bras.,* **34**, 343-358 (2011).

Ananthakrishnan, T.N.: Insect gall systems: Patterns, processes and adaptive diversity. *Curr Sci.,* **75**, 672-676 (1998).

Bates, L.S., R.P. Waldren and I.D. Teare: Rapid determination of free proline for water-stress studies. *Plant and Soil,* **39**, 205-207 (1973).

Biswas, S.M., N. Chakraborty and P. Baidyanath: Foliar gall and antioxidant enzyme responses in *Alstonia scholaris,* R. Br. after psylloid herbivory–an experimental and statistical analysis. *Glob. J. Bot. Sci.,* **2**, 12-20 (2014).

Carneiro, R.G., A.C. Castro and R.M. Isaias: Unique histochemical gradients in a photosynthesis-deficient plant gall. *S. Afr. J. Bot.,* **92**, 97-104 (2014).

Chander, J.: Leaf gall insect, *Pauropsylla tuberculata* attacking fruit of *Alstonia scholaris*. *Indian For.,* **140**, 721-723 (2014).

Datta, O. and S.C. Dhiman: Studies on the histomorphology of leaf galls on *Alstonia scholaris* L. (Apocynaceae) induced by *Pauropsylla tuberculata* Crawford. *Int. Jour. Agric. Inv.,* **1**, 65-70 (2016).

Dhiman, S.C., D. Kumar and V. Malik: Impact of global warming in relation to gall formation by *Pauropsylla tuberculata* (Homoptera: Psyllidae) on the leaves of *Alstonia scholaris* Linn. (Apocynaceae). *J. Env. Bio-Sci.,* **26**, 147-149 (2012).

Dias, G.G., B.G. Ferreira, G.R.P. Moreira and R.M.S. Tsias: Developmental pathway from leaves to galls induced by a sap-feeding insect on *Schinus polygamous* (Cav) Cabrera (Anacardiaceae). *An. Acad. Bras. Cienc.,* **85**, 187-200 (2011).

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith: Colorimetric method for determination of sugars and related substances. *Anal. Chem.,* **28**, 350-356 (1956).

El-Akkad, S.S.: Biochemical changes induced in *Populus nigra* leaves by galling aphids *Pemphigous populi. Int. J. Agric. Biol.,* **6**, 659-664 (2004).

Gailite, A., U. Andersone and G. Ievinsh: Arthropod-induced neoplastic formations on trees change photosynthetic pigment levels and oxidative enzyme activities. *J. Plant Interac.,* **1**, 61-67 (2005).

Gonzales, W.L., P.P. Cabalerro and R. Medel: Galler induced reduction of shoot growth and fruit production in the shrub *Colliguaja integerrima* (Euphorbiaceae). *Rev. Chil. Hist. Nat.,* **78**, 393-399 (2005).

Hartley, S.E.: The chemical composition of plant galls: are level of nutrients and secondary compounds controlled by the gall former? *Oecologia,* **113**, 492-501 (1998).

Hiscox, J.D. and G.F. Israelstam: A method for the extraction of chlorophyll from leaf tissue without maceration. *Canad. J. Bot.,* **57**, 1332-1334 (1979).

Hu, B.Y., Y.L. Zhao, D.S. Xiong, Y.J. He, Z.S. Zhou, P.F. Zhu , Z.J. Wang, Y.L. Wang, L.X. Zhao and X.D. Luo: Potent antihyperuricemic triterpenoids based on two unprecedented scaffolds from the leaves of *Alstonia scholaris. Org. Lett.,* **23**, 4158-4162 (2021).

Jain, S. and S.C. Dhiman: Some ecological aspects of *Pauropsylla tuberculata* Crawford induced galls on *Alstonia scholaris* R. Br. *J. Exp. Zool.,* **17**, 431-436 (2014).

Jensen, W.A.: Botanical Histochemistry- Principles and Practice. London and San Francisco, WH Freeman and Company, San Francisco, 408 pages (1962)

Khattab, H. and I. Khattab: Responses of eucalyptus trees to insect feeding (gall forming psyllid). *Int. J. Agri. Biol.,* **7**, 979-984 (2005).

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall: Protein measurement with folin-phenol reagent. *J. Biol. Chem.,* **193**, 265-275 (1951).

Mandal, S., B. Suparna, M. Biswas, S.M. Biswas, N. Chakraborty and B. Pal: Foliar gall and antioxidant enzyme responses in *Alstonia scholaris* R. Br. after Psylloid herbivory-An experimental and statistical analysis. *Global J. Bot. Sci.,* **2**, 12-20 (2014).

Manna, S.: Morphogenesis and histopathology of fruit galls induced by *Pseudophacopteran* sp. on *Alstonia scholaris.* U.P. *J. Zool.,* **41**, 1-8 (2020).

Moura, M.Z.D., G.L.G. Soares and R.M.S. Isaias: Onto-gênese da folha e das galhas induzidas por *Aceria lantanae* Cook (Acarina: Eriophyidae) em *Lantana camara* L. (Verbenaceae). *Rev. Bras. Bot.,* **32**, 271-282 (2009).

Moura, M.Z.D., G.L.G. Soares and R.M.S. Isaias: Species-specific changes in tissue morphogenesis induced by two arthropod leaf gallers in *Lantana camara* (Verbenaceae). *Aust. J. Bot.,* **56**, 153-160 (2008).

Muhammad, A., N. Kumar, I.I. Dangora, I.I. Muhammad, A. Mathur and M.A. Sarki: Incidence, severity and biochemical studies of *Alstonia scholaris* (L.) R. Br. Foliar Galls. *Int. J. Pl. Pathol. Microbiol.,* **3**, 07-12 (2023)

Mukherjee, S., G. Lokesh, A.S. Aruna, S.P. Sharma and A. Sahay: Studies on the foliar biochemical changes in the gall (*Trioza fletcheri* minor) infested tasar food plants *Terminalia arjuna* and *Terminalia tomentosa*. *J. Ent. Zoo. Stud.,* **4**, 154-158 (2016).

Oliveira, D.C. and R.M.S. Isaias: Redifferentiation of leaflet tissues during midrib gall development in *Copaifera langsdorffi* (Fabaceae). S. *Afr. J. Bot.,* **76**, 239-248 (2010).

Pandey, K. and A.S. Kate: Comparative analysis of foliar galls and ungalled leaves of *Alstonia scholaris* with a focus on tissue ultrastructure and phytochemistry. *Biochem. Syst. Ecol.,* **115**, 104851 (2024)

Pasayat, B. and M.K. Tripathy: Biology of psyllid, *Pauropsylla tuberculata* Crawford infesting the devil tree *Alstonia scholaris* (L.) R.Br. studied at Bhubaneswar, Odisha. *J. Entomol. Zool. Stud.,* **8**, 282-286 (2020).

Rajput, K. and K. Saini: Seasonal incidence of gall forming psyllid, *Pauropsylla tuberculata* infesting *Alstonia scholaris* L., at Meerut Region. *Int. J. Entomol. Res.,* **10**, 155-159 (2025).

Raman, A.: Insect-induced plant galls of India: Unresolved questions. *Curr. Sci.,* **92**, 748-757 (2007).

Rasool, M., F. Ahmad, A. Akhter, H.A. Ali khan, M. Khurshid, W. Anwar, M.S. Asif, M. Waqar and M.S. Haider: Biochemical and molecular analysis of *Alstonia scholaris* leaf galls induced by *Pauropsylla tuberculata* (Psyllidae). *Mycopath,* **17**, 79-88 (2019).

Reinbothe, S., R. Mollenbauer and C. Reinbothe: JIPs and RIPs: the regulations of plant gene expression by jasmonates in response to environmental cues and pathogens. *Plant Cell,* **6**, 1197-1209 (1994).

Rohfritsch, O.: Patterns in gall development. In: Biology of Insect Induced Galls (Eds.: J.D. Shorthouse and O. Rohfritsch). Oxford University Press, New York, pp. 60-86 (1992).

Samsone, I., U. Andersone and G. Ievinsh: Variable effect of arthropod-induced galls on the photochemistry of photosynthesis, oxidative enzyme activity and ethylene production in tree leaf tissues. *Environ. Exp. Biol.,* **10**, 15-26 (2012).

Samsone, I., U. Andersone and G. Ievinsh: Gall midge *Rhabdophaga rosaria*-induced rosette galls on Salix: morphology, photochemistry of photosynthesis and defence enzyme activity. *Environ. Exp. Biol.,* **9**, 29-36 (2011).

Sass, J.E.: Botanical Microtechnique. 3rd Edn., The Iowa State University Press, Ames, Iowa, 240 pages (1958)

Sawarkar A.B. and G.S. Mahajan: Periodic infestation and biology of gall psylids, *Pauropsylla tuberculata* (Crawford) on Saptaparna plant, *Alstonia scholaris. Int. J. Entomol. Res.,* **9**, 14-18 (2024).

Scareli-Santos, C. and E.M. Varanda: Morphological and histochemical study of leaf galls of *Tabebuia ochracea* (Cham.) Standl. (Bignoniaceae). *Phytomorphology,* **53**, 207-214 (2003).

Shang, J.H., X.H. Cai, Y.L. Zhao, T. Feng and X.D. Luo: Pharmacological evaluation of *Alstonia scholaris*: anti-tussive, anti-asthmatic and expectorant activities. *J. Ethnopharmacol.,* **129**, 293-298 (2010).

Sharma, A. and A. Raman: Gall-inducing *Psylloidea* (Insecta: Hemiptera) – plant interactions. *J. Plant Interact.,* **17**, 580-594 (2022).

Singh, J. and K.S. Sangha: Developmental Stages and Seasonal History of *Pauropsylla tuberculata* Crawford on *Alstonia scholaris* (L.) R. Br. *Indian For.,* **144**, 381-386 (2018).

Souza, S.C.P.M., J.E. Kraus, R.M.S. Isaias and L.J. Neves: Anatomical and ultrastructural aspects of leaf galls in *Ficus microcarpa* L.F. (Moracea) induced by *Gynakothrips ficorum* Marchal (Thysanoptera). *Acta. Bot. Bras.,* **14**, 57-69 (2000).

Tripathi, M.K., B. Pasayat, A. Tripathy and N. Bhol: Seasonal Incidence of Gall Forming Psyllid *Pseudophacopteron tuberculatum*, Crawford Infesting *Alstonia scholaris* R. Brown at Bhubaneswar, Odisha. *J. Tree Sci.,* **37**, 14-21(2018).

Wang, C.M., H.T. Chen, Z.Y. Wu, Y.L. Jhan, C.L. Shyu and C.H. Chou: Antibacterial and synergistic activity of pentacyclic triterpenoids isolated from *Alstonia scholaris. Molecules,* **21**, 139 (2016).

Yang, C.M., M.M. Yang, M.Y. Huang, J.M. Hsu and W.N. Jane: Lifetime deficiency of photosynthetic pigment-protein complexes CP1, A1, AB1, and AB2 in two cecidomyiid galls derived from *Machilus thunbergii* leaves. *Photosynthetica,* **45**, 589-593 (2007).

Yu, H.F., C.F. Ding, L.C. Zhang, X. Wei, G.G. Cheng, Y.P. Liu, R.P. Zhang and X.D. Luo: Alstoscholarisine K, an antimicrobial indole from gall-induced leaves of *Alstonia scholaris Org. Lett.,* **23**, 5782-5786 (2021).

Zehra, S. and M.M. Sanaye: Evaluation of anti-urolithiatic potential of leaves of *Alstonia scholaris* and its isolated pentacyclic triterpenoids in ethylene glycol-induced renal calculi rat model. *Indian J. Pharm. Educ.,* **55**, 232-239 (2021).

Zhao, Y.L., M. Su, J.H. Shang, X. Wang, G.L. Bao, J. Ma, Q.D. Sun, F. Yuan, J.K. Wang and X.D. Luo: Acute and sub-chronic toxicity of indole alkaloids extract from leaves of *Alstonia scholaris* (L.) R. Br. in beagle dogs. *Nat. Prod. Bioprospect.,* **10**, 209–220 (2020).

Zhao, Y.L., S.B. Pu, Y.Qi, B.F. Wu, J.H. Shang, Y.P. Liu, D. Hu and X.D. Luo: Pharmacological effects of indole alkaloids from *Alstonia scholaris* (L.) R. Br. on pulmonary fibrosis *in vivo. J. Ethnopharmacol.,* **267**,113506 (2021).