POPULATION DYNAMICS AND MANAGEMENT OF *Pauropsylla tuberculata* CRAWFORD ON *Alstonia scholaris* (L.) R. BR.

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
ENTOMOLOGY
(Minor Subject: Plant Pathology)

By

Jaideep Singh
(L-2014-A-41-M)

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LUDHIANA – 141 004

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CERTIFICATE – I

This is to certify that the thesis entitled, **"Population dynamics and management of *Pauropsylla tuberculata* Crawford on *Alstonia scholaris* (L.) R. BR."** submitted for the degree of **Master of Science**, in the subject of **Entomology** (Minor subject: **Plant Pathology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Jaideep Singh (L-2014-A-41-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled, **"Population dynamics and management of *Pauropsylla tuberculata* Crawford on *Alstonia scholaris* (L.) R. BR."** submitted by **Jaideep Singh (L-2014-A-41-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science**, in the subject of **Entomology** (Minor subject: **Plant Pathology**) has been approved by the Student's Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

The studies on the "Population dynamics and management of *Pauropsylla tuberculata* Crawford on *Alstonia scholaris* (L.) R.Br." were conducted at the Punjab Agricultural University, Ludhiana during 2015 and 2016. Variation in population of psyllids during the year, morphological and anatomical changes brought about by the infestation of psyllids and the efficacy of different insecticides against the psyllids was studied at different locations. The pooled mean number of galls/leaf and galls/pod varied 10.17 to 47.37 and 3.04 to 44.48, respectively during the different months of the year. The mean psyllids emerged from galls on leaves and galls on pods ranged from 7.91 to 42.03 and 2.48 to 35.60, respectively. The population of psyllids with respect to galls per leaf and pods were positively correlated with the temperature (maximum and minimum), rainfall and negatively correlated with the relative humidity. *P. tuberculata* has five nymphal instars in its life cycle. The diameter of the gall chamber on *Alstonia* leaves of *P. tuberculata* ranged from 0.1 cm to 1 cm. The thickness of upper epidermis and lower epidermis was more in healthy leaves than infested leaves. In the mesophyll cells, the thickness of palisade tissues, spongy tissues and vascular bundles was more in the infested leaves as compared to healthy leaves. The total soluble sugar content, total soluble protein content and proline content was significantly higher in the infested leaves and pods as compared to healthy leaves and pods. Whereas, the chlorophyll content (both chlorophyll a and chlorophyll b) was significantly higher in healthy leaves and pods. Among the selected insecticides, Thiamethoxam 25 WG @ 0.3 g, 0.6 g and 0.9 g per litre of water resulted in 99.99 per cent nymphal mortality after 10 days of exposure during 2nd spray under field conditions.

Keywords: *Pauropsylla tuberculata*, *Alstonia scholaris*, leaf galls, pod galls, total soluble sugar content, total soluble protein content, proline content, chlorophyll content, nymphal mortality, insecticides

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Signature of Major Advisor Signature of the Student

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ਸਾਰ

ਅਲਸਟੋਨੀਆ ਸਕੋਲੈਰਿਸ (ਐਲ.) ਆਰ.ਬੀ.ਆਰ. ਉੱਤੇ *ਪੌਰੋਸੀਲਾ ਟਿਊਬਰਕਉਲੇਟਾ* ਕਰਾਅਫੋਰਡ ਦੀ ਜੰਨਸੰਖਿਆ ਦਾ ਆਂਕਲਣ ਅਤੇ ਰੋਕਥਾਮ ਉੱਤੇ ਅਧਿਐਨ ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ ਵਿਖੇ ਸਾਲ 2015-2016 ਦੌਰਾਨ ਕੀਤਾ ਗਿਆ। ਵੱਖ-ਵੱਖ ਥਾਵਾਂ ਤੇ ਸਾਲ ਦੌਰਾਨ ਸਿਲਿਡ ਦੀ ਸੰਖਿਆ ਵਿੱਚ ਉਤਰਾਅ ਚੜਾਅ, ਸਿਲਿਡ ਦੇ ਹਮਲੇ ਕਾਰਨ ਹੋਇਆ ਬੂਟੇ ਆਕਾਰ ਵਿੱਚ ਅਤੇ ਕੌਸ਼ ਪੱਧਰ ਤੇ ਬਦਲਾਅ ਅਤੇ ਵੱਖ-ਵੱਖ ਕੀਟਨਾਸ਼ਕਾਂ ਦੇ ਪ੍ਰਭਾਵ ਬਾਰੇ ਨਰੀਖਣ ਕੀਤਾ ਗਿਆ। ਸਾਲ ਦੇ ਵੱਖ-ਵੱਖ ਮਹੀਨਿਆਂ ਦੌਰਾਨ ਕੁੱਲ ਮਿਲਾ ਕੇ ਛਾਲਿਆਂ ਦੀ ਪ੍ਰਤੀ ਪੱਤਾ ਗਿਣਤੀ 7.91 ਤੋਂ ਲੈ ਕੇ 42.03 ਅਤੇ ਪ੍ਰਤੀ ਫ਼ਲੀ ਗਿਣਤੀ 2.48 ਤੋਂ ਲੈ ਕੇ 35.60 ਸੀ। ਸਿਲਿਡ ਦੀ ਜੰਨਸੰਖਿਆ ਪ੍ਰਤੀ ਪੱਤਾ ਅਤੇ ਫ਼ਲੀ ਤਾਪਮਾਨ (ਅਧਿਕਤਮ ਅਤੇ ਨਿਉਨਤਮ), ਵਰਖਾ ਨਾਲ ਨਿਸ਼ਚਿਤ ਤੌਰ ਤੇ ਵੱਧਦੀ ਅਤੇ ਤੁਲਨਾਤਮਕ ਨਮੀ ਨਾਲ ਨਕਰਾਤਮਕ ਤੌਰ ਤੇ ਘਟਦੀ ਸੀ। *ਪੀ. ਟਿਊਬਰਕਉਲੇਟਾ* ਦੇ ਪੂਰੇ ਜੀਵਨ ਚੱਕਰ ਦੌਰਾਨ ਨਿੰਫ ਦੀਆਂ ਪੰਜ ਤਰ੍ਹਾਂ ਦੀਆਂ ਸਥਿਤੀਆਂ ਜਾਂ ਪੜਾਅ ਸਨ। *ਪੀ. ਟਿਊਬਰਕਉਲੇਟਾ* ਦੇ ਛਾਲਿਆਂ ਦਾ ਵਿਆਸ *ਅਲਸਟੋਨੀਆ* ਪੱਤਿਆਂ ਉਪਰ 0.1 ਸੈ.ਮੀ. ਤੋਂ ਲੈ ਕੇ 1 ਸੈ.ਮੀ. ਸੀ। ਉਪਰੀ ਪਰਤ ਅਤੇ ਹੇਠਲੀ ਪਰਤ ਦੀ ਮੋਟਾਈ ਰੋਗ ਰਹਿਤ ਪੱਤੇ ਵਿੱਚ ਹਮਲੇ ਵਾਲੇ ਪੱਤੇ ਨਾਲੋਂ ਜ਼ਿਆਦਾ ਸੀ। ਵਿਚਲੀ ਪਰਤ (ਮਿਜ਼ੋਫਿਲ) ਦੇ ਕੋਸ਼ਾਂ ਵਿੱਚ, ਪੈਲੀਸੇਡ ਤੰਤੂਆਂ, ਸਪੰਜ ਵਰਗੇ ਤੰਤੂਆਂ ਅਤੇ ਵੈਸਕੁਲਰ ਗੱਠਾਂ ਦੀ ਮੋਟਾਈ ਹਮਲੇ ਵਾਲੇ ਪੱਤੇ ਵਿੱਚ ਰੋਗ ਰਹਿਤ ਪੱਤਿਆਂ ਤੋਂ ਜ਼ਿਆਦਾ ਸੀ। ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਖੰਡ, ਪ੍ਰੋਟੀਨ ਅਤੇ ਪ੍ਰੋਲੀਨ ਦੀ ਮਾਤਰਾ ਹਮਲੇ ਵਾਲੇ ਪੱਤਿਆਂ ਅਤੇ ਫ਼ਲੀਆਂ ਵਿੱਚ ਰੋਗ ਰਹਿਤ ਪੱਤਿਆਂ ਅਤੇ ਫ਼ਲੀਆਂ ਤੋਂ ਨਿਸ਼ਚਿਤ ਤੌਰ ਤੇ ਜ਼ਿਆਦਾ ਸੀ। ਜਦਕਿ ਕਲੋਰੋਫਿਲ ਦੀ ਮਾਤਰਾ ਰੋਗ ਰਹਿਤ ਪੱਤਿਆਂ ਅਤੇ ਫ਼ਲੀਆਂ ਵਿੱਚ ਜ਼ਿਆਦਾ ਸੀ। ਚੁਣੇ ਹੋਏ ਕੀਟਨਾਸ਼ਕਾਂ ਵਿੱਚੋਂ ਥਾਇਆਮਿਥੋਕਜ਼ਾਮ 25 ਡਬਲਯੂ ਜੀ ਦਾ 0.3, 0.6 ਅਤੇ 0.9 ਗ੍ਰਾਮ ਪ੍ਰਤੀ ਲਿਟਰ ਪਾਣੀ ਦੀ ਦਰ ਤੇ ਦੂਸਰੀ ਵਾਰੀ ਛਿੜਕਾਅ ਕਰਨ ਤੋਂ 10 ਦਿਨ ਬਾਅਦ 99.99 ਪ੍ਰਤੀਸ਼ਤ ਨਿੰਫ ਦਾ ਨਾਸ਼ ਹੋਇਆ।

ਮੁੱਖ ਸ਼ਬਦ: *ਅਲਸਟੋਨੀਆ ਸਕੋਲਰਿਸ*, *ਪੌਰੋਸੀਲਾ ਟਿਊਬਰਕਉਲੇਟਾ*, ਪੱਤਿਆਂ ਉਪਰ ਛਾਲੇ, ਫ਼ਲੀਆਂ ਉਪਰ ਛਾਲੇ, ਘੁਲਣਸ਼ੀਲ ਖੰਡ ਦੀ ਮਾਤਰਾ, ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ, ਪ੍ਰੋਲੀਨ ਦੀ ਮਾਤਰਾ, ਕਲੋਰੋਫਿਲ ਦੀ ਮਾਤਰਾ, ਨਿੰਫ ਦਾ ਨਾਸ਼, ਕੀਟਨਾਸ਼ਕ

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ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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CHAPTER I

INTRODUCTION

Alstonia scholaris (L.) R. Br. is an evergreen landscape tree belonging to family Apocynaceae, commonly called scholar tree, blackboard tree, Indian devil tree, ditabark, milkwood pine, white cheesewood, saptparni, satvan, chativan and satpatia. It is a beautiful foliage tree with large canopy and is one of the most popular ornamental trees in landscapes, garden as well as roadside plantations in warm and temperate regions. *Alstonia* is widely distributed in various geographical regions of India. Its generic name *'Alstonia'* has been coined in the honour of Professor C. Alston who was a distinguished Botanist of Edinburgh University (UK). It has been traditionally used in India as wooden planks *(patties* or *phatties*) for writing or tracing letters by school children. Its leaves are used traditionally as medicine for relieving dysentery, fever, tumour, jaundice, hepatitis, malaria and skin diseases (Chander 2014). One of the most important alkaloid present in the plant called alstonine (Pratyush *et al* 2011) was reported to have anticancerous properties (Beljanski *et al* 1982). However, the beauty of the tree is being impaired by the attack of several insect and mite pests.

Several gall inducing thrips and eriophyid mites have been documented in India on different host plants. Limited notes on gall inducing psyllids are available in India. The gall inducing species of Hemiptera are distributed into four superfamilies namely Aphidoidea, Psylloidea, Coccoidea and Aleyrodoidea (Raman 2003). Within family Psyllidae, there are nearly 350 gall inducing species occurring mainly on the leaves of dicotyledonous plants (Hodkinson 1984). Psyllid galls usually contain only one nymph per chamber, but some galls have been found to house more than one nymph. Psyllid galls may have either simple or complex structures, and can be found either as isolated or aggregated (Hodkinson 1984, Dreger-Jauffret and Shorthouse 1992, Raman 2003) in distribution.

Development and formation of leaf galls by homopterans is generally associated with the feeding habits of these insects which extract sap from the xylem, phloem and non conducting tissues of the plant. Covering growth, pouch galls are formed on the both sides of the leaves which open with an ostiole which is very small in immature galls but enlarges with the development of insect and opens to liberate the adults (Saini and Sarin 2012).

A. scholaris is attacked by several insect pests like leaf skeletonizer, lactid borer and leaf gall former. Leaf gall former, *Pauropsylla tuberculata* Crawford (Psyllidae: Homoptera) is one of the major pest of *A. scholaris*. It induces gall formation on each and every part (stem, leaves, inflorescence etc.) of this highly prized tree, which adversely affects the looks and its economic value. Galls on the leaves of *A. scholaris* were noticed in 2005 in

Chandigarh (Chander 2014), however, the attack at that time was not severe, and the insect and its damage on leaves were not taken seriously till 2009. In 2010, there was an epidemic of black brown galls on *A. scholaris* leaves in Punjab, Haryana, Chandigarh and Delhi. The causal organism of *Alstonia* leaf galls was identified as a psyllid (jumping plant louse*), P. tuberculata* (Albert *et al* 2011).

The psyllids has five nymphal instars, from eclosion of the egg to the adult instar, displaying mechanisms that protect them from loss of humidity (Raman 2003, Gullan and Martin 2003). Free feeding psyllids are more active during periods of high humidity while others produce wax as a strategy to avoid water loss (Hodkinson 1984).

Till 2013, galls of the insect were restricted to the leaves. However, towards the end of March, 2014, some elongated structures were also observed for the first time on follicles (fruits) of *A. scholaris* in Punjab, Chandigarh and Haryana (Chander 2014). These elongated structures took the shape of galls and were similar to those formed on the leaves. Ninety per cent fruits were found to be attacked by the galls (Chander 2014). The adults emerged from the fruits in the end of April and most of the affected follicles did not crack open for releasing air borne winged seeds in the first fortnight of May.

Galls gradually affect the growth and development of *A. scholaris*. Galled leaves become unfit to photosynthetic activity due to which leaves are distorted, growth is stunted and *Alstonia* tree dries up permanently. Galls were also observed on the newly developing leaves. Such leaves do not reach to maturity. *P. tuberculata* also makes galls on the inflorescence and follicle fruits of *A. scholaris* due to which reproductivity of plant is also affected (Jain and Dhiman 2014).

The female of *P. tuberculata* lays eggs on the abaxial surface of the developing leaves singly or in groups at more than one place. The eggs appear white, oblong, narrow and acute at one end. It is presumed that the insect alongwith the eggs, deposits some fluid which acts as a stimulant for the initiation of the gall (Albert *et al* 2011). Lysis of the cells occurs leading to the formation of a depression. This process stimulates hypertrophic response resulting in hyperplasia (cell division) and subsequent formation of the gall. The nymph (the first instar) undergoes moulting to reach the adult stage (imago). The nymph is yellow and all the instars are developed inside the zoocecidia. At the time of emergence, the exit hole is made either on the dorsal or ventral side of the leaf. Characteristic white waxy secretion of the nymph occurs in abundance surrounding it. Moulted exuviae of second and third instars are often found on the leaf surface.

The affected plants give ugly look and as a consequence, its planting is being abandoned by several organizations and the young plants are also being removed from urban

landscape leading a big setback to urban forestry. Keeping these facts in view, present study on the population dynamics and management of *P. tuberculata* on *A. scholaris* was planned to achieve the following objectives:

a) To study the population dynamics of *P. tuberculata* on *A. scholaris.*

b) To study the effect of *P. tuberculata* galls on the anatomy, morphology and biochemical composition of *Alstonia* leaves and pods.

c) To develop the chemical control strategy for the management of *P. tuberculata.*

CHAPTER II

REVIEW OF LITERATURE

The review of work pertaining to the study entitled "Population dynamics and management of *Pauropsylla tuberculata* Crawford on *A. scholaris* (L.) R. Br. carried out at Punjab Agricultural University has been presented and discussed under the following different sub headings:

2.1 Population dynamics

2.2 Anatomical alterations

2.3 Morphological alterations

2.4 Biochemical alterations

- 2.4.1 Chlorophyll
- 2.4.2 Carbohydrates
- 2.4.3 Total Soluble Proteins
- 2.4.4 Proline

2.5 Management

- 2.5.1 Monitoring
- 2.5.2 Cultural Control
- 2.5.3 Chemical Control

With about 2000 different galls (attributable to same number of inducing-insect species), the Indian subcontinent displays a rich variety in gall flora (Raman 2007). Among the insects various Hemipteran are known to be capable of inducing galls (Rohfritsch 1992, Wool *et al* 1999, Raman 2003). In the Psyllidae, there are close to 350 species of gall inducers which attack primarily the leaves of dicotyledons (Hodkinson 1984).

Valuable information is available on the different aspects of plant galls induced by the gallmaker Psyllidae, *P. tuberculata* in India, viz. cecidogenetic behavior of some gall inducing insects and morphogenesis of their galls. The morphoanatomical and biochemical changes taking place during the different developmental stages of foliar galls in *A. scholaris* have also been documented. Further information on infested foliar tissues compared with non-infested ones and interactions between gallmakers and the host plants have been studied by Raman (2003).

P. tuberculata, a gall inducer on leaves of *A. scholaris*, is an example of this group of galling insects. Galls on *A. scholaris* caused by *P. tuberculata* are typically covering pouch

galls in which the plant tissue grows around and above enclosing the cecidozoa that lies initially exposed on the surface of the organ. Many authors have reported different morphological types of galls on the same plant caused by different insects (Gonçalves Alvim and Fernandes 2001, Scareli-Santos and Varanda 2003). In *A. scholaris* only one type of gall was observed. According to Rohfritsch (1992), young plant tissues are recorded to present responses against inducing insects when compared to already differentiated tissues. Galls in *A. scholaris* occur on both young and mature leaf tissues indicating that tissues in such species react against the inducing insects regardless of the leaf age of *Baccharis dracunculifolia* (Arduin and Kraus 2005), in *Tabebuia ochracea* (Scareli-Santos and Varanda 2003) and in *Pouteria torta* (Scareli- Santos *et al* 2008).

2.1 Population dynamics

Jain and Dhiman (2014) reported that the gall insect, *P. tuberculata* Crawford which forms galls on *A. scholaris*, begins in the last week of March which coincides with the emergence of new flush of leaves. Adult female of *P. tuberculata* laid eggs at the side of midribs and veins on the ventral surface of the leaves. On an average 170.4-186.2 eggs were observed on a single leaf. The eggs hatched and thereafter went through five wingless nymphal stages (i.e. N1, N2, N3, N4 and N5) before becoming winged adults. Nymphs of all five instars were found at the same time in the gall.

Adults of *P. tuberculata* appear in late March and these oviposited eggs on leaves. Due to this, gall formation increases from late April onwards. Maximum number of gall were observed from August to October at 19-32^0 C and 55-93% R.H. From late November to February, no new gall formation occurred and adults died after depositing eggs.

2.2 Anatomical alterations

Eggs of *P. tuberculata* deposited on the leaves of *A. scholaris* trigger the induction of gall. The nymphal stages feed on the leaf, where the eggs are deposited and it stimulates the gall development by translocating a chemical stimulus on the adaxial and abaxial side of the *A. scholaris* leaves. Mc Calla *et al* (1962), working on the leaf galls of willow induced by sawflies, observed that the initial stimulus for the initiation of gall formation comes from the fluid formed in accessory glands of female which is injected into the plant by the female at the time of egg laying. By depositing multiple eggs at the same host location, multichambered galls develop and such a development is considered as an evolved trait among gall inducing insects (Stone *et al* 2002). The saliva causes the lysis of the upper epidermis and mesophyll or it may lead to infolding of the papillate lower epidermis. This stimulus results in hypertrophy of cells next to the location of the deposited eggs.

Hypertrophy is followed by intense hyperplasia which brings about elevation of palisade parenchyma which undergoes division by anticlinal division. The psyllid has three nymphal instars, from eclosion of the egg to the mature insect, displaying mechanisms that protect them from loss of humidity (Gullan and Martin 2003, Raman 2003). Free feeding psyllids are more active during periods of higher humidity while others produce wax as a strategy to avoid water loss (Arduin *et al* 2005). In contrast, psyllids galls on *A. scholaris* are entirely closed and chambers are not lined by any waxy secretion. However the nymphs appear to produce waxy deposit around it once it comes out of the chamber. Within Psyllidae galls, with few exceptions, nutritive tissue formations were not observed by Rohfritsch (1992).

Hemipteran insects introduce stylets into the tissue and they take some time exploring the tissue and injecting viscose substances forming a salivary sheath (Arduin *et al* 2005) which facilitates the penetration of the insect proboscis or prevents the plant unleashing its hypersensitivity reactions to combat the damage inflicted upon it (Fernandes 1990, Milles 1999). Raman (2003) considered the penetration to be intercellular with the dissolution of middle lamella of the adjacent cells, though Spiller *et al* (1985) and Tjallingh and Hogen Esch (1993) revealed that this always was not the case, because the aphid stylet could penetrate between the wall and the plasmatic membrane and may even display an intracellular path. In *A. scholaris*, the salivary sheath was observed extended into the palisade parenchyma and reaching the xylem element of the galls. The same type of observation was noted by Arduin *et al* (2005) on *B. dracunculifolia* (DC.). In hemipteran galls, the vascular system may be altered. Notably gall vascular bundles were predominately phloematic in *A. scholaris* as observed in galls induced by *Schizomyia macrocapillata* Maia on *Bauhinia brevipes* Vogel (Sá *et al* 2009) and by the gall midge *Izeniola obesula* Dorchin on *Suaeda monoica* Forssk (Dorchin *et al* 2002). In the galls induced by *Geoica wertheimae* Brown and Blackman (Pemphigidae: Forminae) on turpentine tree, *Pistacia palestina* Boiss (Anacardiaceae), seven layers of cells were identified as phloematic which facilitated the access of the inducers to alimentary resources (Wool *et al* 1999). An increase in phloem tissues was also observed in *Baccharopelma dracunculifoliae* (DC) gall. Most of them showed a lack of xylem cells (Arduin *et al* 2005). The non differentiation of these lignified cells was also observed in the ambrosia galls induced by an unidentified species of Cecidomyidae on leaves of *Baccharis concinna* (L.) (Arduin *et al* 2005) and may indicate that the energy otherwise used to differentiate lignified cells is deviated to other gall requirements. Phloematic bundles indicates the establishment of a photosynthtates drain to the gall tissues (Rohfritsch 1992, Sá *et al* 2009). Induction followed by enlargement of the nymphal chamber is seen close to phloem cells in *P. tuberculata* galls on *A. scholaris* (Albert *et al* 2011). Predominant

phloematic bundles were reported for other galls (Kraus *et al* 2003, Arduin *et al* 2005) and it plays an important role in nutrient supply of the growing gall at the expense of the other plant tissue (Vandevyvere and De Bruyn 1998).

Galls are considered a significant drain on leaf resources (Fay *et al* 1993, Nyman and Julkunen-Tiitto 2001). Accumulation of food materials in cells around the nymphal chamber is commonly observed in the foliar galls (Arduin *et al* 2005). Shrinkage and dying up of cells lining the opening in the mature gall has been observed. The size of the ostiole increases facilitating the escape of the insect nymph. The nymph moves through this cleaved passage towards the exit, moults and escapes as an adult winged insect. At gall senescence, around the insect chamber and the exit channel a healing tissue is formed (Albert *et al* 2011).

2.3 Morphological alterations

Albert *et al* (2011) reported some morphological changes in *A. scholaris.* Galls were formed on both abaxial and adaxial surfaces of the leaves. But they were abundant on the abaxial surface. Initiation of the gall formation results from the oviposition on leaves by *P. tuberculata*. The first visible change is a slight discolouration on the areas where the eggs are deposited. Chemical stimulus brings about degeneration of surrounding cells forming a small cavity within which the egg lies. Gradually the discoloured area increases in size and forms a small outgrowth on an adaxial side where the gall appears enlarged and placed in a depression or a small circular spindle shaped pit. At initial stage the gall grows towards the abaxial side of the leaf but later on its growth is towards the adaxial side. A little bulge appears on the adaxial side of the leaf which further develops into a dome shaped structure. The galls are mainly formed on the lateral veins. The nymph at initial stage is enclosed within the gall tissue. A cover cone rapidly grows over the insects from the tissue surrounding and enclosing it completely. The maximum number of galls were found lateral to the second order veins. These were atriate galls with cavities within. The cavity was lined by closely packed mass of chlorophyll lacking cells.

Cells are surrounded by a very thick mass of parenchyma with some conductive tissues. When the leaves are heavily galled the lamina has been completely reduced to a single agglomerate mass of cells. Both young and mature leaves are affected. With an increase of galls in number all the leaves appeared crumbled and completely deformed. The leaf galls occured scattered and isolated or in agglomerated clusters resulting in the crinkling of the leaves. With further development on the surface of the gall a depression/halo was formed. The chamber formed was open to the abaxial side by means of a small opening/ostiole. With the growth of the gall the ostiole becomes indistinct and a well defined cavity is formed. The

mature epiphyllous pouch galls were unilocular with a single chamber or multilocular with 3–4 chambers.

The mature gall was monothalamus subcylindrical, concolorous with the host leaf, except at the apex where it is yellowish. The wall is thick and succulent. The chamber was subcuneate with the sharp edge pointing distal end. The opening was apical, subcircular without any appendage (Alber *et al* 2011). The galls were persistent and remained on the leaf long after the escape of the Cecidozoa. The galls appear multilocular because the adjacent galls coalesce. The number of galls varies from 25 to numerous on a leaf. Depending upon the maturity of the galls the diameter of the gall chamber ranges from 0.1 cm to 1 cm. The mature gall externally does not differ much from the previous stages except in size. The whole gall appears fleshy. The nymph at its third or fourth instar emerged from a small circular opening formed in the centre of the gall. The senescent stage is characterized by the presence of small orifices on the gall surface. After the emergence of the insects the cells close to the chamber and exit canals form a protective layer which appeared as a yellowish brown rim.

The formation of galls on the leaf affects the size and shape of leaf, depending upon the site of gall, shape of gall, structure of gall and the insect causing the gall. Chen *et al* (2011) investigated the different galls formed by aphids. He recorded that galls may occur on the leaf blade, the leaf vein, the petiole, the main axis of a compound leaf, twigs, branches and roots. *Viteus vitifoliae* (Fitch) causes galls on grape leaves and gall-like swellings on grape roots. *Pemphigus bursarius* (Linnaeus) produces galls on the petioles of *Populus simonii*. The galls of *Floraphis meitanensis* Tsai and Tang are located on the main axis of the compound leaf of *Rhus punjabensis* var. *sinica*. Among these galls, the leaf gall is the most common type. Krishnan *et al* (2011) reported the leaf lamina or the leaf-blade as the most favoured organ for galling. Nearly sixty per cent of galls are foliar galls followed by stem galls including shoot apex; other organs are meagre in galling. Reale *et al* (2014) investigated gall development induced by *Dryocosmus kuriphilus* on *Castanea sativa* and they observed small chlorotic zones in the leaf primordia were observed. Chlorotic zones increased in thickness as a consequence of abnormal development of the central rib and lamina which did not occur in normal buds.

Schyzomyia macrocapillata gall in *Bauhinia brevipes* exhibited leaf lamina which was barely thickened, but hypertrophied at the site of larval feeding. This site was characterized by a small invagination from the abaxial to the adaxial side of the lamina. Adaxial epidermal cells were not as globose as in healthy leaf lamina and presented neoformed non-glandular trichomes. The region of connection of the gall to leaf lamina was thickened (Mendes de sa *et al* 2009).

Lara and Fernandes‘s (1994) emphasized that the swelling of the leaf tissue and their histological analysis indicated reduced hyperplasia of the tissues during the folding phase of the gall and an intensification of this process during its swelling phase. The formation of this gall wass a primary consequence of hyperplasia, although cell hypertrophy, largely of the schizogenous secretory cavities, also occurred.

False willow, *B. dracunuculifolia* leaves sustained several tissue alterations as a result of gall induction. On the gall epidermis, the main modification was the neoformation of unicellular or multicellular slit trichomes. According to Meyer and Maresquelle (1983), the trichomes have been shown to play a protective role for the ostiole or slit in various galls. In unaffected leaves of *B. dracunuculifolia,* schizogenous secretory cavities accumulate lipophylic substances when they reach maturity. The accumulation of these substances was not observed in galls caused by *B. dracunuculifolia.* Three processes that affect secretory structures have been observed in many galls: neoformation, hypertrophy and obstruction of the secretory system (Meyer and Maresquell 1983). In the gall studied here, alteration of the secretory cavities best exemplified the process of hypertrophy, although these structures are not apparently functional, due to the absence of lipophylic content in most of them.

Scareli-Santos and Varanda (2003) investigated the morphological and histochemical characteristics of galls induced by insects in leaves of *T. ochracea* (Chem.) Standl. (Bignoniaceae). The cone shaped galls were distributed along the mid vein on the adaxial surface and on the leaves of the abaxial surface. The young galls are isolated, with a velvety appearance and a yellowed chestnut coloration, having only a single chamber. In a longitudinal section, the developed larval chamber is observed with only single inducers larvae. The gall has an epidermis with homogenous cells, followed by a parenchyma with different shaped and sized cells, and a small region of nutritive tissue.

2.4 Biochemical alterations

2.4.1 Chlorophyll

Albert *et al* (2011) reported that the tissues of ungalled leaves of uninfested and infested tree showed a slight variation. The galled tissues showed a decrease in the chlorophyll content with the increase in the growth of the gall becoming very low in the mature gall.

Tissues of ungalled leaves of uninfested and infested trees showed a slight variation. The galled tissues showed a decrease in the chlorophyll content with the increase in the growth of the gall and reaching a very low level in the mature gall. This loss of chlorophyll is responsible for the decolourisation of the area of the leaf where egg was laid in *Ficus* leaves

(Moghe 1980). The low chlorophyll content in galled tissues was due to the loss of palisade tissues, disappearance of chloroplast and modifications of spongy mesophyll.

The *Terminalia arjuna* (Arjun) and *Terminalia tomentosa* leaves with gall infestation showed a decrease in the chlorophyll content which varied in the differentially infested leaves. Higher the number of galls, lower the content of chlorophyll observed in the leaves of both the host plants. The leaves with 28 galls 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 galled and 69.75 mg/g in the ungalled in case of *T. arjuna* (Mukherjee *et al* 2016).

Chlorophyll content of gall tissues generally shows a decrease as growth progresses. This loss of chlorophyll is responsible for the decolourisation of the area of the leaf where egg was laid on leaves (Moghe 1980).

2.4.2 Carbohydrates

Albert *et al* (2011) reported that the level of sugars in the ungalled leaves of uninfested and infested trees show no variation. A steady increase of sugar content is noticed in the galled leaves and the burst gall registered the highest level.

Generally the level of sugars in the ungalled leaves of uninfested and infested trees show little variation. However, steady increase of sugar content is noticed in the galled leaves and the burst gall registered the highest level. The gall tissue usually accumulates starch which is not present in the ungalled tissue. The stimulus of gall forming insects redirects 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 neighboring parts of the gall (Hartley 1998).

Mukherjee *et al* (2016) reported that the level of total sugars in the gall infested leaves showed variation with the number of galls. A steady increase of sugar content was noticed in the galled leaves initially which gradually reduced as the infestation progressed. Comparatively a significant difference was observed in ungalled and the gall infested leaves. The level of total sugars in gall infested leaves increased at initial stages as the tissue accumulates starch which is generally less in the ungalled tissue.

2.4.3 Total soluble protein

Albert *et al* (2011) reported that the protein content in normal leaf of uninfested plant and infested tree was more or less equal. The unburst galled tissue showed almost two fold increases in the protein content. The protein content showed an initial increase and registered the highest level during the young galled stage of their development and declined thereafter in the mature burst galled tissue where in the nymphal stage had already exited out from the chamber.

The total soluble protein content in the normal leaf of uninfested tree as well as infested tree has been reported to be more or less equal. The unburst galled tissue showed almost two fold increases in the protein content. The protein content showed an initial increase and registered the highest level during the young galled stage of their development and declined thereafter in the mature burst galled tissue where in the nymphal stage had already emerged out from the chamber. The higher protein concentration observed in the galled tissue corroborates the observations of Mehalingam (1999) and Scareli-Santos and Varanda (2003). It is also in accordance with Arora and Patni (2001) and El-Akkad (2004).

The histochemical analysis revealed the presence of proteins and tannis in galled and ungalled tisses, with higher concentrations in the galls. Starch grains were only observed in the galls. The *T. ochracea* galls are characterized by extensive structural and physiological alterations, as well as by the abnormal growth of its cells, typical of tumors. The alterations, such as modified tissue with starch grains, higher proteins and tannin concentration, resulted from the stimulus from gall-forming insect observed by Scareli-Santos and Varanda (2003).

Higher protein content was recorded in the infested leaf of *T. arjuna* and *T. tomentosa*. 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 (Mukherjee *et al* 2016).

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 importance in plant defense mechanisms (Reinbothe *et al* 1994). The increased secretion of defensive proteins by host plants that block the action of proteolytic enzymes from herbivores contributes higher proteins in the infested leaves. 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 (Anathakrishnan 2001). Further, there is a reduced total protein in the highly infested leaf which might be due to the lower protein requirement and also the host plant would have reached the saturation levels of proteins.

2.4.4 Proline

Proline is produced as a defense mechanism to protect from invaders (biotic stress) or other stress factors (abiotic) and is believed to be an adaptive response to the altered conditions. Proline accumulation is known to be a response to stress condition in plants (Gibon *et al* 2000, Bates *et al* (1973). A normal leaf from an uninfected tree does not have any trace of proline whereas a normal leaf of an infested tree contain a very low concentration of proline. The galled tissue showed a very high proline content. In the mature gall the proline content double than the young gall. Induction of proline in galled tissues indicates that this has been probably produced due to the stress.

Albert *et al* (2011) reported that a normal leaf from an uninfected tree showed absolutely no trace of proline. A normal leaf of the infested branch of an infested tree showed a very low concentration of proline. The galled tissue showed a very high increase in the proline content and in the mature gall the content was very high almost double fold than the young gall indicating stressed condition of the galled tissue.

Significant elevated levels of proline were recorded in the gall infested *T. tomentosa* and *T. arjuna* leaves compared to the proline content in the ungalled leaves. The ungalled leaves of *T. tomentosa* recorded 8.0±1μg/ml and 10.3±1.9 μg/ml in *T. arjuna* plant. The gall infested leaves showed higher proline, viz. 12±2 μg/ml in 5 galled leaf, 20±2 μg/ml in 15 galled and 35±2 μg/ml in 28 galled leaf of *T. tomentosa* plant. Similar trend was also observed in the *T. arjuna* plants (Mukherjee *et al* 2016). Increase in proline content was observed in galled leaves of *Populus* (El-Akkad 2004). It is also reported that when galls are formed, secondary chemicals get isolated from nutritive tissue and are concentrated in the peripheral tissues at proportions ten times higher than that in non-galled leaves.

2.5 Management

Most psyllids on landscape trees and shrubs do not need to be managed to protect plant health. Species warranting control action are the invasive psyllid species that cause intolerable damage (Dreistadt *et al* 2004).

2.5.1 Monitoring

Monitoring of psyllid abundance on susceptible plants on a regular basis leads to initiate control actions before psyllid abundance or when the damage approaches the level that is found to be intolerable (Grafton-Cardwell and Daugherty's 2013). Psyllids are generally monitored by using sticky traps to capture adults, beat or shake foliage to dislodge adults so they can be counted and susceptible plant parts can be inspected for eggs, nymphs and adults. Regular monitoring helps to determine whether natural enemies are becoming more abundant

and may provide the needed levels of biological control or the most effective time to take action, if management with pesticides is effective.

2.5.2 Cultural Control

To suppress populations of phloem-sucking insects, such as psyllids, appropriate irrigation should be provided and nitrogen fertilizer should not be applied to established woody plants, unless foliage appearance or plant growth is unsatisfactory because of a confirmed nutrient deficiency. For eucalyptus, drought stress increases damage from both lerp psyllids and longhorned borers (Halbert *et al* 2006). For established plants, apply water beneath the outer canopy, not near trunks. A general recommendation is to irrigate established trees infrequently (possibly once a month during drought periods) but with sufficient amounts so that the water penetrates deeply into soil (perhaps about 1 foot or more below the surface). Shearing or clipping of terminals should be minimized to retard new growth, which is preferred by psyllids for feeding and egg laying (Paine *et al* 2009). Prune by cutting plants just above branch crotches and nodes instead of shearing off terminals. Avoid planting problem-prone plants and consider replacing them with pest-resistant species and cultivars that are well adapted to local conditions.

2.5.3 Chemical Control

Non-residual contact insecticides, short-residual translaminar insecticides, and long-lasting systemic insecticides can be a part of effective management of psyllids (Hoddle 2013). These insecticides are most compatible with Integrated Pest Management (IPM). It has the least adverse effect on bee and natural enemy populations. Nonresidual contact insecticides include azadirachtin (AzaMax, Safer Brand BioNeem), neem oil (Green Light Neem, Schultz Garden Safe Brand Neem) and insecticidal soap (Safer).

Systemic neonicotinoids are the most practical insecticides for controlling psyllids infesting large plants and where the more IPM-compatible products are inadequate. Neonicotinoids such as dinotefuran (Safari) and imidacloprid are absorbed by one plant part (e.g., roots or trunks) and moved (translocated) to other plant parts (Percy *et al* 2012). Some products can be sprayed onto plant foliage, but drenching or injecting soil, or for woody species injecting or spraying trunks, minimizes environmental contamination and may be more effective than spraying foliage. Trunk application of systemic insecticide can provide relatively rapid control. With soil application, there is a longer time delay between application and insecticide action. Carbamates (such as carbaryl), the systemic organophosphate acephate, nonsystemic organophosphates (malathion), and pyrethroids (fluvalinate, permethrin) can also be applied.

If psyllids are observed in home gardens application of broad spectrum foliar sprays of Carbaryl and Malathion can be done rapidly to control adults and protect plants for many weeks. The systemic insecticide, Imidacloprid is available for use as a soil drench, which moves through the roots to the growing tissues of the plant. This systemic insecticide provides good long term control (1-2 months) of the nymphs, which are hard to reach with sprays because they are tucked inside the small leaves of new flush growth.

There are also a number of soft foliar insecticides such as oils and soaps (horticultural spray oil, neem oil, insecticidal soap) that can help to reduce psyllids both by killing them and by deterring them from laying eggs. These insecticides are generally lower risk to beneficial insects (natural enemies and pollinators). However, oil and soap insecticides must be applied to sufficiently coat the psyllids to kill it, and the residues don't last long. Thus, thorough applications are especially important and they must be made every 7-10 days when psyllids are observed (Rust and Choe 2012).

CHAPTER III

MATERIAL AND METHODS

The details of materials and methodology adopted during the course of this investigation are presented in this chapter. There were three experiments which are mentioned below:

1. Population dynamics of *P. tuberculata* on *Alstonia* tree
2. Effect of *P. tuberculata* galls on the morphology, anatomy and biochemical composition of *Alstonia* leaves and pods
3. Evaluation of insecticides for the management of *P. tuberculata*

The methodology followed in each experiment is presented under the following sub headings:

3.1. Experimental site

The present studies on the "Population dynamics and management of *P. tuberculata* on *A. scholaris* (L.) R. Br." was conducted in the Biocontrol Laboratory at the Entomological Research Farm of Punjab Agricultural University (PAU), Ludhiana during 2015 and 2016. Ludhiana is situated in the central plain zone of Punjab at 30°54' North latitude and 75°48' East longitude at an altitude of 247 m above the mean sea level.

3.2. Climatic conditions

Ludhiana features a semi-arid climate. The temperature from April to June tends to be very hot and dry with average highs in May and June hovering around 40^{o} C. The monsoon season which runs from July to August, sees a slight decrease in average temperature but an increase in humidity. Ludhiana on average receives roughly 730 mm of precipitation annually with bulk of the city's annual precipitation received during the monsoon season. During 2015, the mean maximum and minimum temperature varied from 19.5 to 32.6^{o} C and 19.2 to 31.2^{o}C during the study period from April to October months, respectively. The mean temperature was maximum in May (39.6^{o} C) and minimum in October (19.2^{o} C). The total rainfall received was 737.3 mm with 40 rainy days.

3.3 Population dynamics of *P. tuberculata* on *Alstonia* tree

Population counts of *Alstonia* psyllid were recorded at different locations. A total of five different locations were selected in Punjab Agricultural University (4) and Guru Angad Dev Veterinary and Animal Sciences University (1) for recording the number of galls per leaf and number of galls per pod. Care was exercised in the selection of locations such that the trees selected were of different age, height and girth classes. The mean tree height (m) and girth (m) of the trees in these locations is presented below.

Table 1. Mean tree height (m) and girth (m) of the trees at different locations

Location	Site	Tree height (m)	Girth of tree (m)
A	Agronomy Department	19.50	2.50
B	Biotechnology Department	5.50	0.80
C	Floriculture Department	11.00	1.20
D	Gate number four	11.25	2.15
E	Guru Angad Dev Veterinary and Animal Sciences University campus	10.00	1.25

Various agrometerological parameters such as temperature, relative humidity and rainfall were obtained from School of Agrometerology and Climate Change lab, Punjab Agricultural University, Ludhiana. The effect of maximum temperature, minimum

Pate 1: Glass jars with *Alstonia* leaves for emergence of psyllids

Plate 2: *Alstonia scholaris* tree

temperature, rainfall and relative humidity on the population dynamics of *P. tuberculata* was recorded and correlation coefficients worked out. For this purpose, 25 leaves from five branches of each tree were randomly collected. The total number of galls/leaf and total number of galls/pods were counted. These leaves were placed in glass jars and covered with muslin for recording the emergence of psyllids from galls on leaves and galls on pods during May 2015 to April 2016 (Plate 1).

3.4 The effect of *P. tuberculata* galls on the morphology, anatomy and biochemical composition of *Alstonia* leaves and pods

3.4.1 Morphology of *A. scholaris*

A. scholaris (Apocynaceae) can be around 40 feet tal (Plate 2)l with greenish white fragrant flowers. It is commonly called as the "devil tree". The bark is greyish, branchlets are copiously lenticellate. Leaves occur in whorls of 3-10; petioles are 1-3 cm; the leathery leaves are narrowly obovate to very narrowly spathulate, base cuneate, apex usually rounded; lateral veins occur in 25-50 pairs, at 80-90° to midvein. the upperside of the leaves are glossy, while the underside is greyish. The infected plant shows crumpled and disfigured leaves.

Sampling:

Mature galled and ungalled leaves of *A. scholaris* were collected from infested and uninfested trees growing on different locations of Punjab Agricultural University, Ludhiana and GADVASU. Samples of healthy and galled leaves at different developmental stages were collected from the canopy of five *A. scholaris* trees. Ten mature galled leaves were collected from each tree randomly. The youngest gall developmental stage was determined based on the smallest diameter, observed as a small spot bulged on the leaf blade. The mature ungalled leaves and galled leaves of different developmental stages were taken to the laboratory for morphological and anatomical analysis. The galls on leaves were dissected daily to observe the various developmental stages of the psyllids (Plate 2(a) and 2(b)).

Morphological analysis

Galls were observed morphologically under dissecting microscope in the laboratory. Photographs of different developmental stages of leaf galls were taken out in the laboratory. The live insect and its nymphal stages were collected along with the gall.

3.4.2 Anatomical analysis

To study the structure of healthy and gall infested leaves, these were stored in Formalin-acetic acid-alcohol (FAA) solution which was prepared according to Sass (1958). Ethyl alcohol (95%) = 50 ml, Glacial acetic acid = 50 ml, Formaldehyde (90%) = 10 ml and Water = 35 ml.

Dehydration:

The fixed material was thoroughly washed in distilled water and then dehydrated using ethyl alcohol series which consisted of 10, 30, 50, 70, 90, 95% alcohol and two changes in absolute alcohol. In each change of ethyl alcohol, material was left for a period of 2 hours. This was then subjected to ethyl alcohol and tertiary butyl alcohol (TBA) series (3:1, 1:1 and 1:3). Finally the material was brought to pure TBA and two changes of 6 hours each of pure TBA were given.

Infiltration:

Infiltration of plant material was undertaken using paraffin-tertiary butyl alcohol (Para-TBA) mixture which was prepared by mixing equal volumes of molten paraffin and TBA. The flakes of para-TBA, which is solid at room temperature, were added to pass through tubes containing plant material at 58-62˚ C in the oven. More flakes of para-TBA were added at an interval of 4 hours for four days. Then flakes of pure paraffin were added for 2 days. After that the corks were opened to allow evaporation of TBA. Two changes of molten paraffin were given. The embedding of this material was undertaken in pure paraffin wax in the lid of coupling jar. Small rectangular blocks of the embedded material were mounted on wooden block for microtomy.

Microtomy:

Serial sections of the plant material were cut on a rotary microtome at 10 µm thickness. Ribbons containing serials sections were mounted on glass slides using Haupt's adhesive which was prepared by dissolving 1 g gelatin in 100 ml of water at 90˚C. This mixture was cooled at 30˚ C and then 15 ml of glycerine was added. 2 gm of phenol was added as preservative (Jensen 1962). To mount sections, slides were flooded with 2% formalin solution and ribbons were floated and spread properly. Excess of formalin solution from the slides was drained off on blotting paper. Slides with section in paraffin ribbons were kept at a 40˚ C in the oven for drying.

Dewaxing:

Before following staining procedure, the slides were dewaxed using xylene. Two changes of xylene were undertaken at 2 hours each. The sections were hydrated using downward series of xylene: alcohol (3:1, 1:1 and 1:3) and alcohol (absolute 95,70,50,30 and 10%) series. Then slides were brought to water and stained with erythrosine and crystal violet (Jensen 1962).

3.5 Biochemical analysis

For biochemical analysis, fresh samples from the same study area were collected. For convenience the collected samples of leaves were categorized into: (A) ungalled leaves; (B) galled leaves.

3.5.1 Total Soluble Sugars

Total soluble sugars were estimated by the method of Dubois *et al* (1956).

Principle:

Sugars react with concentrated sulphuric acid to form a dehydration product i.e. furfural or 5-hydroxymethyl furfural. This dehydration product then reacts with phenol which acts as a chromophore and gives orange yellow colour.

Reagents:

A. 80% Ethanol

B. 5% Phenol

C. Concentrated H_2SO_4

Extraction:

Leaf sample (500 mg) collected at desired developmental stages was homogenized in 80% ethanol and then centrifuged at 5000 rpm for ten minutes. The residue was extracted with 80% ethanol to ensure complete extraction. The supernatants were pooled and the ethanol was evaporated. Aqueous syrup was diluted to a known volume with distilled water and used for estimation of total soluble sugars.

Estimation:

To 0.2 ml of leaf extract 1 ml of 5% phenol was added and kept for ten minutes at room temperature followed by addition of 5 ml of conc.H_2SO_4. The sulphuric acid was poured directly in the middle of the test tube to ensure proper mixing of solutions. After ten minutes, the tubes were cooled to room temperature under running water. After another 20 minutes, the absorbance was measured at 490 nm against reagent blank. The concentration of total soluble sugars was calculated from the glucose standards (10-60 µg) run simultaneously. The total soluble sugar content was expressed as mg g^{-1} dry weight.

3.5.2 Total Soluble Proteins

Total proteins were estimated by the method of Lowry *et al* (1951).

Principle:

Protein (peptide bonds) in the sample reacts with copper tartarate complex in the alkaline solution. The protein-copper complex then reduces phosphomolybdate of folin reagent to a blue-coloured complex having maximum absorbance at 620 nm.

Reagents:

A. Alkaline Na_2CO_3 was dissolved in 0.1N NaOH and final volume was adjusted to 100 ml with 0.1N NaOH.

B. Copper sulphate reagent: 0.5% solution of copper sulphate was prepared in 1% solution of sodium potassium tartarate.

C. 50 ml of reagent A mixed with 1 ml of reagent B at the time of use.

D. Folin's reagent: Folin Ciocalteau's phenol reagent was diluted with water in 1:1 ratio.

20% TCA: 20 g of TCA dissolved in water and volume was made to 100 ml.

Extraction:

100 mg of dried leaf sample was homogenized in 50 ml of 0.1 N NaOH followed by centrifugation and the supernatant was collected. The residue was again washed in 3 ml of 0.1 N NaOH. The two supernatants were pooled and the final volume was adjusted to 10 ml. The supernatant was treated with 20% trichlorocaetic acid (TCA) and kept at 4° C for 24 hours. This extract was later centrifuged for 20 minutes at 5000 rpm and precipitates so obtained were dissolved in 0.1 N NaOH. This served as protein extract.

Estimation:

To 0.2 ml of protein extract, 5 ml of solution C was added and kept at room temperature for 10 minutes. To this, 0.5 ml of Folin's reagent was added and kept at 37° C for 30 minutes. The absorbance of blue colour so obtained was measured at 620 nm against a blank. Protein was quantified from standard curve prepared by using bovine serum albumin (BSA) in the concentration range of 20-100 µg.

3.5.3 Total chlorophyll content

Chlorophyll content was estimated by Dimethyl sulphoxide (DMSO) method (Hiscox and Israelstam 1979).

Procedure:

Freshly removed leaf was finely chopped and 50 mg portion was dipped in a test tube containing 5 ml of DMSO. The tube was then placed in a preheated oven at 60° C for about two hours or more (if required) to facilitate the extraction of the pigments. After requisite period, the extract was allowed to reach the room temperature and the absorbance was read at 663 nm and 645 nm in a spectrophotometer. The chlorophyll content was determined using the following equation (Anderson and Boardman 1964).

$$\text{Chlorophyll a} = [12.7(A_{663}) - 2.69(A_{645})] \times \frac{V}{1000 \times W}$$

Chlorophyll b = $[22.9(A_{645}) - 4.86(A_{663})] \times \frac{V}{1000 \times W}$

Total Chlorophyll = $[20.2(A_{645}) + 8.02(A_{663})] \times \frac{V}{1000 \times W}$

Where,

A_{663} = Absorbance at 663 nm

A_{645} = Absorbance at 645 nm

V= Total volume of the extract (ml)

W= Weight of the sample (g)).

The chlorophyll content was expressed in terms of mg chlorophyll g^{-1} fresh weight.

3.5.4 Proline content

Proline content was estimated by the method of Bates *et al* (1973).

Reagents:

A. Methanol : Chloroform : Water
B. Acid ninhydrin (125 mg of ninhydrin mixed in 3 ml of acetic acid and 2 ml of orthophosphoric acid, then kept in oven at 70˚ C till a clear solution was formed).
C. Benzene

Extraction:

The leaf sample was weighted (0.1 g) and extracted in 6 ml of methanol : chloroform: water (5:12:1) by volume at room temperature. It was then centrifuged for 10 minutes and the supernatant was collected. Again the contents were centrifuged after adding 4 ml of methanol: chloroform : water. Supernatant was pooled and final volume was made up to 10 ml with the same solvent. To this 6 ml chloroform and 4 ml of distilled water was added. After stirring it was allowed to stand for 15 minutes in separating funnel till the two layers get separated. Lower layer containing pigments was rejected and upper layer was collected. The final volume of upper layer was made 10 ml by adding distilled water.

Estimation:

Five ml of this solution was added to 2.5 ml of acid ninhydrin. The mixture was boiled for 45 minutes in boiling water bath till a pink colour was formed. On cooling 5 ml of benzene was added and shaken. Again allowed it to stand in separating funnel for another 30 minutes till the two layers get separated. Lower layer was discarded and upper pink layer was collected. OD was recorded at 515 nm by using benzene as blank. Proline was used as standard to make standard curve. The proline content was expressed as mg g^{-1} fresh weight.

3.6 Evaluation of insecticides for the management of *P. tuberculata*

The experiment was conducted at Biocontrol laboratory, Department of Entomology and five different locations of Punjab Agricultural University, Ludhiana during June and July 2015. The following insecticides of varying concentrations were tested

Insecticide	Concentrations (ml or g per litre)		
Imidacloprid 17.8 SL	0.4	0.8	1.2
Thiamethoxam 25 WG	0.3	0.6	0.9
Spinosad 45 SC	0.5	1.0	1.5
Buprofezin 25 EC	2.0	3.0	4.0
Azadirachtin 1%	3.0	4.0	5.0

3.6.1 Field evaluation

Different concentrations of different insecticides found effective in laboratory conditions were further evaluated under field conditions during July 2015. The insecticides namely Imidacloprid 17.8 SL @ 0.4, 0.8 and 1.2 ml per litre, Thiamethoxam 25 WG @ 0.3, 0.6 and 0.9 g per litre, Spinosad 45 SC @ 0.5, 1.0 and 1.5 ml per litre, Buprofezin 25 EC @ 2.0, 3.0 and 4.0 ml per litre, Azadirachtin 1% @ 3.0, 4.0 and 5.0 ml per litre and control were sprayed with foot sprayer on *Alstonia* trees at the five different locations. The trees were sprayed at the peak period of infestation of the pest i.e. June-July. The data on nymphal mortality were recorded at 0, 2, 7 and 14 days after spray by taking 25 leaves per tree selected randomly from all the four directions of tree.

3.7 Statistical analysis

The data in experiment no.1 were subjected to ANOVA using Complete Block Design (CRD) in CPCS1 programme. The significance of differences between treatments means were compared using critical difference (CD) at 5 per cent probability level. The correlations were calculated between the number of galls, the psyllids emerged from galls on leaves and pods and different climatic factors by using MS-excel function (p=0.05). The data were transformed using square root transformation. The data in experiment no. 2 were subjected to Mean±SE and per cent (%) increase or decrease over healthy leaf/pod. The significance was tested with t-test. The data in experiment no. 3 were subjected to ANOVA using Randomized Complete Block Design (RCBD) in CPCS1 programme. The significance of differences between treatments means were compared using Critical difference (CD) at 5 per cent probability level.

CHAPTER IV

REULTS AND DISCUSSION

The results and discussion of the present studies on the population dynamics and management of *Pauropsylla tuberculata* Crawford on *A. scholaris* (L.) R. Br. are detailed as under:

4.1 Population dynamics of *P. tuberculata* on *A. scholaris* tree

- 4.1.1 Number of *P. tuberculata* psyllid galls/ leaves at different locations during 2015-16
- 4.1.2 Number of *P. tuberculata* psyllid galls/pods at different locations during 2015-16
- 4.1.3 Number of *P. tuberculata* psyllids emerged from galls/leaves at different locations during 2015-16
- 4.1.4 Number of *P. tuberculata* psyllids emerged from galls/pods at different locations during 2015-16
- 4.1.5 Population parameters of *P. tuberculata* on *A. scholaris* during different months of 2015-16 (pooled data)
- 4.1.6 Correlation of maximum temperature, minimum temperature, relative humidity and rainfall with different parameters of population indices

4.2 Developmental stages of *P. tuberculata*

4.3 Effect of *P. tuberculata* galls on the morphology, anatomy and biochemical composition of *Alstonia* leaves and pods

- 4.3.1 Morphological alterations due to *P. tuberculata*
- 4.3.2 Anatomical alterations due to *P. tuberculata*
- 4.3.3 Biochemical alterations due to *P. tuberculata*

4.4 Evaluation of insecticides for the management of *P. tuberculata*

- 4.4.1 Efficacy of different insecticides against nymphs of *P. tuberculata* in field (1st spray) during 2015
- 4.4.2 Efficacy of different insecticides against nymphs of *P. tuberculata* in field (2nd spray) during 2015

4.1 Population dynamics of *P. tuberculata* on *A. scholaris* tree

4.1.1 Number of *P. tuberculata* psyllid galls on *A. scholaris* leaves at different locations during 2015-16

Significant differences were observed at different locations in different months.

Location A (Agronomy Department)

The number of psyllid galls/leaf varied from a minimum of 12.90 (February 2016) to a maximum of 47.40 (July 2015). Significantly higher infestations (44.93- August 2015, 41.20- September 2015) were recorded during July to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 2).

Table 2. Number of *P. tuberculata* psyllid galls/leaf at different locations during 2015-16

Month	Number of psyllid galls/leaf (Mean)				
	Location A	Location B	Location C	Location D	Location E
May 2015	34.00 (5.73)*	28.93 (5.11)	28.97 (5.21)	27.23 (5.05)	25.87 (4.95)
June 2015	28.80 (5.16)	33.67 (5.40)	34.70 (5.71)	33.73 (5.67)	29.98 (5.33)
July 2015	47.40 (6.70)	58.80 (7.29)	45.13 (6.45)	42.17 (6.27)	43.33 (6.33)
August 2015	44.93 (6.56)	52.33 (6.94)	42.13 (6.24)	36.10 (5.78)	39.03 (6.03)
September 2015	41.20 (6.26)	43.27 (6.34)	37.60 (5.95)	31.66 (5.47)	35.47 (5.78)
October 2015	34.37 (5.73)	29.27 (5.24)	32.43 (5.54)	30.07 (5.31)	32.00 (5.52)
November 2015	25.73 (5.01)	21.87 (4.62)	24.13 (4.87)	21.67 (4.60)	20.13 (4.38)
December 2015	13.77 (3.83)	14.50 (3.88)	13.43 (3.72)	11.73 (3.52)	15.03 (3.93)
January 2016	14.00 (3.78)	12.60 (3.61)	10.03 (3.25)	7.23 (2.77)	7.00 (2.73)
February 2016	12.90 (3.67)	12.30 (3.60)	11.63 (3.42)	8.60 (2.96)	7.73 (2.84)
March 2016	18.53 (4.37)	21.00 (4.59)	17.20 (4.20)	20.06 (4.50)	19.87 (4.44)
April 2016	28.47 (5.26)	29.53 (5.26)	25.37 (4.94)	23.73 (4.75)	24.47 (4.87)
CD(p=0.05)	(1.94)	(2.42)	(2.11)	(2.03)	(2.03)

*Figures in the parentheses are square root $\sqrt{n+1}$ transformed values

Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

Location B (Biotechnology Department)

The number of psyllid galls/leaf varied from a minimum of 12.30 (February 2016) to a maximum of 58.80 (July 2015). Significantly higher infestations (52.33- August 2015, 43.27- September 2015) were recorded during July to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 2).

Location C (Floriculture Department)

The number of psyllid galls/leaf varied from a minimum of 10.03 (January 2016) to a maximum of 45.13 (July 2015). Significantly higher infestations (42.13- August 2015, 37.60- September 2015) were recorded during July to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 2).

Location D (Gate number four)

The number of psyllid galls/leaf varied from a minimum of 7.23 (January 2016) to a maximum of 42.17 (July 2015). Significantly higher infestations (36.10- August 2015, 33.73- June 2015) were recorded during June to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 2).

Location E (GADVASU)

The number of psyllid galls/leaf varied from a minimum of 7.00 (January 2016) to a maximum of 43.33 (July 2015). Significantly higher infestations (39.03- August 2015, 35.47- September 2015) were recorded during July to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 2).

4.1.2 Number of *P. tuberculata* psyllid galls/pods at different locations during 2015-16

Significant differences were observed for different locations in different months.

Location A (Agronomy Department)

The number of psyllid galls/pod varied from a minimum of 2.80 (December 2015 and February 2016) to a maximum of 42.80 (July 2015). Significantly higher infestations (34.20- May 2015 and August 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 3).

Location B (Biotechnology Department)

The number of psyllid galls/pod varied from a minimum of 2.60 (December 2015) to a maximum of 45.20 (July 2015). Significantly higher infestations (35.20- August 2015, 30.80- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 3).

Location C (Floriculture Department)

The number of psyllid galls/pod varied from a minimum of 3.40 (December 2015, January 2016 and February 2016) to a maximum of 41.60 (July 2015). Significantly higher infestations (31.80- August 2015, 29.60- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 3).

Table 3. Number of *P. tuberculata* psyllids galls/pod at different locations during 2015-16

Month	Number of psyllid galls/pod (Mean)				
	Location A	Location B	Location C	Location D	Location E
May 2015	34.20 (5.49)*	30.80 (5.16)	29.60 (5.04)	28.00 (4.97)	25.00 (4.70)
June 2015	23.20 (4.47)	28.20 (4.96)	27.60 (5.00)	26.00 (4.78)	24.40 (4.67)
July 2015	42.80 (6.25)	45.20 (6.46)	41.60 (6.18)	39.20 (6.02)	38.60 (5.91)
August 2015	34.20 (5.66)	35.20 (5.73)	31.80 (5.46)	29.60 (5.28)	30.40 (5.34)
September 2015	26.20 (4.85)	29.00 (5.19)	23.60 (4.71)	23.60 (4.70)	23.40 (4.74)
October 2015	18.60 (4.13)	22.00 (4.57)	15.80 (3.98)	11.60 (3.46)	15.00 (3.91)
November 2015	8.20 (2.94)	10.80 (3.33)	8.80 (3.05)	10.40 (3.33)	11.00 (3.34)
December 2015	2.80 (1.90)	2.60 (1.84)	3.40 (2.01)	3.20 (1.99)	3.20 (2.01)
January 2016	3.40 (2.05)	3.40 (2.07)	3.40 (2.01)	3.60 (2.09)	2.60 (1.83)
February 2016	2.80 (1.92)	3.60 (2.04)	3.40 (2.01)	3.40 (2.07)	2.60 (1.83)
March 2016	15.80 (3.80)	14.60 (3.74)	20.00 (4.36)	16.40 (3.98)	15.20 (3.88)
April 2016	25.00 (4.76)	20.00 (4.34)	23.80 (4.68)	21.80 (4.48)	18.20 (4.18)
CD(p=0.05)	(2.25)	(2.17)	(2.08)	(1.98)	(1.91)

*Figures in the parentheses are square root $\sqrt{n+1}$ transformed values

Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

Location D (Gate number four)

The number of psyllid galls/pod varied from a minimum of 3.20 (December 2015) to a maximum of 39.20 (July 2015). Significantly higher infestations (29.60- August 2015, 28.00- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 3).

Location E (GADVASU)

The number of psyllid galls/pod varied from a minimum of 2.60 (January 2016 and February 2016) to a maximum of 38.60 (July 2015). Significantly higher infestations (30.40- August 2015, 25.00- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 3).

4.1.3 Number of *P. tuberculata* psyllids emerged from galls/leaves at different locations during 2015-16

Significant differences were observed for different locations in different months.

Location A (Agronomy Department)

The number of psyllids emerged from galls/leaf varied from a minimum of 9.00 (December 2015) to a maximum of 47.30 (July 2015). Significantly higher infestations (44.80- August 2015, 39.60- September 2015) were recorded during June to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 4).

Location B (Biotechnology Department)

The number of psyllids emerged from galls/leaf varied from a minimum of 12.06 (February 2016) to a maximum of 51.03 (July 2015). Significantly higher infestations (45.33- August 2015, 37.03- September 2015) were recorded during June to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 4).

Location C (Floriculture Department)

The number of psyllids emerged from galls/leaf varied from a minimum of 7.00 (January 2016) to a maximum of 39.77 (July 2015). Significantly higher infestations (35.87- August 2015, 32.70- September 2015) were recorded during June to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 4).

Location D (Gate number four)

The number of psyllids emerged from galls/leaf varied from a minimum of 5.33 (January 2016) to a maximum of 37.67 (July 2015). Significantly higher infestations (32.30- August 2015, 28.33- September 2015) were recorded during June to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 4).

Location E (GADVASU)

The number of psyllids emerged from galls/leaf varied from a minimum of 5.40 (February 2016) to a maximum of 34.30 (July 2015). Significantly higher infestations (33.83- August 2015, 32.40- September 2015) were recorded during June to September months and the infestation was significantly lower during December 2015 to February 2016 period (Table 4).

Table 4. Number of *P. tuberculata* psyllids emerged from galls/leaf at different locations during 2015-16

Month	Number of psyllid emerged from galls/leaf (Mean)				
	Location A	Location B	Location C	Location D	Location E
May 2015	27.20 (4.89)*	26.10 (5.02)	25.27 (4.86)	23.10 (4.64)	21.13 (4.47)
June 2015	28.20 (4.97	28.93 (5.21)	28.93 (5.23)	27.83 (5.15)	24.67 (4.83)
July 2015	47.30 (6.51)	51.03 (6.88)	39.77 (6.07)	37.67 (5.95)	34.30 (5.64)
August 2015	44.80 (6.38)	45.33 (6.51)	35.87 (5.77)	32.30 (5.49)	33.83 (5.64)
September 2015	39.60 (6.03)	37.03 (5.90)	32.70 (5.55)	28.33 (5.18)	32.40 (5.50)
October 2015	33.40 (5.53)	26.10 (4.99)	27.97 (5.18)	23.53 (4.68)	29.73 (5.29)
November 2015	22.20 (4.58)	20.80 (4.45)	20.33 (4.44)	17.87 (4.22)	19.93 (4.26)
December 2015	9.00 (3.10)	13.40 (3.70)	9.23 (3.13)	8.20 (3.00)	10.60 (3.33)
January 2016	9.40 (3.09)	12.40 (3.53)	7.00 (2.75)	5.33 (2.39)	5.40 (2.42)
February 2016	10.00 (3.15)	12.06 (3.45)	8.80 (3.05)	8.43 (2.90)	6.20 (2.56)
March 2016	16.60 (4.08)	20.33 (4.47)	14.13 (3.80)	16.03 (4.04)	12.73 (3.61)
April 2016	26.40 (4.94)	24.66 (4.84)	22.96 (4.72)	20.73 (4.45)	18.60 (4.27)
CD(p=0.05)	(2.46)	(2.04)	(1.96)	(1.91)	(1.98)

*Figures in the parentheses are square root $\sqrt{n+1}$ transformed values

Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

4.1.4 Number of *P. tuberculata* psyllids emerged from galls/pods at different locations during 2015-16

Significant differences were observed for different locations in different months.

Location A (Agronomy Department)

The number of psyllids emerged from galls/pod varied from a minimum of 2.20 (February 2016) to a maximum of 35.60 (July 2015). Significantly higher infestations (29.80- August 2015, 25.00- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 5).

Location B (Biotechnology Department)

The number of psyllids emerged from galls/pod varied from a minimum of 2.20 (December 2015) to a maximum of 39.00 (July 2015). Significantly higher infestations (29.60- August 2015, 26.20- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 5).

Location C (Floriculture Department)

The number of psyllids emerged from galls/pod varied from a minimum of 3.00 (December 2015 and January 2016) to a maximum of 36.00 (July 2015). Significantly higher infestations (26.00- August 2015, 24.80- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 5).

For location D (Gate number four)

The number of psyllids emerged from galls/pod varied from a minimum of 2.20 (February 2016) to a maximum of 33.80 (July 2015). Significantly higher infestations (27.20- August 2015, 23.60- May 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 5).

Location E (GADVASU)

The number of psyllids emerged from galls/pod varied from a minimum of 1.80 (February 2016) to a maximum of 33.60 (July 2015). Significantly higher infestations (26.60- August 2015, 21.80- June 2015) were recorded during May to August months and the infestation was significantly lower during December 2015 to February 2016 period (Table 5).

Table 5. Number of *P. tuberculata* psyllids emerged from galls/pod at different locations during 2015-16

Month	Number of psyllid emerged from galls/pod (Mean)				
	Location A	Location B	Location C	Location D	Location E
May 2015	25.00 (4.73)*	26.20 (4.75)	24.80 (4.63)	23.60 (4.56)	20.20 (4.22)
June 2015	20.60 (4.21)	24.80 (4.68)	23.20 (4.64)	21.80 (4.40)	21.80 (4.42)
July 2015	35.60 (5.69)	39.00 (6.02)	36.00 (5.77)	33.80 (5.60)	33.60 (5.49)
August 2015	29.80 (5.28)	29.60 (5.23)	26.00 (4.97)	27.20 (5.03)	26.60 (4.99)
September 2015	23.00 (4.56)	25.80 (4.89)	21.40 (4.49)	20.60 (4.39)	20.20 (4.45)
October 2015	16.40 (3.90)	16.00 (3.95)	12.40 (3.56)	10.20 (3.28)	11.00 (3.37)
November 2015	6.40 (2.66)	8.80 (3.06)	7.60 (2.87)	8.80 (3.08)	9.20 (3.10)
December 2015	2.40 (1.81)	2.20 (1.72)	3.00 (1.92)	3.00 (1.92)	2.80 (1.91)
January 2016	2.60 (1.86)	3.20 (2.02)	3.00 (1.90)	3.00 (1.92)	2.20 (1.74)
February 2016	2.20 (1.76)	3.00 (1.92)	3.20 (2.01)	2.20 (1.74)	1.80 (1.61)
March 2016	12.80 (3.45)	12.00 (3.43)	16.40 (4.00)	13.00 (3.55)	12.00 (3.46)
April 2016	20.60 (4.32)	17.20 (4.04)	19.20 (4.24)	18.60 (4.16)	16.00 (3.88)
CD(p=0.05)	(2.07)	(2.01)	(1.87)	(1.87)	(1.83)

*Figures in the parentheses are square root $\sqrt{n+1}$ transformed values Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

4.1.5 Population parameters of *P. tuberculata* on *A. scholaris* during different months of 2015-2016 (pooled data)

The significantly higher total mean number of galls on leaves was observed in month of July 2015 (47.37) and the significantly lower total mean number of galls on leaves was observed in month of January 2016 (10.17). Similarly significantly higher total mean number of galls on pods was observed in month of July 2015 (41.48) and the significantly lower total mean number of galls on pods in the month of December 2015 (3.04) (Table 6).

The significantly higher total mean number of psyllids emerged from galls on leaves in the month of July 2015 (42.03) and the significantly lower total mean number of psyllids emerged from galls on leaves in the month of January 2016 (7.91). Similarly, significantly higher total mean number of psyllids emerged from galls on pods in the month of July 2015 (35.60) and the significantly lower total mean number of psyllids emerged from galls on pods in the month of February 2016 (2.48) (p= 0.54, f=0.63).

It can be concluded that in terms of total number of galls on leaves and pods and also total number of psyllids emerging from galls on leaves or pods there is definite pattern. Peak period of activity corresponds to the July-September months with peak population during July. The population declines gradually, starting from October and extends upto February with minimum population recorded during December and January (Fig. 1-4, Table 6)

Jain and Dhiman (2014) studied that adult of *P. tuberculata* appear in late March and these oviposit eggs on leaves Due to this, gall formation increases from late April onwards. The maximum number of galls were observed from August to October. Although reduction in number of gall formation occurred from December 2015 to February 2016, due to cold climatic conditions.

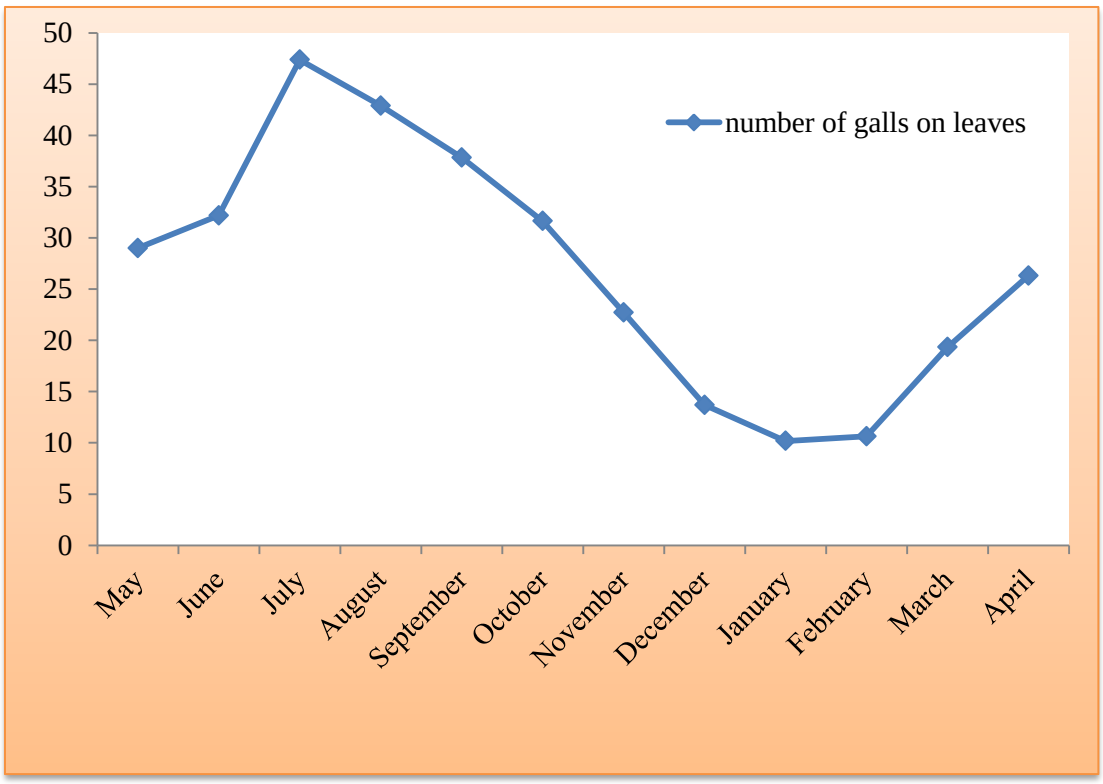


Fig. 1. Mean number of *P. tuberculata* galls on *A. scholaris* leaves during different months of 2015-16 (pooled data)

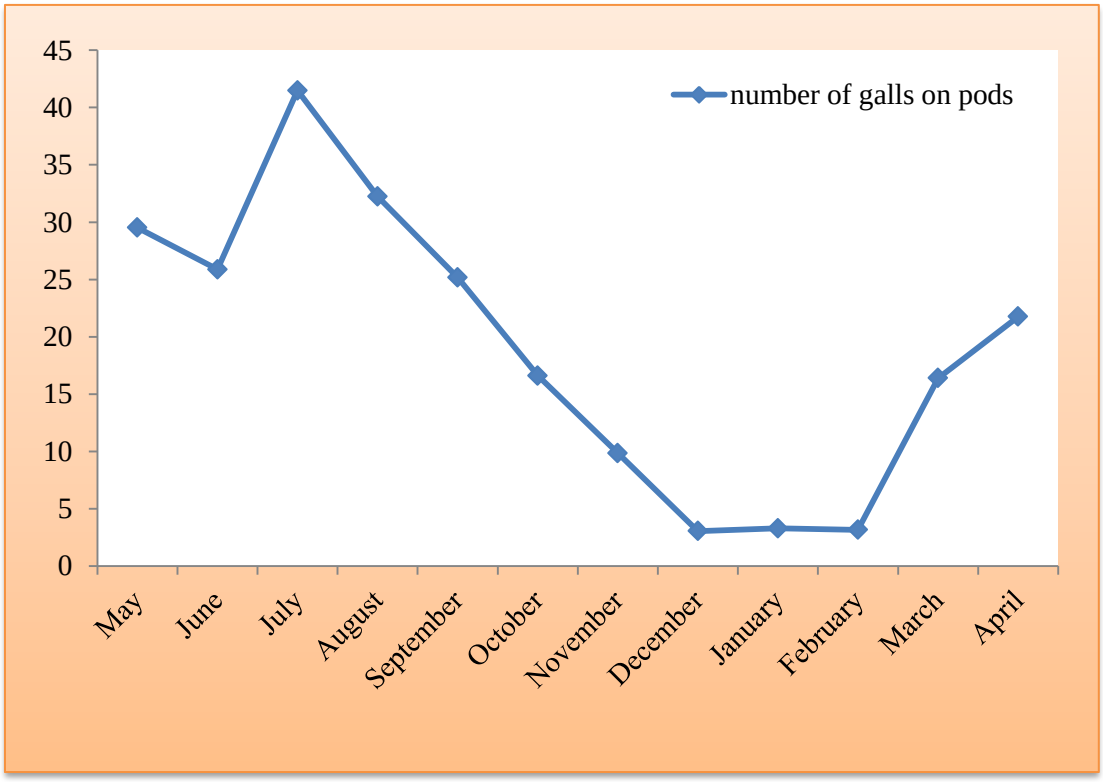


Fig. 2. Mean number of *P. tuberculata* galls on *A. scholaris pods* during different months of 2015-16 (pooled data)

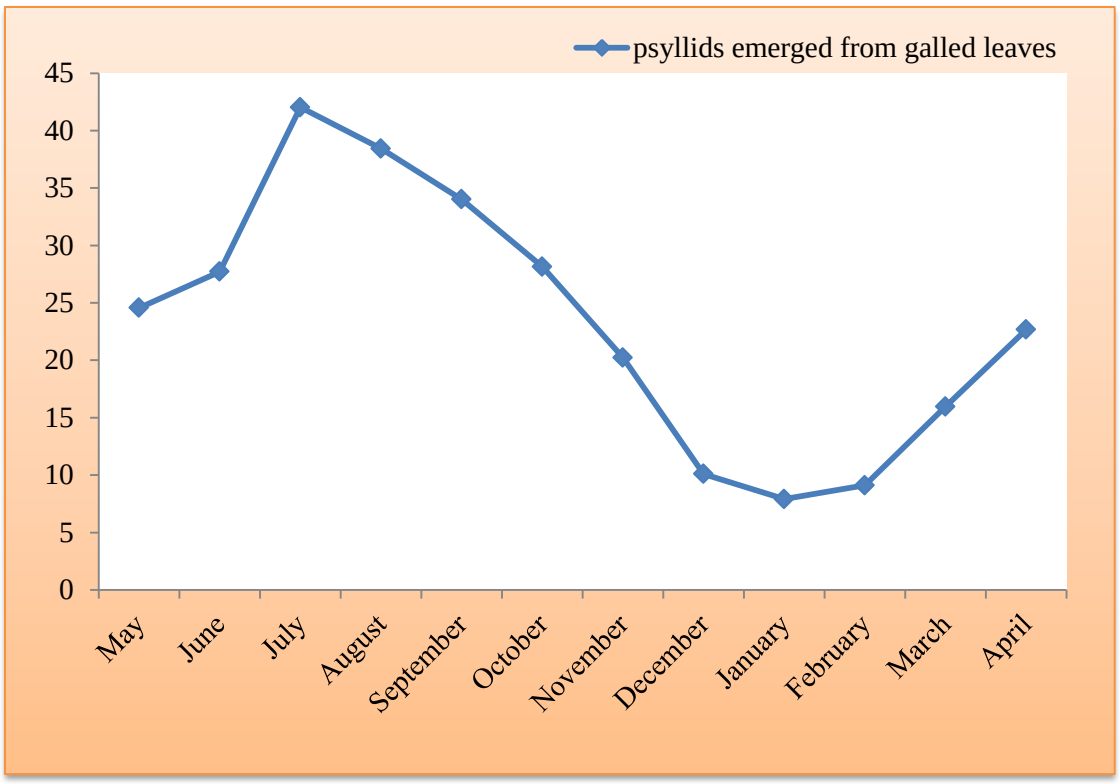


Fig. 3. Mean number of *P. tuberculata* psyllids emerged from galls on *A. scholaris* leaves during different months of 2015-16 (pooled data)

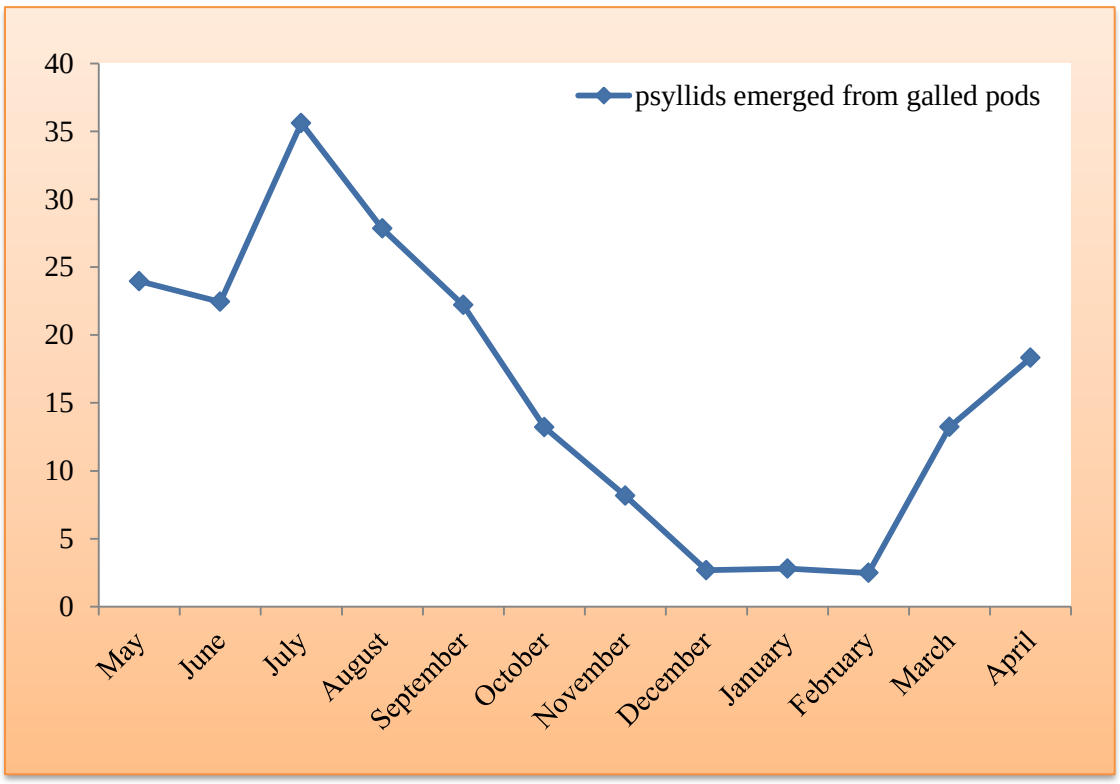


Fig. 4. Mean number of *P. tuberculata* psyllids emerged from galls on *A. scholaris* pods during different months of 2015-16 (pooled data)

Table 6: Population parameters of *P. tuberculata* on *A. scholaris* during different months of 2015-16 (pooled data)

Month	Total number of galls on leaves	Total number of galls on pods	Total number of psyllids on leaves	Total number of psyllids on pods
May 2015	29.00 (5.47)*	29.52 (5.52)	24.56 (5.05)	23.96 (4.99)
June 2015	32.18 (5.76)	25.88 (5.18)	27.71 (5.36)	22.44 (4.84)
July 2015	47.37 (6.94)	41.48 (6.52)	42.03 (6.54)	35.60 (6.05)
August 2015	42.90 (6.61)	32.24 (5.76)	38.43 (6.26)	27.84 (5.37)
September 2015	37.84 (6.22)	25.16 (5.11)	34.01 (5.91)	22.20 (4.81)
October 2015	31.63 (5.71)	16.60 (4.17)	28.15 (5.39)	13.20 (3.75)
November 2015	22.71 (4.86)	9.84 (3.29)	20.23 (4.60)	8.16 (3.02)
December 2015	13.69 (3.83)	3.04 (2.01)	10.09 (3.32)	2.68 (1.92)
January 2016	10.17 (3.32)	3.28 (2.07)	7.91 (2.95)	2.80 (1.95)
February 2016	10.63 (3.40)	3.16 (2.04)	9.10 (3.16)	2.48 (1.86)
March 2016	19.33 (4.51)	16.40 (4.17)	15.96 (4.11)	13.24 (3.77)
April 2016	26.31 (5.22)	21.76 (4.76)	22.67 (4.86)	18.32 (4.39)
CD(p=0.05)	(0.41)	(0.31)	(0.46)	(0.26)

*Figures in the parentheses are square root $\sqrt{n+1}$ transformed values

Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

4.1.6 Correlation of Maximum temperature, Minimum temperature, Relative humidity and Rainfall with different parameters of population indices

The total numbers of galls on leaves correlate positively with maximum temperature (0.83), minimum temperature (0.94) and rainfall (0.26). The total numbers of galls on leaves were negatively correlated with relative humidity (-0.32) (Figure 5, Table 7).

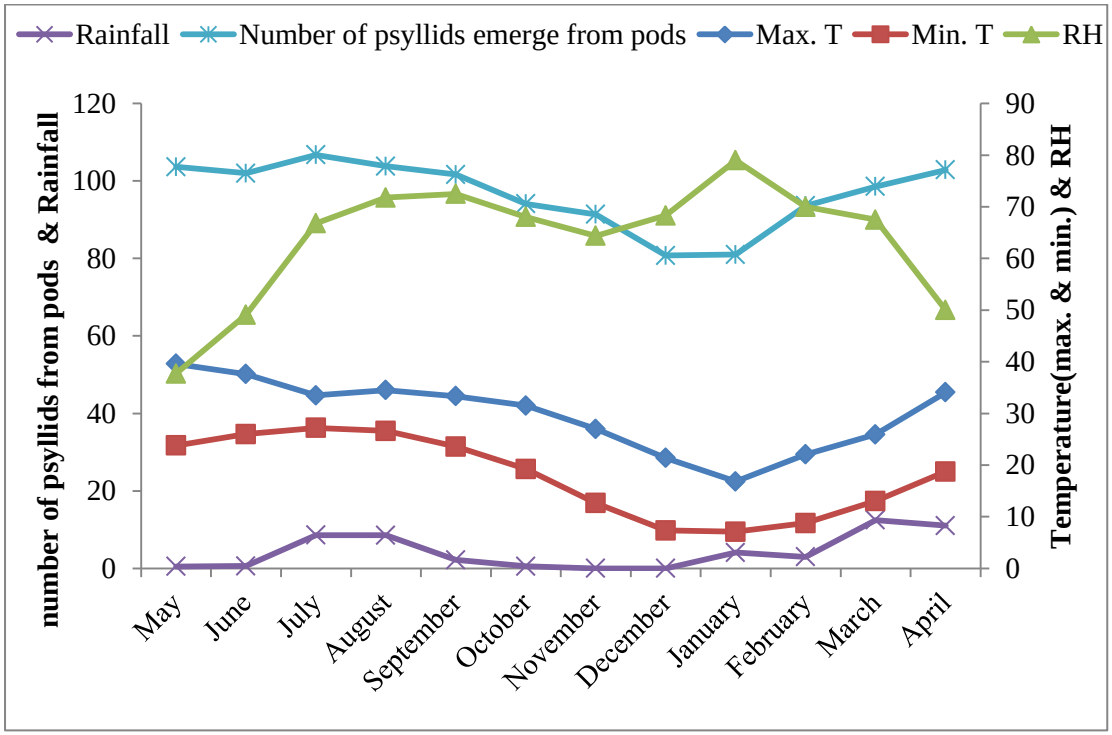


Fig. 5. Total number of galls on leaves and Maximum temperature, Minimum temperature, Rainfall and Relative humidity during May 2015 to April 2016

The total numbers of galls on pods correlate positively with maximum temperature (0.86), minimum temperature (0.96) and rainfall (0.25). The total numbers of galls on pods were negatively correlated with relative humidity (-0.34) (Figure 6, Table 7).

The total numbers of galls on leaves correlate positively with maximum temperature (0.89) and minimum temperature (0.95) and rainfall (0.22). The total numbers of psyllids emerged from galls on leaves were negatively correlated with relative humidity (-0.50) (Figure 7, Table 7).

The total numbers of galls on pods correlated positively with maximum temperature (0.89) and minimum temperature (0.91) and rainfall (0.34). The total numbers of psyllids emerged from galls on pods were negatively correlated with relative humidity (-0.57) (Figure 8, Table 7).

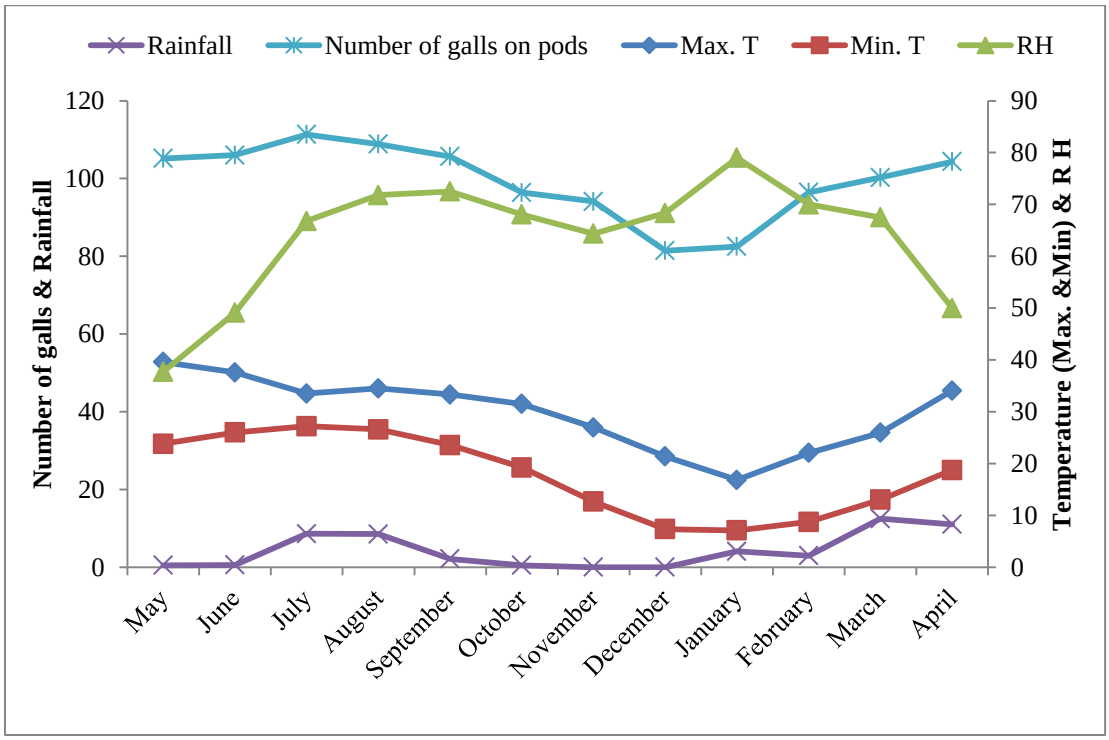


Fig. 6. **Total number of galls on pods and Maximum temperature, Minimum temperature, Rainfall and Relative humidity during May 2015 to April 2016**

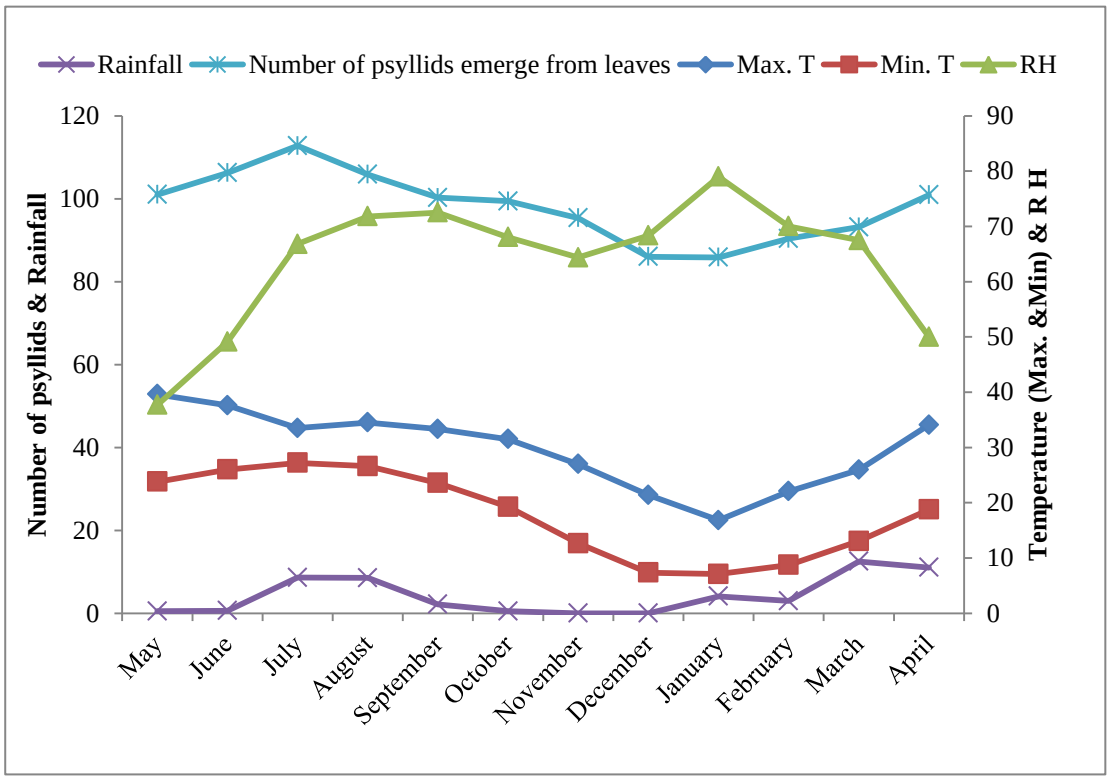


Fig. 7. **Total numbers of psyllids emerged from galls on leaves and Maximum temperature, Minimum temperature, Rainfall and Relative humidity during May 2015 to April 2016**

Jain and Dhiman (2014) studied that number of galls on the *A. scholaris* leaves increases gradually with the increase in temperature up to an optimum level, further decrease with low temperature. Temperature (maximum and minimum) influences the various physiological functions such as reproduction and development and this explains the positive correlations (p=0.54, f=0.63) and the population trends imitating the variations in climatic factors. The lowest population were recorded during December, January and February, when the relative humidity is high (negative correlations p=0.54, f=0.63, n=12)

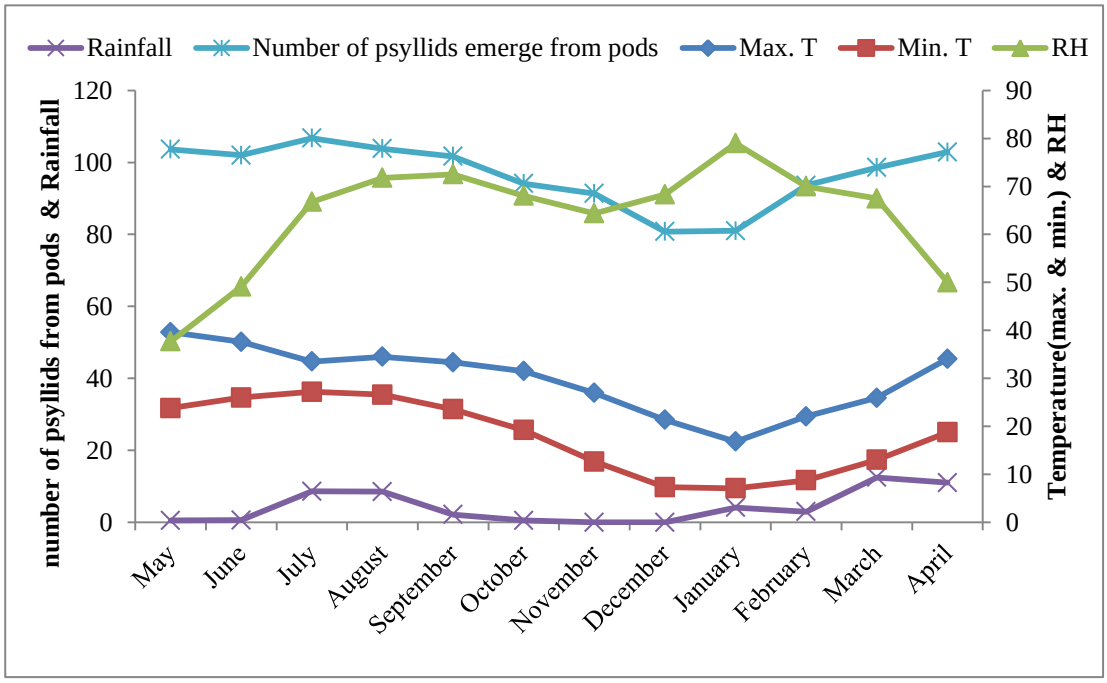


Fig. 8. Total numbers of psyllids emerged from galls on pods and Maximum temperature, Minimum temperature, Rainfall and Relative humidity during May 2015 to April 2016

Table 7. Correlation between population indices and climatic conditions (May 2015-April 2016)

Correlation	Maximum temperature	Minimum temperature	Relative humidity (RH)	Rainfall
Total number of galls on leaves	0.83*	0.94*	-0.32	0.26
Total number of galls on pods	0.86*	0.96*	-0.34	0.25
Psyllids emerged from gall on leaves	0.89*	0.95*	-.050	0.22
Psyllids emerged from gall on pods	0.89*	0.91*	-0.57	0.34

*Significant at p=0.54 f value= 0.63 n=12

4.2 Developmental stages of *P. tuberculata*

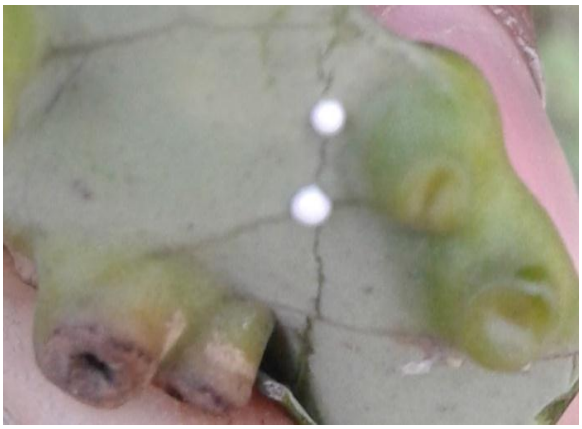
The adult of *P. tuberculata* laid eggs on the lower surface of the developing leaves. Eggs are laid singly or in groups at more than one place. The eggs appear white, oblong, narrow and acute at one end (Plate 2a(i)). It is presumed that the insect along with the egg deposits some physiologic fluid which acts as a stimulant for the initiation of the gall. The hypertrophic response of the plant results in cell division and subsequent formation of the gall. The first nymphal instar undergoes moulting to reach the adult stage (imago). The nymphs are yellow and all the instars are developed inside the gall. At the time of emergence the exit hole is made either on the dorsal or ventral side of the leaf. Characteristic white waxy secretion of the nymph occurs in abundance surrounding it (Plate 2a(ii)). Second and third instars are often found on the leaf surface outside gall (Plate 2a(iii) and 2a(iv)).

P. tuberculata being a hemimetabolous insect and had no pupal stage. The wings developed as small white buds on the outside of their body which could be distinctly observed as buds on the body of the nymph.

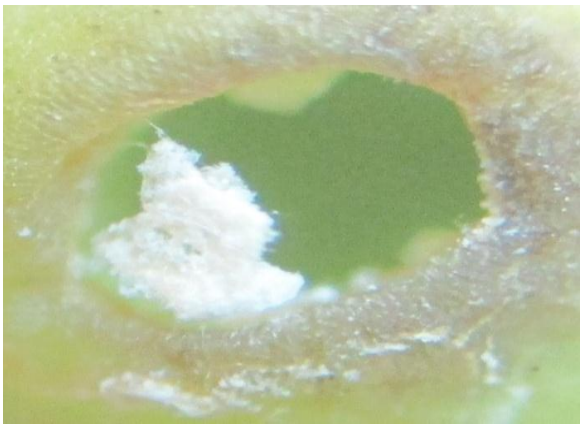
Adult psyllid body is ovate with distinct thorax and abdomen. Setae are present all over the body. The dorsal side of the body is clothed with long setae and the setae on the ventral side are small and spine like. The antennae are long, tapering and straight (Plate 2b(i)) and the fifth instar is yellow in colour (Plate 2b(ii)). The mature insect is colourful, winged and with bulging eyes (Plate 2b(iii)) and the female is longer than male in size (Plate 2b(iv)).

Albert *et al* (2011) reported that *P. tuberculata* laid eggs singly or in groups at many places. The depression was formed when the lysis of cell occurs. *P. tuberculata* contains egg, nymphal and adult stage in its life cycle. The mature insect was yellow in colour, winged and with bulging eyes. These insects after nuptial flight appear to probably ovipost eggs on the galled leaves.

Jain and Dhiman (2014) reported that *P. tuberculata* female lays eggs on the leaves and the young shoot in scattered groups. The eggs hatch and thereafter went through five wingless nymphal stages before becoming winged adults. The nymph is yellow and all the instars developed inside the zoocecidia. Male and female are of approximately same size, although, female has been observed slightly longer than male. The length of the fully mature male varies from 0.95 mm to 1.05 mm whereas; the length of female varies from 1.30 mm to 1.80 mm.



(i) Eggs deposited linearly and cracked epidermis



(ii) First nymphal instar

(iii) Second nymphal instar

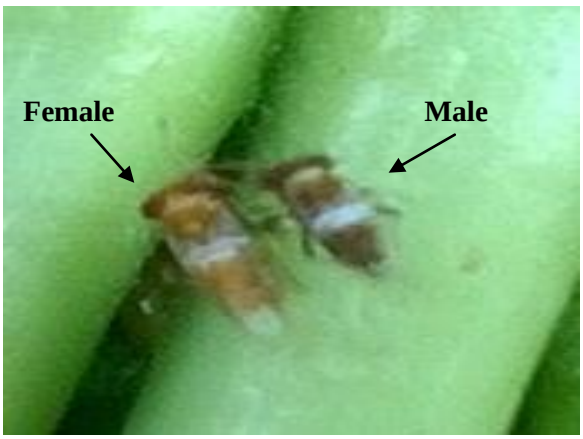
(iv) Third nymphal instar

Plates 3(a): Different life stages of *P. tuberculata*

(i) Fourth nymphal instar

(ii) Fifth nymphal instar

(iii) Adult (Mature insect)



(iv) **Female and male psyllid**

Plates 3(b): Different life stages of *P. tuberculata*

(i) Galls on upper surface of leaf

(ii) Galls on lower surface of leaf

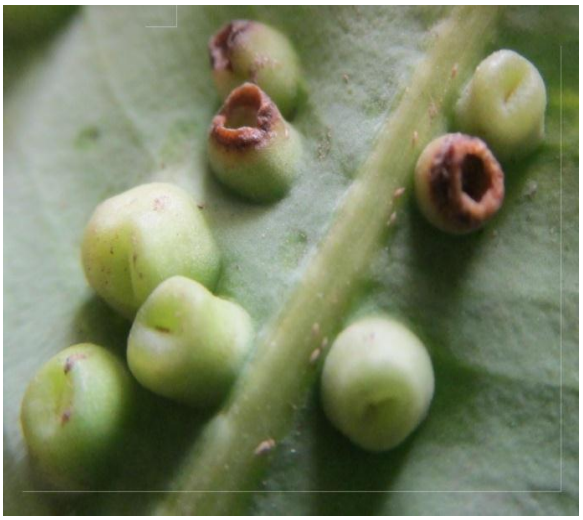
(iii) Galls on lateral vein (upper surface)

(iv) Galls on lateral vein (lower surface)

Plate 4: 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

(iv) Yellowish brown periderm formed at the rim of the opening

Plates 5: Morphological changes in *Alstonia* leaves

4.3 Effect of *P. tuberculata* galls on the morphology, anatomy and biochemical composition of *Alstonia* leaves and pods

4.3.1 Morphological alterations due to *P. tuberculata*

Galls were formed on both the upper and lower surfaces of the leaves. But they were abundant on the lower surface (Plate 4(i) and 4(ii)). Gall formation was initiated when *P. tuberculata* oviposits on the leaves. Slight decolorization was visible on the areas where the eggs were deposited. The discoloured surface enlarges and forms a small outgrowth on the upper side of the leaf where the gall appears enlarged and is placed in a depression. Initially the gall grows towards the lower side of the leaf but later on its growth is towards the upper side. Majority of the galls are formed on the lateral veins (Plate 4(iii) and 4(iv)). Initially the nymph is enclosed within the gall tissue, which grows over the insect and enclosed it completely. The maximum number of galls were found lateral to the second order veins. When the leaves are heavily galled the lamina was completely reduced into a mass of cells. Both young and mature leaves are equally affected. With an increase of galls in number all the leaves appear crumbled and completely deformed (Plate 5-i), scattered and isolated (Plate 5-ii).

During the later stage of the gall, a depression/halo is formed (Plate 5-iii). The chamber formed is opened to the lower side by means of a small opening. Apparently, the ostiole becomes indistinct and a well defined cavity is formed. The mature epiphyllous pouch galls are unilocular with a single chamber or multilocular with 3–4 chambers. The galls appear multilocular because the adjacent galls coalesce. The mature gall is monothalamus subcylindrical, similar in colour with the host leaf, except at the apex which is yellowish. The wall is thick and succulent. The chamber is subcuneate with the sharp pointing edges at the distal end. The opening of gall is apical, subcircular without any appendage. The galls are persistent remaining on the leaf long after the escape of the psyllid adult. The number of galls varies from 25 to numerous on a single leaf. Depending upon the maturity of the galls the diameter of the gall chamber ranges from 0.1 cm to 1 cm. The mature gall externally does not differ much from the previous stages except in size.

The gall at senescent stage is characterized by the presence of small orifices on the gall surface. After the emergence of the insects, the cells close to the chamber and exit canals form a protective layer which appears as a yellowish brown rim (Plate 5-iv).

4.3.2 Anatomical alterations due to *P. tuberculata*

The data pertaining to the anatomical alterations in the galled leaves caused by *P. tuberculata* is presented in Table 8, Plate 6.

A. Thickness of upper epidermis

The thickness of upper epidermal layer was more in healthy leaves (18.74 µm) than the infected leaves (18.15 µm) (Plate 6). The percentage decrease in the thickness of upper epidermal layer over healthy was 3.25 per cent (Table 8).

In response to the stimuli of gall induction, the parenchyma cells recover their ability to divide and hypertrophy. Cell divisions occur in several planes, increasing the number of cell layers and the thickness of the mesophyll, and originating the homogeneous parenchyma cortex. Both the homogenization of the parenchyma and cell hypertrophy are common phenomena in the formation of galls (Souza *et al* 2000, Moura *et al* 2008, Moura *et al* 2009, Oliveira and Isaias 2010). These phenomena are linked to reduced photosynthetic capacity in some galls (Moura et al. 2008), and are related to the new functions of the mesophyll, such as a feeding site for the insect and a protective barrier (Rohfritsch 1992, Moura *et al* 2008). Some plants infested with galls have shown less growth and productivity (Gonzáles *et al* 2005) and ornamental value.

B. Thickness of lower epidermis

The thickness of lower epidermal layer was more in healthy leaves (15.04 µm) than the infected leaves (12.62 µm) (Plate 6). The percentage decrease in the thickness of lower epidermal layer over healthy was 19.18 per cent. The thickness of the epidermis covering the nymphal chamber is greatly reduced (Dias *et al* 2011) (Table 8).

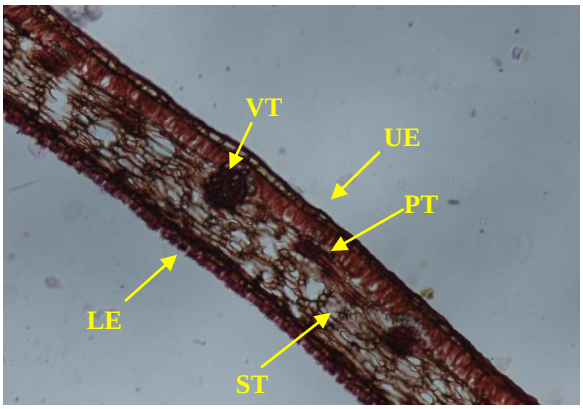
Table 8. Anatomical changes in leaf tissues due to galls caused by *P. tuberculata*

Parameters	Healthy leaves (µm)*	Infected leaves (µm)*	Per cent increase /decrease over healthy
Thickness of Upper epidermis	18.74±1.71	18.15±2.71	(-)3.25
Thickness of Lower epidermis	15.04±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

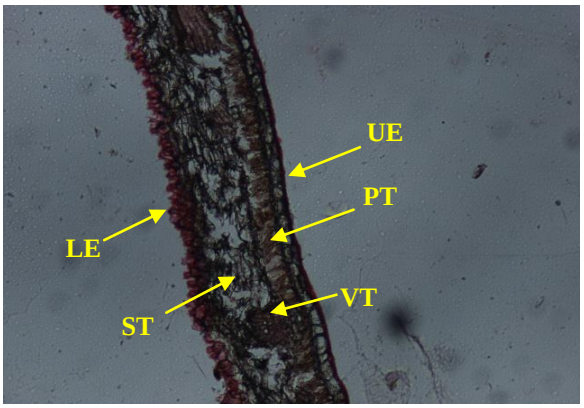
*Mean of four replications, Figures are Mean±SE

C. Palisade tissues thickness

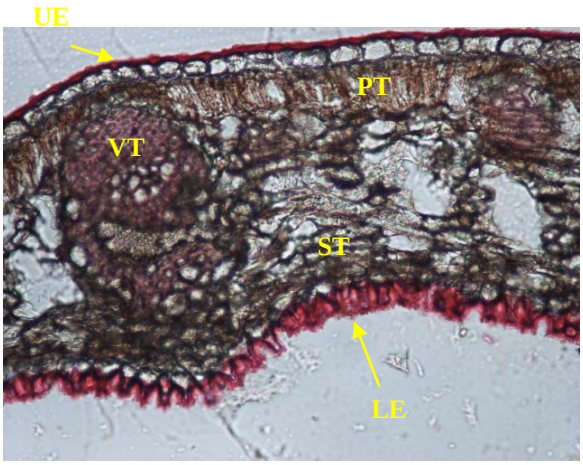
In the mesophyll cells, the palisade tissues thickness was recorded to be lesser in healthy leaves (41.19 µm) than the infected leaves (47.21 µm). The percentage increase in the



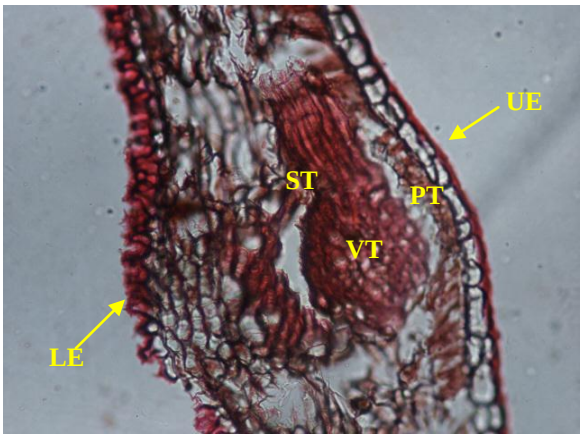
Healthy leaf



Initial enlargement of PT, ST and VT



VT enlarged and distorted LE and UE



Bulging of UE and LE

Plate 6: Anatomical changes in gall and healthy leaves

LE: Lower epidermis; UE: Upper epidermis; ST: Spongy tissues;

VT: Vascular tissues; PT: Palisade tissues

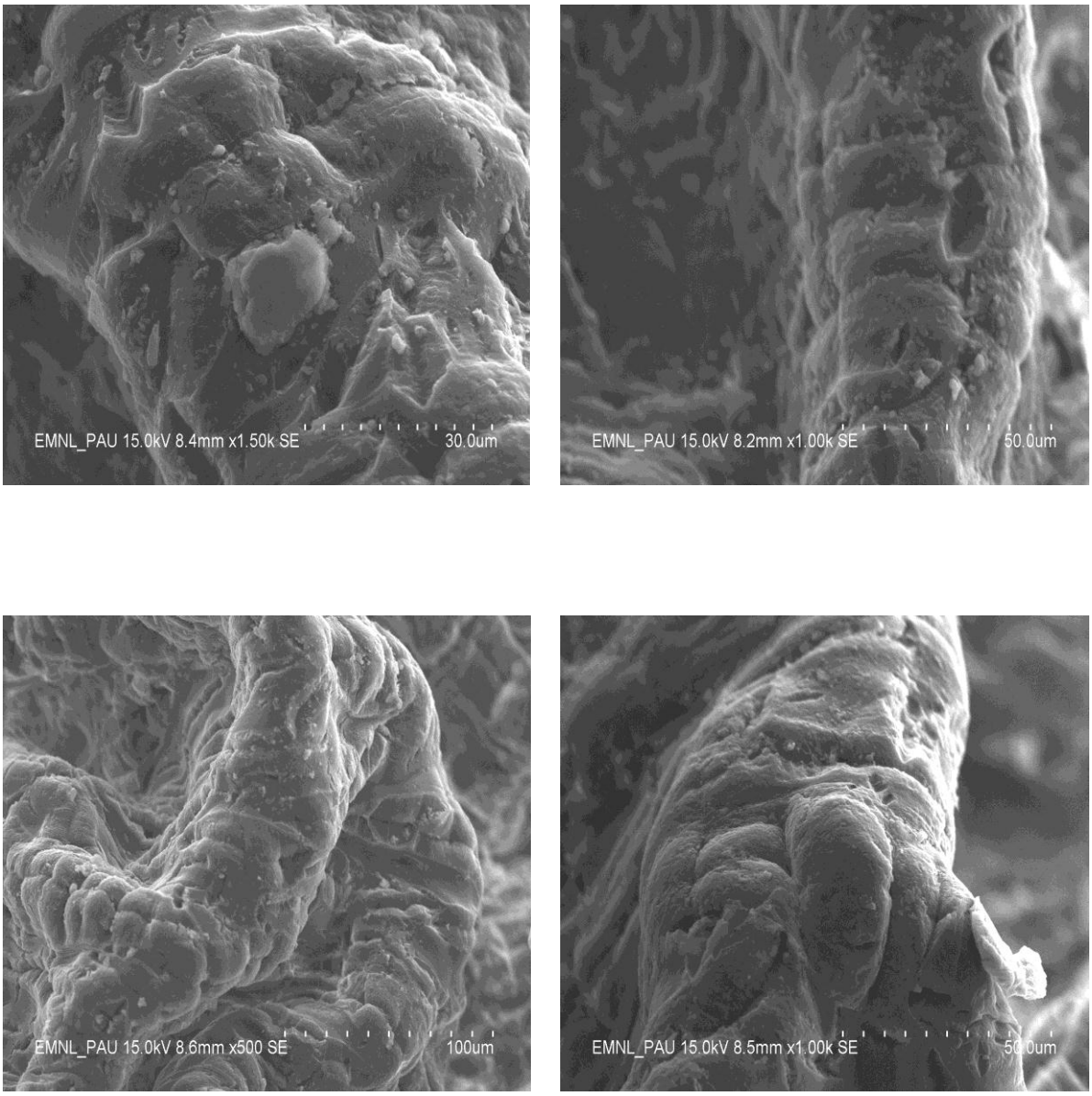


Plate 7: Stereo Electron Microscope (SEM) images of sections of *A. scholaris* galls

thickness of palisade tissues over healthy was 14.62 per cent (Table 8). Cells of the epidermis and the spongy cells proliferate and become hypertrophied (Albert *et al* 2011).

D. Spongy tissues thickness

The spongy tissues thickness was recorded lesser in the healthy leaves (138.37 µm) than the infected leaves (163.13 µm) (Plate 6). The percentage increase in the thickness of spongy tissues over healthy was 17.89 per cent (Table 8). The mesophyll tissues develop, involving the nymph and by divisions of the cells of the lower epidermis and of the adjacent parenchyma. During gall development, the cells of the spongy parenchyma become homogeneous and divide, resulting in an increase in gall size. The upper surface of the epidermis has isodiametric cells with a thick cuticle (Dias *et al* 2011).

E. Vascular tissues thickness

The vascular bundle thickness was recorded lesser in the healthy leaves (84.03 µm) as compared to the infected leaves (119.87 µm) (Plate 6). The percentage increase in the thickness of vascular bundles over healthy was 42.65 per cent (Table 8). The number of cell layers and vascular bundles, and the mesophyll thickness in the infected galls was higher than healthy leaves (Plate 6). The increase in cell number and thickness of the mesophyll and vascular bundles, as well as the considerable increase in the area of parenchyma cells, reflect the hyperplasia and hypertrophy typical of gall formation [Mani (1964), Oliveira and Isaias (2010), Moura *et al* (2009)]. Due to the feeding activity of the galling insect, vascular bundles malformation and the scarce xylem differentiation was observed in the vascular system of healthy leaves. The inhibition in vascular system development and the collapse of protoxylem cells of young galls, in general could happen in function of cell hypertrophy reported by Souza *et al* (2000).

The anatomically studies are supported by the Stereo Electron Microscope (SEM) studies undertaken on galled leaves support hypertrophy induced by *P. tuberculata* in *Alstonia* leaves (Plate 7). Also indicating an increase in mesophyll palisade tissues and mesophyll spongy tissues due to gall infection.

4.3.3 Biochemical alterations due to *P. tuberculata*

A. Total Chlorophyll

(i) Leaf galls

The chlorophyll a, chlorophyll b and total chlorophyll content was recorded to be higher in healthy as compared to gall infected leaves (Table 9).

Table 9: Changes in Chlorophyll Content of healthy and gall infected leaves*

Location	Chlorophyll a (mg/ g fresh weight)		Per cent decrease over healthy	Chlorophyll b (mg/ g fresh weight)		Per cent decrease over healthy	Total Chlorophyll (mg/ g fresh weight)		Per cent decrease over healthy	Plant height (m)	Girth of tree (m)
	Healthy	Infected		Healthy	Infected		Healthy	Infected			
A	1.06±0.25	0.22±0.02	79.35	0.89±0.01	0.04±0.01	95.00	1.96±0.26	0.27±0.04	86.45	19.5	2.5
B	1.33±0.15	0.19±0.01	85.90	0.36±0.05	0.08±0.04	77.55	1.70±0.20	0.27±0.06	83.80	5.5	0.80
C	1.54±0.22	0.16±0.04	89.25	1.01±0.16	0.04±0.02	96.40	2.58±0.37	0.18±0.06	92.80	11	1.20
D	1.53±0.16	0.23±0.14	85.35	1.28±0.47	0.05±0.04	95.10	2.83±0.31	0.29±0.18	89.30	11.25	2.15
E	1.25±0.06	0.22±0.06	82.80	0.80±0.23	0.04±0.02	94.00	2.07±0.16	0.26±0.09	87.50	10	1.55

*Mean of five replications, Figures are Mean±SE

t calculated value (0.05) ('t' test)

Chlorophyll a = 12.6

Chlorophyll b = 5.4

Total Chlorophyll = 34.0

t table value = 1.86 (df = 8)

Location A= Agronomy Department Location B= Biotechnology Department

Location C= Floriculture Department Location D= Gate number four

Location E= GADVASU campus

The maximum total chlorophyll (2.83 mg /g fresh weight) and chlorophyll b (1.28 mg/g fresh weight) was recorded in location in D (Gate number four). However, maximum chlorophyll a (1.54 mg/g fresh weight) was recorded in location C (Floriculture Department) (Table 9).

The percentage decrease in chlorophyll a content over healthy varied between 79.35 to 89.25 per cent, the chlorophyll b varied between 77.55 to 96.40 per cent and total chlorophyll varied between 83.80 to 92.80 per cent.

Albert *et al* (2011) observed that tissues of healthy leaves of uninfested and infested tree showed a slight variation in the chlorophyll content. The galled tissues showed a decrease in the chlorophyll content with the increase in the growth of the gall.

This loss of chlorophyll is responsible for the decolourisation of the area of the leaf where egg was laid on leaves (Moghe 1980). The low chlorophyll content in galled tissues was due to the loss of palisade tissues, disappearance of chloroplast and modifications of spongy mesophyll (Plates 1-3).

Similar decrease in chlorophyll content in gall infected leaves of *T. arjuna* and *T. tomentosa* have earlier been reported by Mukherjee *et al* (2016). The leaves with gall infestation showed a decrease in chlorophyll content. There was a significant variation in the chlorophyll content of the differentially infested leaves. The galled leaves 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 gall infected leaves and 69.75 mg/g in ungalled leaves in *T. arjuna.*

Khattab and Khattab (2005) studied that the chlorophyll a, chlorophyll b and total chlorophyll content was recorded to be higher in healthy as compared to gall infected leaves of *Eucalyptus oblique.*

(ii) Pod Galls

The chlorophyll a, chlorophyll b and total chlorophyll content was recorded to be higher in healthy as compared to gall infected pods (Tale 10).

The maximum total chlorophyll (2.57 mg /g fresh weight), chlorophyll a (1.45 mg/g fresh weight) and chlorophyll b (1.10 mg/g fresh weight) was recorded in location in D (Gate number four). The percentage decrease in chlorophyll a content over healthy varied between 81.80 to 86.70 per cent, the chlorophyll b varied between 81.30 to 98.10 per cent and total chlorophyll varied between 83.50 to 90.30 per cent (Table 10).

Table 10. Changes in Chlorophyll Content of healthy and gall infected pods*

Location	Chlorophyll a (mg/ g fresh weight)		Per cent decrease over healthy	Chlorophyll b (mg/ g fresh weight)		Per cent decrease over healthy	Total Chlorophyll (mg/ g fresh weight)		Per cent decrease over healthy	Plant height (m)	Girth of tree (m)
	Healthy	Infected		Healthy	Infected		Healthy	Infected			
A	1.19±0.09	0.22±0.01	81.80	1.02±0.18	0.06±0.02	94.70	2.22±0.08	0.28±0.02	87.60	19.5	2.5
B	1.25±0.06	0.20±0.01	83.95	0.38±0.08	0.08±0.01	81.30	1.64±0.03	0.27±0.03	83.50	5.5	0.80
C	1.40±0.06	0.21±0.07	85.05	0.99±0.28	0.02±0.01	98.10	2.42±0.23	0.23±0.06	90.30	11	1.20
D	1.45±0.08	0.25±0.11	82.90	1.10±0.20	0.05±0.01	95.80	2.57±0.12	0.30±0.13	88.20	11.25	2.15
E	1.38±0.10	0.19±0.06	86.70	0.70±0.49	0.07±0.03	84.75	2.11±0.40	0.26±0.09	87.25	10	1.55

*Mean of five replications, Figures are Mean±SE

t calculated value (0.05) ('t' test)

Chlorophyll a = 42.99

Chlorophyll b = 2.70

Total Chlorophyll = 12.1

t table value = 1.86 (df = 8)

Location A= Agronomy Department Location B= Biotechnology Department

Location C= Floriculture Department Location D= Gate number four

Location E= GADVASU campus

B. Total Soluble Protein

(i) Leaf Galls

The total soluble protein content was recorded to be lesser in healthy as compared to gall infected leaves (Table 11).

The maximum total soluble proteins (1.75 mg/g fresh weight) were recorded in location C (Floriculture Department) in healthy leaves but the maximum total soluble proteins (3.33 mg/g fresh weight) were recorded in location D (Gate number four) in gall infected leaves. The percentage increase in total soluble proteins content over healthy varied between 40.15 to 53.45 per cent (Table 11).

Albert *et al* (2011) reported that the total protein content in normal leaf of uninfested plant and infested tree was more or less equal. The unburst galled tissue showed almost two fold increases in the protein content. The protein content showed an initial increase and registered the highest level during the young galled stage of their development and declined thereafter in the mature burst galled tissue where the nymphal stage had already exited out from the chamber.

Table 11. Total soluble protein content of healthy and gall infected leaves

Location	Total_Soluble_Proteins (mg/g fresh weight)*		Per cent increase over healthy
	Healthy	Infected	
A	1.56±0.07	3.01±0.21	48.05
B	1.51±0.24	2.89±0.16	47.80
C	1.84±0.11	3.10±0.17	40.65
D	1.56±0.33	3.05±0.08	48.95
E	1.53±0.13	2.84±0.33	45.50

*Mean of five replications, Figures are Mean±SE
t calculated value (0.05) = 14.8 ('t' test)
t table value = 1.86 (df = 8)
Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

Similar increase in total soluble proteins content in gall infected leaves of *T. arjuna* and *T. tomentosa* have earlier been reported by Mukherjee *et al* (2016). Higher protein content was recorded in the infested leaf and significant change compared to control. The galled leaves recorded 186.50 mg/ g tissue of total soluble proteins content as compared to the

ungalled leaves 148.20 mg/g in *T. tomentosa.* Similarly it was 157.10 mg/g in gall infected leaves and 134.33 mg/g in ungalled leaves in *T. arjuna.*

El-Akkad (2004) observed the total soluble proteins content in gall infected leaves of *Populus nigra*. The galled leaves recorded higher total soluble protein content (175 mg/ g tissue) as compared to the ungalled leaves (164 mg/g tissue).

The higher protein concentration observed in the galled tissue corroborates the observations of earlier reports (Mehalingam 1999 and Scareli-Santos *et al* 2008). 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 mechanism of the plant (Reinbothe *et al* 1994). The increased secretion of defensive proteins by host plants blocks the action of proteolytic enzymes from herbivores which contributes to higher proteins in the leaves on infestation. Similar observations were reported in many plant defence mechanisms. These enzymes, 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 (Ananthakrishnan 1998).

(ii) Pod Galls

The total soluble proteins content was recorded to be lesser in healthy as compared to gall infected pods (Table 12).

Table 12. Total Soluble Protein content of healthy and gall infected pods

Location	Total Soluble Protein (mg/g fresh weight)*		Per cent increase over healthy
	Healthy	Infected	
A	1.52±0.43	3.27±0.01	53.45
B	1.69±0.38	2.81±0.52	40.15
C	1.75±0.09	3.10±0.60	42.75
D	1.66±0.20	3.33±0.33	49.70
E	1.45±0.48	2.64±0.49	45.70

*Mean of five replications, Figures are Mean±SE

t calculated value (0.05) = 9.6 ('t' test)

t table value = 1.86 (df = 8)

Location A= Agronomy Department Location B= Biotechnology Department

Location C= Floriculture Department Location D= Gate number four

Location E= GADVASU campus

The maximum total soluble proteins content was recorded in healthy pods (1.75 mg/g fresh weight) and the maximum total soluble protein content in gall infected (3.10 mg/g fresh weight) were recorded in location C (Floriculture Department). The percentage increase in total soluble proteins content over healthy was varied between 40.65 to 48.95 per cent.

C. Total Soluble Sugars

(i) Leaf Galls

The total sugars soluble content was recorded to be lesser in healthy as compared to gall infected leaves (Table 13).

The maximum total soluble sugars (9.26 mg/g fresh weight) were recorded in location A (Agronomy Department) in healthy leaves but the maximum total soluble sugars (10.80 mg/g fresh weight) were recorded in location C (Floriculture Department) in gall infected leaves. The percentage increase in total sugars content over healthy was varied between 7.24 to 51.76 per cent.

The level of total soluble sugars in ungalled leaves of uninfested and infested tree showed no variation as reported by Albert *et al* (2011). A steady increase of sugar content was noticed in the galled leaves and the burst gall registered the highest level as compared to uninfected leaves.

Table 13. Total Soluble Sugars of healthy and gall infected leaves

Location	Total Soluble Sugars Content (mg/g fresh weight)*		Per cent increase over healthy
	Healthy	Infected	
A	9.26±1.22	9.97±0.23	7.24
B	8.59±1.02	9.83±0.01	12.58
C	5.19±1.73	10.80±0.26	51.76
D	6.02±0.21	8.54±2.62	26.43
E	6.87±0.91	9.64±0.81	28.88

*Mean of five replications, Figures are Mean±SE
t calculated value (0.05) = 3.88 ('t' test)

t table value = 1.86 (df = 8)

Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

Hartley (1998) studied that the level of total soluble sugars in gall infested and reported that it increased in initial stages due to the tissue accumulated starch which is generally less in the ungalled tissue. The stimulus of gall forming insects redirected growth

and differentiation of cells which acted as a sink of nutritive substances from the host plants by normal flow of resources and/ or by the active mobilization of neighboring parts of the gall. Shannon and Brewer (1980) studied that differences in gall starch and sugar levels reflected morphological differences in the mouthparts of the insects concerned.

Similar increase in total soluble sugars content in gall infected leaves of *T. arjuna* and *T. tomentosa* have earlier been reported by Mukherjee *et al* (2016). The level of total soluble 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 as compared to uninfected leaves. Comparatively a significant difference was observed in ungalled and the gall infested leaves. The galled leaves recorded 195.13 mg/ g tissue of total sugars content as compared to the ungalled leaves 188.53 mg/g in *T. tomentosa.* Similarly it was 205.46 mg/g in gall infected leaves and 210.50 mg/g in ungalled leaves in *T. arjuna.*

(ii) Pod Galls

The total soluble sugars content was recorded to be lesser in healthy as compared to gall infected pods (Table 14).

The maximum total soluble sugars content (8.47 mg/g fresh weight) was recorded in location A (Agronomy Department) in healthy pods whereas the maximum total soluble sugars content (10.02 mg/g fresh weight) was recorded in infected pods at location C (Floriculture Department). The percentage increase in total soluble sugars content over healthy varied between 4.04 to 44.20 per cent.

Table 14. Total Soluble Sugars of healthy and gall infected pods

Location	Total Soluble Sugars (mg/g fresh weight)*		Per cent increase over healthy
	Healthy	Infected	
A	8.47±0.93	8.87±0.40	4.71
B	8.10±0.41	8.46±0.82	4.04
C	5.45±1.63	10.02±1.26	44.20
D	6.82±0.23	8.77±1.42	21.37
E	7.18±0.29	8.96±0.65	19.60

*Mean of five replications, Figures are Mean±SE
t calculated value (0.05) = 3.9 (‘t’ test)
t table value = 1.86 (df = 8)
Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

D. Total Proline

(i) Leaf Galls

The total proline content was recorded to be lesser in healthy as compared to gall infected leaves (Table 15).

The maximum total proline (0.0151 µg/g fresh weight) was recorded in location C (Floriculture Department) in healthy leaves but the maximum total proline (0.0266 µg/g fresh weight) was recorded in location E (GADVASU) in gall infected leaves. The percentage increase in total proline content over healthy varied between 48.40 to 65.75 per cent (Table 15).

Table 15. Total Proline Content of healthy and gall infected leaves

Location	Total Proline content (µg/g fresh weight)*		Per cent increase over healthy
	Healthy	Infected	
A	0.0062±0.0006	0.0181±0.0004	65.75
B	0.0106±0.0021	0.0205±0.0024	48.50
C	0.0151±0.0005	0.0311±0.0023	51.55
D	0.0082±0.0004	0.0189±0.0007	56.65
E	0.0137±0.0008	0.0266±0.0030	48.40

*Mean of five replications, Figures are Mean±SE
t calculated value (0.05) = 142.6 ('t' test)
t table value = 1.86 (df = 8)
Location A= Agronomy Department Location B= Biotechnology Department
Location C= Floriculture Department Location D= Gate number four
Location E= GADVASU campus

Albert *et al* (2011) reported that the normal leaf from an uninfected tree showed absolutely no trace of proline. A normal leaf of the infested branch of an infested tree showed a very low concentration of proline. The galled tissue showed a very high increase in the proline content and in the mature gall the content was very high almost double fold than the young gall indicating stressed condition of the galled tissue.

Similar increase in total proline content in gall infected leaves of *T. arjuna* and *T. tomentosa* have earlier been reported by Mukherjee *et al* (2016). Higher proline content was recorded in the infested leaf and significant change compared to control. The galled leaves recorded 26 µg/ml of total proline content as compared to the ungalled leaves 8 µg/ml in *T. tomentosa.* Similarly, it was 20 µg/ml in gall infected leaves and 10 µg/ml in ungalled leaves in *T. arjuna.*

An increase of proline content in the galled leaves of *T. arjuna* and *T. tomentosa* has been reported as compared to the ungalled leaves. Proline is produced as a defense 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 has been observed in galled leaves of *Populus* (El-Akkad 2004). Proline accumulation is known to be a response to stress condition in plants (Bates *et al* 1973).

El-Akkad (2004) reported the total proline content in gall infected leaves of *P. nigra*. The galled leaves recorded 1.183 µmol/g tissue of total proline content as compared to the ungalled leaves 0.589 µmol/g tissue. The increased level of proline in galled leaves might confirm the idea that galling has stressful effect on the infested leaves of *P. nigra*. Proline accumulation in response to stress is widely reported, and may play a role in stress adaptation within the cell, which is of great interest to those studying stresses in plants (Gibon *et al* 2000).

Khattab and Khattab (2005) reported that the total proline content was recorded to be lesser in healthy as compared to gall infected leaves of *Eucalyptus oblique.* The galled leaves recorded 0.91 µmol/g fresh weight of total proline content as compared to ungalled leaves 0.51 µmol/g fresh weight.

(ii) Pod Galls

The total proline content was recorded to be lesser in healthy as compared to gall infected pods (Table 16).

Table 16. Changes in Total Proline Content of healthy and gall infected pods

Location	Total_Proline_content (µg/g fresh weight)*		Per cent increase over healthy
	Healthy	Infected	
A	0.0093±0.0012	0.0273±0.0031	65.65
B	0.0154±0.0006	0.0213±0.0027	27.15
C	0.0286±0.0004	0.0381±0.0002	24.95
D	0.0085±0.0005	0.0174±0.0005	50.85
E	0.0128±0.0013	0.0234±0.0065	42.35

*Mean of five replications, Figures are Mean±SE

t calculated value (0.05) = 10.476 ('t' test)

t table value = 1.86 (df = 8)

Location A= Agronomy Department Location B= Biotechnology Department

Location C= Floriculture Department Location D= Gate number four

Location E= GADVASU campus

The maximum total proline content in healthy pods (0.0286 µg/g fresh weight) and in infected pods (0.0381 µg/g fresh weight) was recorded at location C (Floriculture Department). The percentage increase in total proline content over healthy varied between 24.95 to 65.65 per cent (Table 16).

4.4 Evaluation of insecticides for the management of *P. tuberculata*

4.4.1 Efficacy of different insecticides against nymphs of *P. tuberculata* in field (1st spray) during July 2015

The data revealed that among the different insecticides evaluated against nymphs of *P. tuberculata* in field (1st spray) during 2015 (Table 17). Among the lower doses after 3 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (81.83%) and minimum mortality causes by buprofezin 25 EC (66.10%). After 7 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (84.16%) and minimum mortality was caused by buprofezin 25 EC (67.46%). After 10 days of spray maximum mortality of nymphs causes by thiamethoxam 25 WG (90.23%) and minimum mortality was caused by buprofezin 25 EC (70.40%).

Among the middle doses after 3 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (96.02%) and minimum mortality was caused by buprofezin 25 EC (70.03%). After 7 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (96.72%) and minimum mortality was caused by buprofezin 25 EC (71.00%). After 10 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.35%) and minimum mortality was caused by buprofezin 25 EC (74.80%).

Among the higher doses after 3 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by spinosad 45 SC (75.33%). After 7 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by spinosad 45 SC (80.56%). After 10 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by buprofezin 25 EC (85.16%).

Among all the doses thiamethoxam 25 WG resulted in maximum nymphal mortality (99.39%) @ 0.3 g, 0.6 g and 0.9 g and buprofezin 25 EC @ 2.0 ml resulted in minimum nymphal mortality (66.10). In control, 22.23, 25.00 and 26.03 per cent nymphal mortality after 3, 7 and 10 days respectively, were recorded.

Table 17. Efficacy of different insecticides to nymphs of *P. tuberculata* in field (1st spray) during July 2015

*Mean cumulative nymph mortality (%) at different days of exposure				
Treatment	Dose	Mortality (%)		
		3 days	7 days	10 days
Lower Doses				
Imidacloprid 17.8 SL	0.4 ml	74.80 (59.94)	76.53 (61.17)	77.90 (61.95)
Spinosad 45 SC	0.5 ml	68.60 (55.91)	71.89 (57.97)	72.06 (58.08)
Buprofezin 25 EC	2.0 ml	66.10 (54.37)	67.46 (55.21)	70.40 (57.03)
Thiamethoxam 25 WG	0.3 g	81.83 (65.00)	84.16 (66.57)	90.23 (72.93)
Azadirachtin 1%	3.0 ml	76.33 (60.86)	77.40 (61.60)	80.90 (64.06)
Middle Doses				
Imidacloprid 17.8 SL	0.8 ml	75.26 (60.24)	78.63 (62.59)	85.26 (67.69)
Spinosad 45 SC	1.0 ml	70.40 (57.02)	75.96 (60.68)	80.60 (63.91)
Buprofezin 25 EC	3.0 ml	70.03 (56.81)	71.00 (57.39)	74.80 (59.87)
Thiamethoxam 25 WG	0.6 g	96.02 (82.85)	96.72 (83.50)	99.35 (86.94)
Azadirachtin 1%	4.0 ml	79.06 (62.75)	79.36 (62.97)	82.77 (65.46)
Higher Doses				
Imidacloprid 17.8 SL	1.2 ml	83.76 (66.25)	86.46 (68.42)	86.69 (68.82)
Spinosad 45 SC	1.5 ml	75.33 (60.22)	80.56 (63.84)	85.36 (67.64)
Buprofezin 25 EC	4.0 ml	76.20 (60.82)	80.90 (64.17)	85.16 (67.44)
Thiamethoxam 25 WG	0.9 g	99.99 (89.39)	99.99 (89.39)	99.99 (89.39)
Azadirachtin 1%	5.0 ml	83.63 (66.17)	83.66 (66.97)	87.07 (69.04)
Control		22.23 (28.11)	25.00 (29.96)	26.03 (30.61)
CD (p=0.05)		(6.52)	(6.51)	(4.53)

*Mean of three replications, Figures in the parentheses are arc sine $\sqrt{\text{percentage}}$ transformed values

4.4.2 Efficacy of different insecticides against nymphs of *P. tuberculata* in field (2nd spray) during August 2015

Among the different insecticides evaluated against nymphs of *P. tuberculata* in field (2nd spray) during 2015 (Table 18). Among the lower doses after 3 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (98.13%) and minimum mortality was caused by buprofezin 25 EC (76.70%). After 7 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.39%) and minimum mortality was caused by buprofezin 25 EC (80.03%). After 10 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.39%) and minimum mortality was caused by buprofezin 25 EC (80.97%).

Among the middle doses after 3 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.39%) and minimum mortality was caused by buprofezin 25 EC (78.46%). After 7 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (96.72%) and minimum mortality was caused by buprofezin 25 EC (80.97%). After 10 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by buprofezin 25 EC (82.57%).

Among the higher doses after 3 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by buprofezin 25 EC (81.87%). After 7 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by buprofezin 25 EC (83.93%). After 10 days of spray maximum mortality of nymphs was caused by thiamethoxam 25 WG (99.99%) and minimum mortality was caused by buprofezin 25 EC (84.50%).

Among all the doses thiamethoxam 25 WG resulted in maximum nymphal mortality (99.39%) @ 0.3 g, 0.6 g and 0.9 g and buprofezin 25 EC @ 2.0 ml resulted in minimum nymphal mortality (76.70%). In control, 21.46, 21.80 and 23.73 per cent nymphal mortality after 3, 7 and 10 days respectively, were recorded.

Table 18. Efficacy of different insecticides to nymphs of *P. tuberculata* in field (2nd spray) during August 2015

*Mean cumulative nymph mortality (%) at different days of exposure				
Treatment	Dose	Mortality (%)		
		3 days	7 days	10 days
Lower Doses				
Imidacloprid 17.8 SL	0.4 ml	81.40 (64.42)	84.56 (66.93)	86.70 (68.62)
Spinosad 45 SC	0.5 ml	77.67 (61.79)	80.47 (63.75)	82.86 (65.52)
Buprofezin 25 EC	2.0 ml	76.70 (61.11)	80.03 (63.43)	80.97 (64.11)
Thiamethoxam 25 WG	0.3 g	98.13 (83.43)	99.99 (89.39)	99.99 (89.39)
Azadirachtin 1%	3.0 ml	79.53 (63.28)	88.23 (70.06)	89.50 (71.20)
Middle Doses				
Imidacloprid 17.8 SL	0.8 ml	86.93 (69.00)	88.03 (69.82)	89.13 (70.77)
Spinosad 45 SC	1.0 ml	82.50 (65.24)	83.67 (66.14)	85.23 (67.38)
Buprofezin 25 EC	3.0 ml	78.46 (62.23)	80.97 (64.11)	82.57 (65.30)
Thiamethoxam 25 WG	0.6 g	99.99 (89.39)	99.99 (89.39)	99.99 (89.39)
Azadirachtin 1%	4.0 ml	90.03 (71.68)	91.69 (73.34)	92.17 (73.78)
Higher Doses				
Imidacloprid 17.8 SL	1.2 ml	88.96 (78.75)	89.83 (71.50)	90.36 (72.00)
Spinosad 45 SC	1.5 ml	85.80 (67.91)	88.00 (69.75)	89.27 (70.89)
Buprofezin 25 EC	4.0 ml	81.87 (64.78)	83.93 (66.35)	84.50 (66.80)
Thiamethoxam 25 WG	0.9 g	99.99 (89.39)	99.99 (89.39)	99.99 (89.39)
Azadirachtin 1%	5.0 ml	91.63 (73.29)	92.37 (74.00)	93.10 (74.82)
Control		22.23 (28.11)	25.00 (29.96)	26.03 (30.61)
CD (p=0.05)		(4.34)	(2.93)	(2.63)

*Mean of three replications, Figures in the parentheses are arc sine $\sqrt{\text{percentage}}$ transformed values

CHAPTER V

SUMMARY

Alstonia scholaris is a beautiful evergreen landscape tree belonging to family Apocynaceae. It is a beautiful foliage tree with large canopy and is one of the most popular ornamental trees in landscapes, garden as well as roadside in warm and temperate regions. It is widely distributed in India.

Leaf gall former, *P. tuberculata* Crawford (Psyllidae: Homoptera) is one of the major pest of *A. scholaris*. It induces gall formation on each and every part (Stem, leaves, infloroscence) of this highly prized tree which adversely affects the looks and its economic value.

From the present study of population dynamics of gall formation and psyllids emerged from the galls on leaves or pods during May 2015 to April 2016, it was found that the maximum number of galls caused by *P. tuberculata* on leaves were observed in the month of July 2015 (58.80) at location B (Biotechnology Department) and minimum galls were observed in month of January 2016 (7.00) at location E (GADVASU). The number of galls/pod was maximum in the month of July 2015 (45.20) at location B (Biotechnology Department) and minimum in the month of December 2015 (2.60) at location B (Biotechnology Department), January 2016 (2.60) and February 2016 (2.60) at location E (GADVASU).

Number of psyllids emerged (galls/leaf) were maximum in the month of July 2015 (51.03) at location B (Biotechnology Department) and minimum in the month of January 2016 (5.33) at location D (Gate number four). Similarly, the maximum number of psyllids (galls/pod) emerged in the month of July 2015 (39.00) at location B (Biotechnology Department) and minimum in the month of January 2016 (1.80) at location E (GADVASU).

Pooled data of population parameters of *P. tuberculata* on *A. scholaris* during different months of 2015-16 revealed that, the mean number of galls/leaf were maximum in the month of July 2015 (47.37), minimum in the month of January 2016 (10.17) and the mean number of galls/pod was maximum in the month of July 2015 (44.48), minimum in the month of December 2015 (3.04). Similarly, the mean number of psyllids emerged from the galls were maximum in the month of July 2015 (42.03), minimum in the month of January 2016 (7.91) and the mean number of psyllids emerged from that galls were maximum in the month of July 2015 (35.60), minimum in the month of February 2016 (2.48). The population indices were positively correlated with the temperature (maximum and minimum), rainfall and negatively correlated with the relative humidity (RH).

P. tuberculata has five nymphal instars in its life cycle. The total nymphal period ranges between 22-24 days. The mature insect is colorful, winged and with bulging eyes and the female is larger than male in size. Due to hypertrophy, galls were formed on both the upper and lower surfaces of the leaves. But they were abundant on the lower surface. Slight decolorization was visible on the areas where the eggs were deposited. Majority of the galls were formed on the lateral veins. With an increase of galls in number all the leaves appeared crumbled and completely deformed. The leaf galls occured scattered and isolated. During the later stage of the gall, a depression/halo was formed. The number of galls varied from 25 to numerous on a single leaf. Depending upon the maturity of the galls the diameter of the gall chamber ranges from 0.1 cm to 1 cm.

The thickness of upper epidermis (18.74±1.71 µm) and lower epidermis (15.04±2.24 µm) was higher in healthy leaf than infested leaf. In the mesophyll cells, the thickness of palisade tissues (47.21±9.98 µm), spongy tissues (163.13±69.59 µm) and vascular bundles (119.87±12.22 µm) were higher in the infested leaves as compared to healthy leaves. The total chlorophyll content of healthy leaf (2.83±0.31 mg/g fresh weight) was higher than infested leaf (0.29±0.18 mg/g fresh weight) at location D (Gate number four). The total chlorophyll content of healthy pod (2.57±0.12 mg/g fresh weight) was higher than infested pod (0.30±0.13 mg/g fresh weight) at location D (Gate number four). The total soluble protein content was more in infested leaf (3.10±0.17 mg/g fresh weight) as compared to healthy leaf (1.84±0.11 mg/g fresh weight) at location C (Floriculture Department). Similarly, the total soluble protein content was more in infested pod (3.33±0.33 mg/g fresh weight) at location D (Gate number four) as compared to healthy pod (1.75±0.09 mg/g fresh weight) at location C (Floriculture Department). The total soluble sugars content was more in infested leaf (10.80±0.26 mg/g fresh weight) at location C (Floriculture Department) as compared to healthy leaf (9.26±1.22 mg/g fresh weight) at location A (Agronomy Department). Similarly, the total soluble sugars content was higher in infested pod (10.02±1.26 mg/g fresh weight) at location C (Floriculture Department) as compared to healthy pod (8.47±0.93 mg/g fresh weight) at location A (Agronomy Department).

The total proline content was higher in infested leaf (0.0311±0.0023 µg/g fresh weight) at location C (Floriculture Department) as compared to healthy leaf (0.0151±0.0005 µg/g fresh weight). The total proline content was more in infested leaf (0.0381±0.0002 µg/g fresh weight) at location C (Floriculture Department) as compared to healthy pod (0.0286±0.0004 µg/g fresh weight).

Thiamethoxam 25 WG @ 0.3 g, 0.6 g and 0.9 g caused 99.99 per cent nymphal mortality after 10 days of exposure, and was the most effective for the management of *P. tuberculata* on *Alstonia* trees.

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