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Incidence, severity and biochemical studies of *Alstonia scholaris* (*L.)* R. Br. Foliar Galls

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Abstract
Alstonia scholaris R. Br. is commonly known as saitan ka jhad in Hindi belongs to the family *Apocynaceae,* is a classy and graceful evergreen tall tree grows up to 25m high which widely used as medicine and served as an important shade tree. Gall formation induced by psyllid herbivory *Pauropsylla tuberculata, Crawf* is of growing concern in *A. Scholaris* leaves infested with this insect developed an outgrowth called galls with change the leaf structure and alter physiological processes in the affected plant. A survey was conducted at Mewar University Rajasthan India Campus to access the incidence and severity from May to July 2020 and to study the total chlorophyll content and phenolic compound in the gall-infested leaves and uninfected *viz* (fresh leave, young gall, mature gall, and perforated gall) research survey depicted that incidence and severity of galls increase from May to July 2020 this is due to the onset of rainfall start in mid-June and gall require wet and warm temperature, activity of total chlorophyll were found to exhibit strong negative correlation with phenolic compound at different stages of gall development. The finding of this research can be enough to suggest that rainfall coupled with high-temperature increases galls incidence and severity and phenolic compound acts as a biochemical marker for foliar galls resistance in devil tree *Alstonia scholaris.*

Keywords: *Alstonia scholaris, Pauropsylla tuberculata,* Galls, Incidence, severity.

Introduction
Alstonia scholaris (L.) R.Br. popularly known as Devil tree Satwin, chattin or Saptarapni (from two Sanskrit words sapta meaning seven, and parni meaning leaves) is a beautiful foliage tree which serves the purpose of the avenue plantation. Its large evergreen and grown widely in the landscapes, gardens as well as roadside plantations (Pasayat & Tripathy, 2020) [19]. The branchlets are profusely lenticellate, and the bark is greyish. Plants have 1-3 cm petioles, leathery leaves that are narrowly obovate, round at the apex, and lateral veined to indicate that they are eudicots. The adaxial side of the leaves is glossy, while the abaxial side is greyish (Albert *et al*., 2011) [1].

Gall formation is spreading alarmingly quickly on *A. scholaris* trees and saplings planted throughout the Mewar University Campus (Gangrar Chittorgarh, Rajasthan, India). The plant are seriously affected by gall-inducing psyllid *Pauropsylla tuberculata* Crawf. The formation and characteristics of galls caused by this sucking insect (Homoptera) are usually closely associated with its feeding behaviour. These insects obtain nutrients from either the phloem, which is responsible for transporting sugars in the plant, or the xylem, which transports water, or even from non-conductive plant cells (Majumdar *et al*., 2017) [11]. Insect attack gives bad and ugly appearance and affected parts fails to serve the original purpose (Tripathy *et al*., 2018) [23].

Due to importance of *Alstonia scholaris* as shade tree and medicinal purposes as studies indicated it uses. Its leaves and bark contained echitamine, echitamine chloride, scholarine, scholaricine, monoterpenoid indole alkaloids, as well as iridoids, coumarins, flavonoids, simple phenolics, steroids, saponins, and tannins (Dey, 2011) [8]. Traditional uses of the plant include the treatment of cancer, jaundice, fever, malaria, and skin conditions (Mollik *et al.*, 2010) [13]. Alstonine, an essential alkaloid found in the plant, has been documented to possess anticancer properties (Beljanski & Beljanski, 1982; Beljanski & Beljanski, 1986) [4, 3] due to rich source of phytochemicals of pharmaceutical importance incidence and severity of the gall disease in *Alstonia scholaris* need to be investigated.

Material and Methods

Study Site

The research took place at Mewar University, situated in Gangrar, Chittorgarh, Rajasthan, India. The university spans approximately 30 acres and is located at coordinates 25°02'02.5"N 74°38'07.6"E. The average elevation of the university is 417 meters above sea level. The study was carried out between May 2020 and July 2020.

Survey and sample collection

The survey commenced on May 1, 2020, and concluded on July 15, 2020. Data collection occurred at fifteen-day intervals within the study period. The study area was divided into two plots; Plot A around Bhamasha Hostel, school garden, and veg mess and Plot B surrounding academic and administrative block. Each plot was divided into three replications and data was collected and recorded carefully based on:

Disease incidence: Disease incidence was calculated using the formula below as adopted by (Charles & Amusa, 2015) [7].

$$\text{Disease incidence} = \frac{\text{No. of infected plants}}{\text{Total No. of asses plant}} \times 100$$

Disease severity: Disease severity was calculated using the formula below as adopted by (Horsfall & Barrat, 1945) [10].

$$\text{Disease severity} = \frac{\text{Sum of all disease rating}}{\text{Total No. of rating} \times \text{maximum disease grade}} \times 100$$

Biochemical Analysis of Leaves Sample

To conduct biochemical analysis, fresh samples of mature leaves from both galled and ungalled *Alstonia scholaris* trees were collected. From ten different Alstonia scholaris individuals, samples of healthy leaves and leaves affected by Psyllidae at various developmental stages was collected for further analysis. During collection of samples, care was taken to ensure that the leaves of the same individual (both galled and ungalled) were of the same age so that comparison can be drawn between the two. The leaves obtained were washed thoroughly and wiped with ethanol before extraction as done by (Majumdar *et al*., 2017) [11]. Standard procedures was followed for the analysis of phenolic compound and total chlorophyll content.

Leaf Extract Preparation

To extract was prepared using distilled water as per the standard protocol by (Biswas *et al*., 2014) [5]. To perform biochemical assays, 20 grams of ground leaves were combined with distilled water to achieve a final volume of 100 ml. This process was repeated three times to obtain independent samples, with each batch of leaves ground separately at regular intervals.

Determination of phenolic content

The phenolic content of both fresh and infested leaves of Alstonia was determined using a freshly prepared reagent solution. This solution was created by combining equal volumes of 1% ferric chloride (FeCl3) and 1% potassium ferricyanide [K3Fe(CN)6]. A total of five sets of experiments were conducted, including one set for control leaves, one set for fresh leaves, and three sets for infested leaves with varying ages of galls. Each experiment was repeated three times, resulting in a total of three repetitions for each set.

In the case of the fresh leaf group and the three different infested leaf groups, 5 ml of leaf extracts were utilized, while 5 ml of distilled water was employed for the control group. Following that, 0.5 ml of the reagent solution was added to each set, and the samples were subsequently subjected to colorimetric analysis at wavelengths of 490 nm and 520 nm.

Estimation of total chlorophyll content

Chlorophyll extraction was carried out using 40mg of leaf tissue from various stages of galls. The extraction process involved the use of 3 mL of DMSO. The determination of total chlorophyll content in fresh leaves of *Alstonia*, both infested and non-infested, was conducted according to a standard procedure by (Muhammad *et al*., 2021) [15].

Statistical Analysis

ANOVA was performed on the collected data on incidence and severity using SPSS software, version 20. The mean and standard deviation of the biochemical analysis results were displayed. A one-way analysis of variance (ANOVA) was performed with a significance level of P 0.01 to determine the impact of all variables on the foliar gall in *Alstonia scholaris.*

Results

Incidence and Severity of *Alstonia scholaris* Foliar Galls Based on Location

The results of incidence and severity of *Alstonia scholaris* foliar galls based on location was presented in Table 1

Table 1: Location-wise incidence and severity of *Alstonia scholaris* Foliar Galls at Mewar University 2020.

	Block A			Block B		
	Mean	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**
Incidence	97.72	18	5.02	97.00	18	5.68
Severity	50.04	18	11.95	60.88	18	28.22

From the above table it showed that block A has the highest mean of incidence compared to block B, in terms of severity of the disease block B has the highest mean of severity compared to block A.

Incidence and Severity of *Alstonia scholaris* Foliar Galls Monthly-wise

The results of incidence and severity of *Alstonia scholaris* foliar galls monthly wised was presented in Table 2-

Table 2: Monthly-wise incidence and severity of *Alstonia scholaris* foliar galls at Mewar University 2020.

	May			June			July		
	Mean	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**
Incidence	96.83	12	5.98	97.25	12	6.51	98.00	12	3.19
Severity	48.26	12	23.61	54.13	12	22.88	64.00	12	18.12

From the above table it showed that the incidence and severity of the disease increased with the increase in month from May-July with the highest mean and standard deviation recorded at the month of July.

Incidence and severity of *Alstonia scholaris* foliar galls monthly-wise for Block A

The results of incidence and severity of the *Alstonia scholaris* foliar galls monthly-wise for block A was presented in Table 3 below.

Table 3: Monthly-Wise for Block an Incidence and Severity of *Alstonia scholaris* Foliar Galls At Mewar University 2020.

	May Block A			June Block A			July Block A		
	Mean	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**
Incidence	94.5	6	7.84	100	6	0.00	98.67	6	2.07
Severity	38.4	6	5.53	51.4	6	10.15	60.33	6	7.75

From the above table, it showed that the highest incidence of the disease was recorded in June followed by July and least was recorded in May.
Severity of the disease was highest in July followed by June and the least were recorded in May.

Incidence and Severity of *Alstonia scholaris* Foliar Galls Monthly-Wise for Block B

The results of incidence and severity of the *Alstonia scholaris* foliar galls monthly-wise for block B were presented in Table 4 below.

Table 4: Monthly-wise for block B incidence and severity of *Alstonia scholaris* foliar galls at Mewar University 2020.

	May Block B			June Block B			July Block B		
	Mean	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**	**Mean**	**N**	**Std. Dev.**
Incidence	99.17	6	2.04	94.5	6	8.67	97.33	6	4.13
Severity	58.12	6	31.03	56.85	6	32.10	67.66	6	25.1

From the above table, it showed that the highest incidence of the disease was recorded in May followed by July and the least was recorded in June. Severity of the disease was highest in July followed by May and the least were recorded in June.

Descriptive statistics of biochemical variables

The table below showed descriptive statistics of biochemical parameters at 490 optical density and 520 optical density.

Table 5: Descriptive statistic of biochemical variables

Stage of Leaves		**O.D 490**		**O.D 520**	
		Mean N=3	**Std. Dev.**	**Mean N=3**	**Std. Dev.**
Phenolic compound	Fresh leaves	0.95	0.02	1.18	0.03
	Young galls	0.95	0.02	1.18	0.03
	Matured galls	1.72	0.01	1.9	0.02
	Perforated galls	1.82	0.03	1.98	0.03
Total chlorophyll content	Fresh leaves	1.05	0.04	1.28	0.02
	Young galls	0.82	0.02	1	0.02
	Matured galls	0.14	0.05	0.25	0.11
	Perforated galls	0.08	0.01	0.16	0.04

O.D. Means Optical Density

From the above table at O.D 490 and 520 phenolic compounds were found considerably higher in fresh leaves which shows the resistance of this plant to eating by psyllids the mean values of phenolic compound increase with the increase in a different stage of galled leaves, in contrast to total chlorophyll content it's found that chlorophyll content decrease with increase in gall stages.

Bivariate correlation of biochemical variables at optical density total

Results of bivariate correlation at O.D total was presented in Table 6 below

Table 6: Bivariate correlation of biochemical variables at optical density total

		Phenolic Content	**Total Chlorophyll**
Phenolic Content	Pearson Correlation	1	-.764**
	Sig. (2-tailed)		0.004
	Sum of Squares and Cross-products	2.073	-1.837
	Covariance	0.188	-0.167
	N	12	12
Total Chlorophyll	Pearson Correlation	-.764**	1
	Sig. (2-tailed)	0.004	
	Sum of Squares and Cross-products	-1.837	2.788
	Covariance	-0.167	0.253
	N	12	12

** Correlation is significant at the 0.01 level (2-tailed).

The above table showed the bivariate correlation among two variables (phenolic compound and total chlorophyll content) from the results, it is found that phenolic compound is strongly negatively correlated with total chlorophyll content at all different stages of gall formation and vice versa at 0.01 level of significance.

Bivariate correlation of biochemical variables at optical density 490

Results of bivariate correlation at O.D 490 was presented in Table 7 below.

Table 7: Bivariate correlation of biochemical variables at optical density 490

		Phenolic Content	Total Chlorophyll
Phenolic Content	Pearson Correlation	1	-.960**
	Sig. (2-tailed)		0
	Sum of Squares and Cross-products	1.46	-1.69
	Covariance	0.13	-0.15
	N	12	12
Total Chlorophyll	Pearson Correlation	-.960**	1
	Sig. (2-tailed)	0	
	Sum of Squares and Cross-products	-1.69	2.14
	Covariance	-0.16	0.19
	N	12	12

** Correlation is significant at the 0.01 level (2-tailed).

The above table showed the bivariate correlation among two variables (phenolic compound and total chlorophyll content) at O.D 490 from the results, it is found that phenolic compound is strongly negatively correlated with total chlorophyll content at all different stages of gall formation and vice versa at 0.01 level of significance.

Bivariate correlation of biochemical variables at optical density 520: Results of bivariate correlation at O.D 520 was presented in Table 7 below

Table 8: Bivariate Correlation of Biochemical Variables at Optical Density 520

		Phenolic Content	Total Chlorophyll
Phenolic Content	Pearson Correlation	1	-.764**
	Sig. (2-tailed)		0.004
	Sum of Squares and Cross-products	2.07	-1.84
	Covariance	0.19	-0.17
	N	12	12
Total Chlorophyll	Pearson Correlation	-.764**	1
	Sig. (2-tailed)	0.004	
	Sum of Squares and Cross-products	-1.84	2.79
	Covariance	-0.17	0.25
	N	12	12

** Correlation is significant at the 0.01 level (2-tailed).

The above table showed the bivariate correlation among two variables (phenolic compound and total chlorophyll content) at O.D 520 from the results, it is found that phenolic compound is strongly negatively correlated with total chlorophyll content at all different stages of gall formation and vice versa at 0.01 level of significance.

Discussion

Incidence and Severity of the Disease in the Study Area

Alstonia scholaris is one of the main canopy-producing trees in Mewar University that provide beautification to the environment, this elegant tree was infected by a galls-producing insect which lead to un control neoplastic in the affected leaves. Yet this zoocecidia have not been reported or recognized and they cause a potential threat to plants in the University in particular and in the horticultural industry in this region as large.

The current study was carried out at Mewar University in Gangrar, Chittorgarh, Rajasthan, India, spanning from May to July 2020. The field survey was conducted between May 1, 2020, and July 15, 2020, with data collection taking place at fifteen-day intervals. The study area was divided into two plots: Block A encompassing Bhamasha Hostel, school garden, and veg mess, and Block B surrounding the academic and administrative block. Each plot was further divided into three replications, and data was collected and recorded meticulously, focusing on the incidence and severity of the observed phenomena.

Finding of this research showed that incidence of the disease based on the location as shown in Table 1, block A has the highest mean of incidence with 97.72% when compared to Block B with 97.00% in the area of study while the severity of the disease is high in the block B this is because block B has many saplings that are most affected by the elation, in general, the severity of the disease in both location was severe with 50.04% in block A and 60.88% in block B.

Table 2 showed the monthly wise incidence and severity of *Alstonia scholaris* foliar galls from the results incidence and severity of the disease increased from May to July this is because galls and almost all plant diseases prevail in wet, warm days and nights, no rainfall, low relative humidity and temperature is high in May and onset of rainfall start in mid-June which in turn increase the incidence and severity of the disease this is in line with the work of Ogah *et al*., (2012) [17] to access the effect of abiotic factors (rainfall, relative humidity and temperature) in two site within two years on the incidence of African rice gall midge (AfRGM) management. Abiotic factors such as rainfall, relative humidity, and temperature exerted a significant influence on the incidence of the disease. The percentage of infestation demonstrated an upward trend corresponding to increased rainfall and relative humidity. Pasayat & Tripathy (2020) [19] also noted that the adult longevity and survival of psyllid insects varied seasonally, with the highest recorded during the rainy season. Monthly wise for each block showed that Block A has disease incidence in June 100% and July 98.67% and least were recorded in May 94.5 wherese severity of the disease was highest in July with 60.33% compare to June 51.4% and moderate infection 38.4% in May Block B showed different trend with Block A having

highest incidence of the disease infestation in May with 99.17% followed by July 97.33% and least were recorded in June 94.5, based on the severity of the enation disease in Block B month of July has the highest and must severe disease severity with 67.66% May 2ith 58.12% and least where recorded in June with 56.85%. This is similar with the work of (Ali *et al*., 2010) [2].
that conducted a survey on incidence and severity of crown gall disease in on apple, apricot and cherry caused by *Agrobacterium tumefeciens*, mean incidence of crown gall disease was found to be highest in cherry fruit with 87.96% high in apple fruit with 87.87 and lowest in apple and no single infestation in apricot fruit, the severity of the disease range from severe, moderate and mild in cherry and apple depending on the location.

Response of *Alstonia scholaris* to Psyllid Herbivore on Biochemical Parameters

Gall formation in *Alstonia scholaris* is primarily induced by chemical stimuli from insect herbivores, leading to anatomical and biochemical disturbances in the host tissue, as observed in this study. The biochemical analysis revealed an increase in phenolic compound activity with different stages of gall leaves, while total chlorophyll content decreased as gall formation progressed. These findings are consistent with the research conducted by Mandal *et al*. (2014) [12], who investigated the biochemical changes induced by the *P. tuberculata* psyllid insect in the plant. Their study focused on the chlorophyll content, phenolic compounds (which serve as defence compounds), as well as the activity of two foliar antioxidant enzymes: Catalase and peroxidase. They also observed an increase in the activity of antioxidant enzymes and phenolic compounds with different stages of gall leaves, while the chlorophyll content decreased as gall formation advanced. Based on these findings, it can be inferred that total chlorophyll and phenolic compounds serve as biochemical markers of foliar gall resistance in *Alstonia scholaris*. Cytological and histochemical investigations were carried out by Oliveira *et al*. (2016) [18] on various insect-induced galls. Their research demonstrated that the initiation of plant galls occurs through the recognition of sensitive plant tissues by gall inducers. Subsequent feeding and/or oviposition by these gall inducers trigger a series of events. Reactive oxygen species (ROS) play a crucial role in gall induction and development. Controlled levels of ROS lead to the accumulation of plant hormones, such as cytokinins, and polyphenols in the affected gall sites. The accumulation of plant hormones and polyphenols disrupts biochemical pathways in the plant, ultimately resulting in gall formation (Biswas *et al*., 2014) [5]. Gall-forming insect species have evolved mechanisms to manipulate plants and stimulate the production of harmful chemicals that cause disorganization of plant tissue, leading to the formation of cancer-like structures known as galls (Teixeira *et al*., 2011) [22]. In most cases, galls are harmless to the host plant. However, they can become a significant problem when they affect the young parts of the plant.

Plants, in their natural environment, encounter various internal and external stresses that require them to respond in order to maintain ecological balance. The resistance of plants to diseases is a result of the interaction between the host and the pathogen, which triggers morphological, biochemical, and other responses depending on the type of infection. A. *Scholaris* exhibits both physiological and biochemical responses to psyllid herbivory. In the case of *Alstonia,* the total chlorophyll content in fresh, uninfested leaves was found to be higher compared to galled leaves. This decrease in chlorophyll content may be attributed to the reduction and disruption of the palisade layer caused by the outgrowth or formation of galls. The loss of chlorophyll serves as a visible symptom of psyllid herbivory. Phenolic compound and total chlorophyll were measured at 490 and 520 optical density colorimetrically. Significant negative correlations were observed between phenolic compound and total chlorophyll content at the 0.01 level in the measurements taken at wavelengths 490 nm, 520 nm, and total optical density. Several studies (Carneiro *et al*., 2014; Yang *et al*., 2007; Samsone *et al*., 2012; Samsone *et al*., 2011; Gailite *et al*., 2005) [6, 24, 21, 20, 9] have reported that galled plants generally exhibit lower chlorophyll content. On the other hand, secondary metabolites, particularly phenolics, are well-known for their role in plant defence against insect herbivory by triggering oxidative responses in plants (Mukherjee *et al*., 2016; Ni X *et al*., 2001) [14, 16].

In general, when a plant is diseased, the production of phenolic compounds increases as a defence reaction against the disease. In the case of *Alstonia scholaris* leaves infested by psyllid herbivory, an increase in phenolic content was observed, leading to elevated oxidative stress in the host plants, particularly in gall-infested leaves compared to fresh leaves. Based on the experimental findings, analytical observations, and statistical analysis, it can be concluded that phenolic compounds serve as biochemical markers of foliar gall resistance in *Alstonia scholaris* R. Br.

Conclusion

At the end of this study, the finding of the research revealed that incidence and severity of *Alstonia scholaris* foliar gall is of growing concern in our environment as it distort the leave shape and size there by making trees less attractive and induces physiological stress that reduced chlorophyll content, disease incidence and severity were found highest during spring or rainy season particularly in month of July 2020 in which the disease incidence reach 98.00% and severity of 64.00% (which is very high) , biochemical parameters estimated after colourimetric analysis shows that phenolic compound were found to be highest in perforated galls followed by matured and young galls, fresh leaves has least phenolic compound and this is an indication that defence chemical were produced to contain the infection, chlorophyll content decrease with increase of gall stages.

Recommendation

Majority of plant galls induce least or no harm to host plant health which means that management of its are not needed most, but unfortunately, when young plants are infected it leads to retarded growth and, defoliation and even death as severe cases due to little attention on insect induce galls-makers almost no known based scientific research alone on efficacy of some insecticidal or biopesticides that managed the disease.

In view of the findings from this study, the following recommendations are hereby proposed:

1. Pruning of the affected part of the trees to remove the galled portion and allow for good air circulation will help to keep the foliage dry.
2. Infected leaves, and dead twigs, should be gotten rid of

through raking and other sanitary practices to limit the infection and reduce the total number of innoculum.
3. Funding by some agricultural and environmental organisations to conduct research on appropriate pesticides that can get rid of plant galls.

Declaration of Conflict of Interest Statement

I declared that all authors of this manuscript titled Incidence, Severity and Biochemical Studies of *Alstonia scholaris* (*L.)* R. Br. Foliar Galls, they have no involvement in any organisation, company or institute with financial or non-financial interest in the subject matter.

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