

**STUDYING VARIATION AMONG *EUCALYPTUS* CLONES  
FOR GROWTH TRAITS, FLOWERING, AND TOLERANCE  
TO GALL WASP (*LEPTOCYBE INVASA*)**

**Thesis**

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

**MASTER OF SCIENCE  
in  
FORESTRY  
(Minor Subject: Entomology)**

**By**

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(L-2017-A-80-M)**

**Department of Forestry and Natural Resources  
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**2020**

## **CERTIFICATE – I**

This is to certify that the thesis entitled, “**STUDYING VARIATION AMONG *EUCALYPTUS* CLONES FOR GROWTH TRAITS, FLOWERING, AND TOLERANCE TO GALL WASP (*LEPTOCYBE INVASA*)**” submitted for the degree of **Master of Science**, in the subject of **Forestry** (Minor subject: **Entomology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Akashdeep Singh (L-2017-A-80-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, “**STUDYING VARIATION AMONG *EUCALYPTUS* CLONES FOR GROWTH TRAITS, FLOWERING, AND TOLERANCE TO GALL WASP (*LEPTOCYBE INVASA*)**” submitted by **Akashdeep Singh (L-2017-A-80-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirement for the degree of **Master of Science**, in the subject of **Forestry** (Minor Subject: **Entomology**) 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

Clonal *Eucalyptus* is the widely adopted by farmers of North-Western India and provides raw material for plywood and paper industries. The present study entitled “Studying variation among *Eucalyptus* clones for growth traits, flowering and tolerance to gall wasp *Leptocybe invasa*” was conducted at Department of Forestry & Natural Resources, Punjab Agricultural University, Ludhiana, by recording observations on 3 year old clonal trial, to investigate variation among the fourteen clones for various growth traits, flowering behaviour and incidence of gall wasp. In this study, clone C-413, C-407 and PE-11 were found to be promising ones from growth point of view, whereas, clone PE-11, C-413, C-72 and C-407 were superior in stem quality traits. Profused flowering were recorded in clone PE-7, PE-8 and PE-9, and nil flowering was in case of C-72, PE-12 and PE-14. In clone C-413 and C-407, floral buds were noticed but blossoming did not occurred. The highest honey bee visitation was recorded during afternoon session and maximum visitation on clone PE-9. The maximum incidence was recorded in F-316 and relatively lowest incidence was in case of clone C-413, PE-11, PE-14 and C-72. Gall incidence was recorded to be the highest in the month of April and starts to decrease from May to December and gradually increased January to April. The content of total soluble sugars and total phenol were found higher in the infested leaves whereas the protein content were found higher in the healthy leaves. Clones C-413 and PE-11 were found to be promising ones on basis of combined index score analysis and followed by C-72.

**Keywords:** Clone diversity, *Eucalyptus*, selection, gall wasp

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Signature of Major Advisor

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ਉੱਤਰ-ਪੱਛਮੀ ਭਾਰਤ ਵਿੱਚ ਕਿਸਾਨਾਂ ਨੇ ਕਲੋਨਲ ਯੂਕਲਿਪਟਸ ਦੀ ਕਾਸ਼ਤ ਨੂੰ ਬਹੁਤੇ ਵੱਡੇ ਪੱਧਰ ਉੱਪਰ ਅਪਣਾਇਆ ਹੈ ਅਤੇ ਇਸ ਦੀ ਕਾਸ਼ਤ ਕਰਕੇ ਉਹ ਪਲਾਈ-ਵੁੱਡ ਅਤੇ ਪੇਪਰ ਉਦਯੋਗ ਨੂੰ ਕੱਚਾ ਮਾਲ ਮੁਹੱਈਆ ਕਰਵਾਉਂਦੇ ਹਨ। ਮੌਜੂਦਾ ਅਧਿਐਨ “ਵੱਖੋ-ਵੱਖਰੇ ਵਿਕਾਸ ਗੁਣਾਂ, ਫੁੱਲਾਂ ਦੇ ਵਤੀਰੇ ਅਤੇ ਗੰਡ ਭਰਿੰਡ (*Leptocybe invasa*) ਪ੍ਰਤੀ ਸਹਿਣਸ਼ੀਲਤਾ ਲਈ ਯੂਕਲਿਪਟਸ ਕਲੋਨਾਂ ਵਿੱਚ ਵਿਭਿੰਨਤਾ ਦਾ ਮੁਲਾਂਕਣ” ਸਿਰਲੇਖ ਅਧੀਨ ਪੰਜਾਬ ਐਗਰੀਕਲਚਰਲ ਯੂਨੀਵਰਸਿਟੀ ਲੁਧਿਆਣਾ ਦੇ ਜੰਗਲਾਤ ਅਤੇ ਕੁਦਰਤੀ ਸੋਮੇ ਵਿਭਾਗ ਵਿੱਚ ਤਿੰਨ ਸਾਲ ਦੀ ਉਮਰ ਦੇ ਚੌਦਾਂ ਕਲੋਨਾਂ ਵਿੱਚ ਵਿਭਿੰਨਤਾ ਦਾ ਪਤਾ ਲਗਾਉਣ ਲਈ ਕੀਤਾ ਗਿਆ। ਇਸ ਖੋਜ ਵਿੱਚ ਵਿਕਾਸ ਦੇ ਲਿਹਾਜ਼ ਨਾਲ C-413, C-407 ਅਤੇ PE-11 ਵਧੀਆ ਪਾਏ ਗਏ ਜਦੋਂਕਿ ਤਣੇ ਦੀ ਗੁਣਵਤਾ ਦੇ ਲਿਹਾਜ਼ ਨਾਲ PE-11, C-413, C-72 ਅਤੇ C-407 ਕਲੋਨ ਵਧੀਆ ਸਨ। PE-7, PE-8 ਅਤੇ PE-9 ਕਲੋਨਾਂ ਵਿੱਚ ਬਹੁਤ ਵਧੇਰੇ ਫੁੱਲ ਪਏ ਜਦੋਂ ਕਿ C-72, PE-12 ਅਤੇ PE-14 ਕਲੋਨਾਂ ਵਿੱਚ ਬਿਲਕੁੱਲ ਵੀ ਫੁੱਲ ਨਹੀਂ ਪਏ। C-413 ਅਤੇ C-407 ਕਲੋਨਾਂ ਵਿੱਚ ਫੁੱਲਾਂ ਦੀਆਂ ਕਲੀਆਂ ਤਾਂ ਵੇਖਣ ਨੂੰ ਮਿਲੀਆਂ ਪਰ ਬੂਰ ਨਹੀਂ ਆਈ। ਕਲੋਨ PE-9 ਉੱਪਰ ਮਧੂ ਮੱਖੀਆਂ ਨੇ ਸ਼ਾਮ ਸਮੇਂ ਸਭ ਤੋਂ ਜ਼ਿਆਦਾ ਭ੍ਰਮਣ ਕੀਤਾ। ਗੰਡ ਭਰਿੰਡ ਦਾ ਸਭ ਤੋਂ ਜ਼ਿਆਦਾ ਹਮਲਾ F-316 ਵਿੱਚ ਵੇਖਣ ਨੂੰ ਮਿਲਿਆ ਅਤੇ ਕਲੋਨ C-413, PE-11, PE-14 ਅਤੇ C-72 ਵਿੱਚ ਸਭ ਤੋਂ ਘੱਟ ਤੀਬਰਤਾ ਦਰਜ ਕੀਤੀ ਗਈ। ਗੰਡ ਦੀ ਤੀਬਰਤਾ ਅਪ੍ਰੈਲ ਮਹੀਨੇ ਵਿੱਚ ਸਭ ਤੋਂ ਜ਼ਿਆਦਾ ਦਰਜ ਕੀਤੀ ਗਈ ਅਤੇ ਇਸ ਵਿੱਚ ਮਈ ਤੋਂ ਦਸੰਬਰ ਤੱਕ ਕਮੀ ਆਈ ਅਤੇ ਫਿਰ ਜਨਵਰੀ ਤੋਂ ਅਪ੍ਰੈਲ ਮਹੀਨੇ ਦੌਰਾਨ ਇਸ ਵਿੱਚ ਨਿਰੰਤਰ ਵਾਧਾ ਹੋਇਆ। ਰੋਗੀ ਪੱਤਿਆਂ ਵਿੱਚ ਕੁੱਲ ਘੁਲਣਸ਼ੀਲ ਸੂਗਰ ਅਤੇ ਕੁੱਲ ਫਿਨੋਲ ਦੀ ਮਾਤਰਾ ਸਭ ਤੋਂ ਜ਼ਿਆਦਾ ਸੀ ਜਦੋਂਕਿ ਪ੍ਰੋਟੀਨ ਦੀ ਮਾਤਰਾ ਸਹਿਤਮੰਦ ਪੱਤਿਆਂ ਵਿੱਚ ਸਭ ਤੋਂ ਜ਼ਿਆਦਾ ਦਰਜ ਕੀਤੀ ਗਈ। ਸੰਯੋਕਤਾ ਸੂਚਕਾਂਕ ਅੰਕ ਮੁਲਾਂਕਣ ਦੇ ਅਧਾਰ ਤੇ C-413 ਅਤੇ PE-11 ਕਲੋਨ ਵਧੀਆ ਪਾਏ ਗਏ ਅਤੇ ਇਸ ਮਗਰੋਂ C-72 ਵਧੀਆ ਕਲੋਨ ਸੀ।

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## CHAPTER I

### INTRODUCTION

*Eucalyptus* is one of the most widely planted hardwood genus because they can tolerate and grow well on degraded, unfertile soils, where it is very difficult to establish other tree species. The wood of *Eucalyptus* species can be used for production of paper, which providing income for local people and government. *Eucalyptus* belongs to family Myrtaceae. There are more than 800 species of *Eucalyptus* belongs to two genera *Eucalyptus* and *Corymbia*. All the species are originated from Australia except *Eucalyptus deglupta* Blume and *Eucalyptus urophylla* S.T. Blake which are indigenous to New Guinea and Timor and its adjoining areas respectively (Ohmart and Edwards 1991). Now *Eucalyptus* is cultivated in more than 120 countries and mainly planted in Australia, Thailand, India, Brazil, China, Indonesia and Vietnam (Yao and Chen 2009). The main species of *Eucalyptus* planted in South-East Asia are *E. camaldulensis*, *E. tereticornis*, and *E. urophylla*. In India, *Eucalyptus* is one of those exotic tree which become very popular and dominant in our country due to its wider adaptability, short rotation, straight and fast growth, withstand against drought and waterlogging conditions and variety of uses such as timber, boles, firewood, honeybee foraging, paper, pulp, recreational areas, shelterbelts and windbreaks etc.

In India, *Eucalyptus* was first planted around 1790 at Nandi hills near Bangalore by Tippu Sultan who was the emperor of Mysore in his garden (Sundar 1984). Subsequently, it was planted by Britishers at Nilgiri hills of Tamil Nadu around 1843 for timber purpose (Troup 1932). Commercial plantations of *Eucalyptus tereticornis* (Mysore gum) initiated from 1952 (Tewari 1992). In Punjab, first plantation was taken in 1860 by Director of Canals of Madhopur headworks situated on the Ravi river. In northern western states especially in Punjab, Haryana, Uttarakhand and Uttar Pradesh, many farmers had started *Eucalyptus tereticornis* as an agroforestry in their fields. Other major species planted in India are *Eucalyptus grandis*, *E. citriodora*, *E. torelliana*, *E. pellita*, *E. urophylla* and *E. globulus*. New clonal plantations were initiated from 1992 and 2,50,000 ha land come under clonal plantations and so on (Kulkarni 2004). *Eucalyptus* is adopted under Agroforestry in North-West India such as Punjab, Haryana and Western districts of Uttar Pradesh as boundary or block plantation and boundary plantation, along roadsides and canal banks.

*Eucalyptus* is reported to be attacked by 60 insect species but termites and beetles (*Celosterna scabrator*) were recorded causing considerable damage to *Eucalyptus* plantation (Sen-Sarma and Thakur 1983). In recent times, a gall forming insect gall wasp *Leptocybe invasa* Fisher and La Salle caused heavy damage to *Eucalyptus* plantations throughout the world (Mendel *et al* 2004). *L. invasa* was first reported in Middle East and Mediterranean

countries in 2000 where it caused heavy loss to young foliage (Fatih 2006). Currently this pest is reported in almost the whole tropical regions including India (Aytar 2003). In India, this pest was reported at Mandya district of Karnataka state and later on other states (Kumar *et al* 2007). In Punjab, the gall wasp was noticed first time in 2009 (Sangha *et al* 2011).

*L. invasa* belongs to order Hymenoptera and family Eulophidae. The pest causes severe injury to the young plantations by producing galls on the leaf midrib, petiole and young turgid shoots of seedlings, sapling and coppiced shoots (Mendel *et al* 2004). Severe attack leads to deformation of leaves bump-shaped galls, shoots and reduction in growth. In mature trees, the galls occur only on leaf midribs and upto two years of *Eucalyptus* has been reported more susceptible to the pest. Initially the galls are green and then turn pink.

*Eucalyptus* flowers produce profuse amount of pollen and nectar which attracts a wide range of pollinators such as *Apis dorsata*, *A. mellifera* etc. The foraging activity of honeybee depends upon the floral structure of the tree. *Eucalyptus* species show high variation in the flowering time (Blakely 1965).

Honey is the major non forest timber product (NFTP) and *Eucalyptus* is one of the highly important species for Apiculture. In Australia, 70 to 80 percent honey is derived from *Eucalyptus* trees (Somerville 2000). *Eucalyptus camaldulensis*, *E. delegatensis*, *E. melliodora*, *E. saligna*, *E. tereticornis*, *E. globulus* and *Corymbia citriodora* are important species for honey production and pollen availability for bees. In Australia, *E. paniculata*, *E. polyanthermos* and *E. caleyi* trees are not cut for timber but retained only for honey production (Moncur and Kleinschmidt 1992; Somerville 1998). The trees do not bear flowers until they are 5-6 years old. Therefore early flowering has been considered as a valuable trait in *Eucalyptus* breeding programmes (Chambers *et al* 1997; Wiltshire *et al* 1998). The traditional breeding programmes across the world focused on production of timber, pulp, fuel wood etc. and the value of beekeeping products was overlooked. Rather the plus trees are usually selected having nil or very less flowering.

With the adoption of clonal *Eucalyptus*, the farmers in Punjab and adjoining states shifted to the monoculture of few fast growing clones like C-413, C-72, C-2045 and C-288. Incidentally these clones have negligible flowering and Punjab State Horticulture Department had given feedback that trees of clonal *Eucalyptus* is adversely affecting apiculture in the state. Keeping in view the concerns of beekeepers and the threat of large scale spread of gall wasp, the present study is planned to study variation for tolerance to gall wasp and to study flowering behaviour in the existing germplasm in hand.

The objectives of the present study were:

1. To study the variation among *Eucalyptus* clones for growth and flowering behaviour to explore potential for Apiculture.
2. To study variation among *Eucalyptus* clones for tolerance/resistance to gall wasp.

## CHAPTER II

### REVIEW OF LITERATURE

The already related research work done on study “Studying variation among *Eucalyptus* clones for growth traits, flowering, and tolerance to gall wasp” has been described in this chapter as following:

2.1 Growth traits

2.2 Floral phenology

2.3 Incidence of *Leptocybe invasa*

2.4 Leaf biochemical analysis

#### 2.1 Growth traits

In *Eucalyptus tereticornis*, Krishnaswami *et al* (1982) found significant differences between eight plus tree progenies for height, basal area and size and number of leaves at the age of 6- and 12 months after planting in Andhra Pradesh. Kedharnath (1982) found that heritability estimates for height were 19 to 21 per cent for ages to 3 years and 34 per cent at age of 4 years. Significant differences among eight families of *Eucalyptus tereticornis* were reported in height and diameter at the age of 7 12 months after planting in Tamil Nadu by Krishnaswami *et al* (1984) and the values of genotypic co-efficient of variation (GCV) and heritability were higher for diameter than for height.

A progeny test was conducted by Wu *et al* (2002) with 13 hybrids of *Eucalyptus urophylla* x *E.tereticornis*, 10 hybrids of *E. urophylla* x *E. camaldulensis*, 1 hybrid of *E. urophylla* x (*E. urophylla* x *E. tereticornis*), 5 open-pollinated families from the maternal trees of *E. urophylla*, and 1 open pollinated family from *E. urophylla* x *E. tereticornis*. They found significant differences in height, diameter and volume were found among families and other replicates.

In *Eucalyptus grandis*, Lal (2005) estimated the growth performance of several *Eucalyptus* clones and reported that clones C-413, C- 407, C-411, 112, 72, 105 and 316 were superior with mean annual increment 25 to 30 m<sup>3</sup> per hectare per year at age of 3 years. Progenies of 145 genotypes were evaluated by Sidhu *et al* (2005) under nursery condition. The seedling survival ranged from nil of 16347 plus tree from Australian seed sources to 100 per cent in progeny of OUAT, ET-17. At about 5 month age, the seedling height ranged from 6.05 cm in progeny 16347 of Australian seed source to 87.65 cm in progeny of CU-13 and collar diameter ranged from 0.12 cm in progeny of 16347 to 0.54 cm in progenies of CU-13 and OUAT, ET-10

Kumar *et al* (2010) studied eighteen clones of *Eucalyptus tereticornis* for various

growth parameters at Hoshiarpur, Punjab, India for three years and compared them with two checks. They recorded significant variations for height, diameter at breast height and clear bole height. The broad sense heritability was found to be low to moderate for both height and clear bole height. The average genetic gain for three years was recorded maximum for height (159.60 %) followed by diameter at breast height (110.97 %) and clear bole height (70.34 %). In this study, clone 17, 14 and 5 were evaluated to be the most promising clones for commercial cultivation.

Dutt and Tyagi (2011) studied the morphological characteristics of *Eucalyptus* eleven species and hybrids of *E. alba*, *E. grandis*, *E. tereticornis*, *E. torelliana*, *E. urophylla* and clones of 266, 413, 348 and 471. They observe that *E. grandis* gave significantly better result as compared to other *Eucalyptus* species. On the basis of morphological evaluation, maximum leaf area was recorded in clone P-407. Smooth leaf surface was recorded in all the clones except P-2070 which had bumpy leaf surface. Longest petiole length (3.03 cm) was noticed in clone P-316 and maximum diameter of petiole (1.62 mm) was measured in clone BCM-272. Highest tree height was reported in clone in BCM-2253 (11.62 m) and maximum DBH was reported highest in clone BCM-2253. Clone P-2045 had maximum number of branches. Relatively higher gall infestation was reported in the longer petioles

Gupta (2012) studied morphological characteristics such as height and diameter of several *Eucalyptus* clones and found that significant difference among all the clones. The maximum tree height varied from 15.38m in clone P 316 to minimum tree height 12.36m in clone P 411. The diameter at breast height (DBH) in selected clones varied from 12.19 cm in JKSC 08 to 9.22 cm in BCM 2045

Rawat *et al* (2012) studied the biomass estimation (t/ha) in four forest divisions of Punjab namely Amritsar, Hoshiarpur, Ludhiana and Patiala. Biomass (t/ha) was estimated through complete harvesting of 23 *Eucalyptus* trees of various ages (18-30 years), density (232-740 trees/ha), diameter (12.7 cm to 49.75cm) and height (14.4 to 41.5 m). Total biomass ranged from 112.98 t/ha to 531.09 t/ha and the productivity ranged from 3.77 t/ha. Biomass of various plant components ranged as; leaf biomass (1.08 kg/tree to 29.95 kg/tree), twig biomass (1.62 to 31.92 kg/tree), branch biomass (6.36 to 151.56 kg/tree), bark (8.32 to 168.77 kg/tree), bole biomass (55.88 to 1911.41 kg/tree), root biomass/below ground biomass (BGB) (9.36 to 397.20 kg/tree)

Singh *et al* (2015) showed with respect to growth performance, highest average height (11.42 m) was obtained in P-411 (highly tolerant) and followed by P413 (10.77 m), P-2136 (10.33m), P-526 (11.19 m), B-2153 (10.58 m) and SRO-16 (10.27 m). The highest average DBH (85.64 mm), average volume (22599.96 cm<sup>3</sup>) and average wet biomass

(1129.99 kg) were obtained in P-526 (highly tolerant), which was at par and followed by P-411 (84.23 m, 22168.57 cm<sup>3</sup> and 1108.43 kg, respectively); P-413 (82.89 m, 20426.23 cm<sup>3</sup> and 1021.31 kg, respectively); SRO-16 (81.06 m, 18534.21 cm<sup>3</sup> and 926.71 kg, respectively); JK-08 (80.58 m, 18963.40 cm<sup>3</sup> and 948.17 kg, respectively) and B-2153 (76.89 m, 17926.87 cm<sup>3</sup> and 896.34 kg, respectively). Among all the *Eucalyptus* entries highly tolerant P-411, P-413 and P-526, moderately tolerant B-2153 and SRO-16 and moderately susceptible JK-08 were found to be comparatively higher in biomass production under South Gujarat conditions.

Kumar and Dhillon (2016) estimated the genetic variability, heritability and genetic advance in various clones of *Eucalyptus* spp. and found significant variation among the clones for number of branches, growth and tolerance to *Leptocybe invasa*. The estimates of phenotypic and genotypic coefficients of variation established that reasonable amount of genetic variability existed for plant height, collar diameter and number of branches. They recorded significant positive correlations for all characters except number of galls. From the study, it was found that plant height and collar diameter had high and positive direct and indirect effect whereas number of branches and number of galls had negative direct and indirect effect on volume index

*Eucalyptus tereticornis* clones cultivated on shallow medium soil under dry land conditions were evaluated for five years and compared with the seedling growth (Ilorkar *et al* 2016). Among the clones ITC-413 attained maximum and significant height (6.62 m) followed by ITC-71 (6.33 m) and ITC-316 (6.32 m). ITC-413 and ITC-71 were at par while the lowest height was recorded by ITC-136 (5.26 m). Maximum mean annual height increment (MAI) was observed in ITC-413 (1.324 m). With reference to diameter at breast height (DBH) maximum DBH was attained by ITC-413 (20.13 cm) followed by ITC-316 (16.32 cm) and ITC-71 (15.23 cm). The lowest DBH was attained in ITC-286 (7.12 cm). Maximum mean annual DBH (MAI) was produced by ITC-413 (4.026 cm). *Eucalyptus* clones ITC-413, ITC-316 and ITC-71 suggest their suitability for cultivation under local dry land conditions.

Santos *et al* (2016) studied multi-location clonal tests of *Eucalyptus* species (864 clones at the age of three years). Total height, diameter at breast height and dry weight were recorded. The individual tree heritability was 0.148 and 0.48 for MAI weight and Pilodyn penetration depth, respectively. Whereas, genetic correlation between environments ranged from 0.503 to 0.926 for all traits.

## **2.2 Floral phenology**

Shanks (1969) observed that without any additional pollination a berry developed frequently, although it was be relatively small and may had a tuft of non-pollinated pistils at its centre. Insect activity helped to spread pollen over the pistils and to increase the set of

drupelets. The extent of this increase seemed to vary with the cultivar.

Daubeny (1971) studied floral biology of different varieties of raspberry and concluded that the flowers pollinated with their individual pollen or that from neighbouring flowers set (57 to 77 %) as open pollinated flowers. Majority of commercially cultivated varieties cultivars were found as self-fertile.

Ashton (1975) studied flowering behaviour of *Eucalyptus regnans* and observed that flower buds were protected by a capsular sheath for 12 months and for next 15-17 months. The period of flowering was about 3 months and flowers remained open about 1-2 weeks. Younger trees tend to flower earlier than older trees. Heavy flowering occurs after every fourth year. Litter tray analysis show that good year produce 25 to 65 times more flowers as compared to poor years and mature trees produce 2-15 times more flowers than pole stage trees.

Hodgeson (1976) reported that the major flowering season of *Eucalyptus* is from February to June, but some tree flowered out-of-season. The trees initiated first flowering at 2-3 years old. Three stages were observed in flower-bud development. Flower buds had taken 4 months for anthesis and capsules were mature in five months. The pollination is carried out by insect pollinators, especially by honey bees

Pryor (1976) outlined general floral biology of *Eucalyptus* and mention that the flowers as protandrous with pollen remained available to pollinating agents and stigma remained dry and non-receptive. Nectar is first produced early in anthesis, from immediately post-operculum shed to 1-2 days later (Griffin and Hand 1979). The flower development within inflorescence is sequential and gradual phenomenon, allowing for self pollination and promotes repeated visits to flowers by pollinating agents (House 1997).

Pollination syndromes differentiate plants that are pollinated through insects (bee, moth, wasp or fly) or bird pollinated (Baker and Hurd Jr. 1968; Faegri and van der Pijl 1979). 'Typical' bird-pollinated trees are characterised by having large and tubular flowers that are large, tubular and often overhanging, which ease access to nectar by birds but it deters insect nectar-robbers whereas Insect pollinated trees often have small, open and upright flowers that encourages small insects to access nectar and pollen (Faegri and van der Pijl 1979).

Griffin (1980) studied flowering phenology of *Eucalyptus regnans* at Gippsland Victoria during 1977 and 2<sup>nd</sup> time in 1979. Flowering initiation was observed from 15 February in 1977 and from 30 January in 1979. Peak flowering reached within 40 days in both years. Flowering was almost completed within 90 days in 1977 and 100 days in 1979. Seventy two percent trees flowered in 1977 and 85 per cent recorded flowering in 1979.

There was a highly significant association, between years, for the relative time and duration of flowering of individual trees. Size of the tree had not determinantal effect on flowering attributes. Trees were not randomly distributed with respect to peak flowering time: thus flowering of adjacent trees tended to be synchronous. Detailed observation of flowering within the crowns of three trees showed that within inflorescences the mean period between anthesis of the earliest and latest flowers was 6 days, and within branches 14 days. The degree to which flowering was synchronous within the crown varied consistently between trees at each structural level assessed, which suggested genetic control of this trait.

Variation in flowering timing has been associated with seasonal patterns of rainfall and mean temperature, soil moisture and the intensity of solar radiation, ultimately these variations affects flowering initiation and rate of bud development (Friedel *et al* 1993). The flowering time of a *Eucalyptus* species varied between a few days to two months or more (Cremer *et al* 1978). Shivanna and Johri (1985) stated that pollen germination and pollen tube growth are prerequisite for fertilization and seed development. Pollen viability and stigma receptivity are critical factors for the successful completion of post-pollination events.

There are very few studies available that examine nectar secretion potential of *Eucalyptus* but this trait is also be very important and helps in distinguishing mode of pollination (House 1997). Floral nectar in flowers pollinated by birds may be less concentrated than in insect-visited flowers (Pyke and Waser 1981) but this has not been tested among eucalypts in Australia (House 1997). This species has large, red flowers suggesting that birds may be pollinators. In addition, flowers pollinated by birds often have greater volumes of nectar than do flowers of insect-pollinated species (Cruden *et al* 1983).

Sedgley and Griffin (1989) emphasized the importance of knowing the details of the timing of flower initiation and flower development for tree-crop research and management. Orchard management and cultural practices at this critical time can be optimized with the acknowledgement in order to favour floral initiation.

Creamy-white flowers are dominate in maximum species of genus *Eucalyptus* in South-western Australia (Chippendale 1988) and whereas in south-eastern Australia, brightly coloured forms are rare, with the exception of the red flowering forms of Yellow Gum *E. leucoxylon* (Williams and Brooker 1997).

Dhillon and Khajuria (1994) studied floral biology of *Acacia nilotica* and recorded peak flowering in the month of September to November. The pollination mode in *Acacia nilotica* was zoophilic. The flowering time varied among the different agro-climatic regions.

Gore and Potts (1995) reported that flowering time in an open pollinated base

population trial of *E. globulus* ssp. *globulus* was highly variable and strongly genetically controlled. Races from Furneaux group and East coast of Tasmania flowered almost one month earlier than races from King Island and Victoria. The flowering time of F<sub>1</sub> hybrid of *E. globulus* and *E. nitens* was same to the late flowering of *E. nitens*. In Tasmania, Chambers *et al* (1997) showed that the flowering precocity is highly heritable in four base population trials of *E. globulus* ssp. *globulus* and little genotype by environment interaction.

In *Grewia optiva* floral buds appeared with the commencement of few leaves during March-April. Flowering started during late March and continued till late June, but a little variation in the flowering period from plant to plant and site to site was observed (Pant *et al* 1997).

In Tasmania, Hingston and Potts (1998) studied floral visitors in the stands of *Eucalyptus globulus*. Seven bird and 71 insect species were noticed during this study. Colletid bees (local) and honeybee (exotic) was the dominant insect species whereas anthophilous parrots and wattlebirds were major diurnal contributors for open pollination in *E. globulus*. Geographic variation occurred in the avian species composition, whereas insect communities appeared to vary temporally and according to flowering intensity. However, insects were rarely observed to contact stigmas and showed little evidence of movement between trees.

The study on floral biology of *Acer caesium* conducted in Kamaun, Central Himalayan found that the first floral bud was observed in the last week of February and the flowering was started from the 10th of March on the lower altitude and in higher altitude site flowering occurred about 10 days later. The opening of the flowers started with a slit at the top of the bud, widened gradually and took 2-6 hours to complete opening (Semalty and Sharma 1996)

In *Prosopis juliflora*, floral buds began to appear in early March and began to open by end of March (Dhillon and Hooda 2005). Under subtropical conditions in Punjab, Khajuria and Bajwa (1997) observed that flowering in 6 varieties of *Leucaena* occurred twice in the year first time is March to May and then again in September to October.

Jordan *et al* (1999) studied various open pollinated and controlled cross pollinated field trials of *Eucalyptus globulus* ssp. *globulus* and concluded that first flowering was under moderate to strong additive genetic control with narrow sense heritability of 0.4 to 0.6.

Keatley *et al* (1999) examined the flowering patterns of four species of *Eucalyptus* *tricarpa*, *E. leucoxydon*, *E. microcarpa* and *E. polyanthemos*. Opercula traps were used to examine the timing, intensity and duration of flowering for two years at 3 different sites and recorded that *E. polyanthemos* and *E. tricarpa* did not bears flower in the study period.

Ford (1983) opined that the flowering intensity was determined through various methods or techniques, such as presence or absence of flowering and opercula counts. Some of these methods were boring and time consuming (e.g. opercula counts), while others provided incomplete or little information (e.g. presence or absence of flowering does not show whether there was just single one flower or several hundred flowers) or may provide only general information on flowering intensity (heavy or light flowering).

Information on *Eucalyptus* pollinator types are limited as were most studies based upon observational data of flower visitors on several *Eucalyptus* species (Hingston and McQuillan 2000). These data not be indicative of actual pollinators, as all flower visitors do not necessarily pollinate the flowers. Birds may visit several species, but for some *Eucalyptus* species, birds may be feeding on the insects attracted to the flowers rather than acting as pollinators. They found that the birds visit those *Eucalyptus* species having large flowers but the insect visit both large and small flowered *Eucalyptus* species.

Birtchnell and Gibson (2006) studied the flowering behaviour of various *Eucalyptus* species and reported that flowering period was highly variable. *E. leucoxydon* and *E. gracilis* flowered for the period of 8 months, *E. delegatensis* and *E. polyanthemos* flowered for 2 months. Some tree of *E. baxteri* bears floral buds for 12 to 24 months. Mostly species bears flowering during spring season. They also reported some tree of same species exhibit flowering at one region and at other region it does not bear a single bud and in some species flowering was one month or more earlier or later at different regions. Biwere determined, as were spatial differences within and among species. Data were obtained by face-to face interviews with 25 Victorian apiarists, each of whom had operated a minimum of 350 hives for a minimum of 30 years. Flowering frequency ranged from one to seven years, and most species flowered once every 2–4 years. Long-term flowering frequency, timing and duration were reported as constant, although short-term perturbations could occur. Most melliferous species flowered during spring and summer for a period of three months or more. Only few species had shorter flowering periods. Information provided by apiarists compared well with available published information (e.g. flowering period reported in field guides) and revealed a reliable, largely untapped source of long-term data, the use of which could benefit many ecological research endeavours.

Kumar *et al* (2007) reported more than 40 per cent of *Eucalyptus* plantation affected by attack of *Leptocybe invasa* at Marva JFMC plantation in Godhra district of Gujrat

Branco *et al* (2005) found that *L. invasa* was found on almost the entire *Eucalyptus* plantation in Portugal and also revealed that *Eucalyptus globulus* was most susceptible species host for this gall wasp in Portugal.

Nicolson (2008) adults of the *Eucalyptus* nectar fly, *Drosophila flavohirta* Malloch (Diptera: Drosophilidae), feed on *Eucalyptus* pollen. Flies of both male and female sexes collect pollen with the proboscis and formed a large mass of pollen on the frontal surface of the prolonged proboscis. Pollen collecting alternates with inactive periods when the moistened pollen load is constantly vibrated. Nothing pollen was found in the adult gut, and it was suggested that pollen hydration causes release of nutrients which are then imbibed.

Kaur *et al* (2011) conducted a studied on the floral biology of *Jatropha curcas* at Ludhiana, Punjab, India and reported that there was two time flowering in a year. First flowering time was April to June and second flowering time is July to November. Female and male flowers opened within 7 -10 days after bud formation. Fruiting was visible 26.5 to 30.2 days after bud formation. Fruit setting ranged from 37.0 to 61.6% and the anthesis of female and male flowers occurred between 700 to 800 hours and 600 to 700 hours respectively. Flowers were found as unisexual and female and male flowers were produced in same inflorescence but male flowers were shorter than female flower. The ratio of male to female flowers was 13.4:1. Average number of pollens per anther was 122.3 and average pollen viability was 71.6 per cent. Fruit setting through cross pollination 93.2 %, self-pollination 72.2, through apomixis 36.2 and 79.2% open pollination.

Jones *et al* (2011) monitored the flower initiation and flower opening time in seed orchard of *E. globulus* ssp. *globulus* containing clones of 63 genotypes from 4 different localities. Every year anthesis occurred over a long period. Anthesis occurred earlier for Tasmania and Furneaux than Otways and Strzelecki every year. However, there was a weak genetic control in flower initiation time between regions and genotypes. The average anthesis time varied from year to year and total heat was a major factor of environmental variation. To some extent, anthesis time is controlled by both genetic factors and environmental factors.

Singh (2012) carried out the biochemical analysis of 14 *Eucalyptus* clones for gall wasp in Gujarat, India. The experiment comprised of 14 *Eucalyptus* clones viz.. P-2049, P-2155, P-405,,IK-08, Jk-02, P-286. B-2153. SRO-16.,IK-04, ERK-04, P-411. P-413, P-2136. P-526 and two *Eucalyptus* species viz., *E. torelliana* and *E. pellita* of fifteen months age. Among all the *Eucalyptus* entries *E. torelliana* was found resistant (free of gall). *Eucalyptus* entries P-411. P-413. P-2136. ERK-04, P-526 and *E. pellita* were found highly tolerant, P-286. JK-04, B-2153 and SRO-16 were found moderately tolerant, JK-08 and JK-02 were found moderately susceptible and P-2049,P-2 I 55 and P-405 were found highly susceptible.

Hilgert-Moreira *et al* (2014) checked the utilization of *Eucalyptus* spp. in the diet of *Apis mellifera* L. and evaluated the proportion of pollen that collected from *Eucalyptus* spp. in the regions of Riozinho and Rolante, RS, Brazil and observed that the availability of

flowering *Eucalyptus* spp. throughout the year allowed bees to use pollen and nectar. In this way, *Eucalyptus* is the exclusive source of pollen for honeybees

Behera *et al* (2018) studied the foraging activity of *Apis dorsata* (Rockbee) on fully bloomed *Eucalyptus* plantation during November in South Gujarat condition. For this purpose, 4 fully bloomed trees in different four locations in the NAU campus were marked. Rockbee visitation and visitation percentage were noticed relatively highest during morning hours than the afternoon hours. Highest frequency of visit 334.4 and highest percentage of visitation 16.08% by *Apis dorsata* was noticed during 9-10 am followed by 10-11 AM.

### **2.3 Incidence of *Leptocybe invasa***

The recent outbreak of the invasive gall wasp, *Leptocybe invasa* Fisher & La Salle is threatening the productivity of the *Eucalyptus* plantations and has become a serious constraint in the expanding plantation area under *Eucalyptus* throughout India. The literature related to the incidence, management and bio control aspect of *Eucalyptus* gall wasp (*Leptocybe invasa* Fisher & La Salle) is reviewed.

In Indian subcontinent, around 2000 gall inducer insect species have been identified on various crops, shrubs and tree species. Hemipteran insects are more dominant and have more potential of inducing galls (Rohfritsch 1992, Wool *et al* 1999, Raman 2003). Espirito and Wilson (2007) reviewed the incidence of gall wasp insects in the globe and found to be higher percentage of gall incidence in Panama tropical rain forests, whereas higher gall insect per plant species were found in Cape Floristic province in South Africa.

Durand *et al* (2008) observed the differences in susceptibility between *E. camaldulensis* and *E. globulus*. Differences in susceptibility were also found within the hybrid populations and between families of *Eucalyptus globulus* spp. *globulus* (Labill.). These results demonstrate inter and intra specific susceptibility variations. Physical analysis of *Eucalyptus* leaves by thickness measurements and leaf surface did not differ significantly between susceptible and resistant genotypes, whereas, chemical analysis differed significantly.

Jacob and Kumar (2009) compared the levels of susceptibility and resistance in terms of gall incidence induced by *L. invasa* on seedling of *E. tereticornis* and *E. camaldulensis* collected from 9 different seed sources in South India and variation in number of galls, growth of seedling and percentage of gall incidence were recorded. Under the same environmental conditions, seedlings from the Ongole red, Kennedy river, Pudukkottai and Rudrapur were severely infested with galls on petiole, midrib and new branches, whereas seedlings from Sathyavedu showed resistance with a few galls on petioles or midribs and rarely on stems and seriously infested seedlings shows slowdown of growth.

In Vietnam, Thu *et al* (2009) recorded maximum per cent damage and higher damage index of *L. invasa* in *E. grandis* and *E. tereticornis* with lower survival in the field where as, *E. globulus* and *E. pellita* indicated lower damage severity with higher survival in the field. They revealed that the incidence was higher on the nursery stage than field plantation. Five species recorded resistant in field which were susceptible in nursery. *E. camaldulensis*, *E.tereticornis* and *E. grandis* were most susceptible at both field and nursery conditions.

Dhahri *et al* (2010) reports about the first presence of two gall wasps on the *Eucalyptus* plantation in Tunisia. *Leptocybe invasa* was noticed in 2004 which produce galls on midrib of leaf, petiole and young branches whereas *Ophelimus maskelli* firstly noticed in 2006 which produce galls on limbs. *Eucalyptus camaldulensis* was appearing more susceptible to both pests than other species.

Dhiman *et al* (2010) studied species variation on 15 *E. tereticornis*, 7 *E. camaldulensis*, 2 *E. urograndis* clones and 1 seedling population each of *E. tereticornis* and *E. grandis* against *Leptocybe invasa*. Minimum gall infestation was recorded on *E. urograndis* whereas maximum on *E.tereticornis*. Among clones *E. urograndis*, clone BCM-2135 has shown minimum infection (4%) compared to 100 percent infestation in *E. tereticornis* clones viz., W-36, W-104 and W-B 10. The other *E. tereticornis* clones BCM-10 and W-B 33 showed gall infection 84% and 80% respectively.

Vastrad *et al* (2010) found that 10.83 to 212.13 adults of gall wasp *Leptocybe invasa* were trapped on the yellow sticky trap during the month of May to September in nursery with maximum 98.50 adults/trap in the month of December.

Javaregowda and Prabhu (2010) studied the relative resistance in various *Eucalyptus* clones against *L. invasa* and found that among commercial clones, only one clone GR-330 of *E. grandis* observed minimum number of galls per plant (9.90) with the least damage index. Two *E. camaldulensis* clones viz DLD-31, DLD-IO, one clone SA-2 from *E. urograndis* and *E. pellita* registered moderate incidence of this pest.

Kulkarni (2010) reported that *E. camaldulensis*, *E. tereticornis*, *E. grandis* and their hybrids were seriously attacked by *Leptocybe invasa*. On the other hand, *E. alba*, *E. urophylla*, *E. citriodora* and *E. torelliana* escaped from attack of gall wasp and found gall free due to the presence of pubescent hairs on leaf, petiole and new growing shoot which prohibits *L. invasa* in laying eggs in the plant system. Out of 135 *E. tereticornis* clones screened, 92.6% were found infested. Clone No.10 and 27 were highly susceptible while clone No. 1, 320, 411, 413, 513, 612, 145, 2253, 2254 and 2306 found free from galls.

Kumar *et al* (2010) evaluated the different coloured such as yellow, green, blue white

and red sticky traps to manage *Leptocybe invasa*. Yellow coloured traps was found most effective which traps 146.83 pest per trap followed by green coloured trap 67.33, white coloured 66.33, blue coloured 49.08 and red coloured 48.92. These observations showed that yellow coloured sticky traps used for pest monitoring and mass trapping under protected conditions. Insect gum used as sticky material traps 192 wasps with *Eucalyptus* oil and 169.25 without *Eucalyptus* oil, petroleum jelly 122.5 and automobile grease traps 56.25 wasps per trap. Among different trap shape evaluation flat trap (176.53) glued on both sides appears significantly superior than spherical (92.27), cuboidal (67.33) and flat trap with glue on single side (75.07)

Nyeko *et al* (2010) examined the spatially and temporally variations in *Eucalyptus* gall inducing wasp adult population in Uganda and Kenya. Adults of gall wasp occurred throughout the whole year with overlapping generations having life cycle of less than 7 days. No definite peak in *L. invasa* population occurrence even a decline was recorded in dry months. 35 *Eucalyptus* germplasms evaluated for gall wasp infestations, only *Eucalyptus henryi*, clonal GC 578 and GC581 were noticed resistant to the gall wasp. *E. camaldulensis*, GC540 and GC784 in Uganda, and MAU1, GC10, GC14 and GC15 in Kenya were recorded as highly susceptible.

Rameshbai (2010) carried out surveillance for incidence of *Leptocybe invasa* was in forest nurseries of Bharuch, Navsari, Surat and Vadodara districts during January - June 2009. The incidence varied greatly at each location and different seasons. Except Vadodara, incidence started increasing from April at each location. In Vadodara higher incidence up to 70% was found from January onwards. Peak incidence was recorded during first and second fortnight in June at all four locations. Significant and positive effect of abiotic factors observed on gall incidence 45.63, 88.94 91.37, 92.38 per cent variations at Bharuch, Vadodara, Navsari and Surat, Bharuch respectively. Highest gall incidence i.e. 92.08 % was recorded in Vadodara, while it remained least at Surat i.e. 33.33%.

Ramanagouda *et al* (2010) studied biology of *Leptocybe invasa* Fisher & La Salle under green house during 2009-10. Tissue disruption (stage I) appeared within 10 days and 15 days in summer and winter respectively. Bump shaped green colour stage (Stage II) remains for 45 in summer and 57.4 days during winter. Glossy Pink colour (Stage III) lasted for 29.6 during summer and 30.2 in winter. Dull Pink colour (stage IV) lasted for 20.66 days and 18.80 in summer and winter respectively.

Gall induction on the leaf or petiole effects the size and shape of the leaf, depending upon the site of gall (mid rib, leaf lamina, petiole), shape of the gall, structure of the gall and the type or insect species causing the gall. Chen and Qiao (2011) studied the diversity and

host specificity of various gall forming aphids and recorded that galls may induce on the leaf blades, the leaf veins, the petioles, twigs, branches and even also roots. *Pemphigus bursarius* (Linnaeus) caused galls on the leaf petioles of *Populus simonii*.

Krishnan *et al* (2011) revealed that the leaf lamina or the leaf-blade is the most favourable organ of gall inducer insects. Around 60 percent of galls are the foliar galls, which followed by the stem galls. Reale *et al* (2014) studied the gall development process produced by *Dryocosmus kuriphilus* on *Castanea sativa* and they recorded increase in the thickness of small chlorotic zones in the leaf.

The first incidence of *L. invasa* in Punjab was noticed in 2009 by Sangha *et al* (2011). Visual survey of *Eucalyptus* plantation and nursery indicated the presence of gall wasp, such as bumped shaped galls on the leaf midrib, petioles and stems. The was recorded from the south western districts (Moga, Shri Mukatsar Sahib, Ferozpur), central districts (Ludhiana, Jalandhar, Fatehgarh Sahib) and the sub-mountainous tract (Gurdaspur, Hoshiarpur, Saheed Bhagat Singh Nagar, Ropar) of Punjab. The survey revealed that the *L. invasa* damage was comparatively less in the dry regions of Punjab than the humid sub-mountainous areas. They have found negative correlation between tree girth and gall wasp infestation. Maximum attack was recorded in control seedling plants, clone 413, P-15, P-20, P-11, and P-18 at Pandori Mindo (Hoshiarpur). In other two trials at Hoshiarpur, significant genetic variation was noticed but the clone 413 showed less attacks.

Fang-li *et al* (2012) examined the susceptibility of ten *Eucalyptus* species against *Leptocybe invasa* by determining gall density, gall volume and pest population in Hainan and Guangdong provinces of China. *E. urophylla* x *E. camaldulensis* recorded as most susceptible in Hainan whereas *E. propinqua* was most susceptible at Guangdong. Galls were not recorded on *E. camaldulensis*, *E. dunii* and *E. microcorys*. Highest number of gall wasp was recorded on *E. urophylla* x *E. camaldulensis* whereas least on *E. grandis* x *E. urophylla* at Hainan Province. Highest population of pest on *E. propinqua* and lowest on *E. microcorys* at Guangdong Province.

In Gujrat, Rajpoot (2012) evaluated the performance of 15 *Eucalyptus* clones and two species viz *Eucalyptus citriodora* and *E. camaldulensis* against gall insect *Leptocybe invasa*. Maximum leaf damage and gall intensity was recorded during interval of 24 May to 6 June. Highest number of galls was noticed on leaf midrib and petiole. Significant and positive correlation was found with sunshine, temperature and evaporation while negative correlation with rain. *E. citriodora* and clone BCM-288 and BCM-413 were observed tolerant while clone BCM-271 was reported as highly susceptible against the attack of *Leptocybe invasa*. Higher Incidence of gall wasp was observed higher in lanceolate leaves whereas linear to

lanceolate leaves were escaped from attack of gall wasp.

Zhang *et al* (2012) studied the incidence of *L. invasa* on different *Eucalyptus* clones in relation to the nutrients and secondary metabolites by the grey correlation analysis. The results showed that DH32-29 and QG5 were resistant clones, while QG3, QG5 and DH201-2 were highly susceptible clones. The soluble sugar, total phenols, flavonoids and protein's content of petioles in resistant clones were higher than susceptible clones.

In Portugal, Rocha *et al* (2013) studied the interaction between host plant and gall inducer pest *Leptocybe invasa*. In field observations infested with galls *E. camaldulensis* was found less susceptible against cold injury as compared to neighbouring non galled plants. In the laboratory conditions gall infested plants show higher resistance to frost injury than healthy ones. Gall wasp induced physiological changes on *Eucalyptus* foliage were examined by chemical analysis of plant tissue. Anthocyanin, chlorophyll, total protein and total soluble sugars were found higher in galled infested plants as compared to non-infested plants showed defence against cold injury. These increasing biochemicals in plant showed positive indirect effect on *Eucalyptus* plantation and promoting resistance to cold injury.

Senthilkumar *et al* (2013) reported on the basis of a survey was conducted in *Eucalyptus* plantations and nurseries of TAFORN and TNPL covering 5 Agro-climatic zones of Tamil Nadu. Out of the 19 clones planted, clones like C7, C10, C271, C283 and T61 were found infested with *Leptocybe invasa* ranging from 80-100 per cent. Clone C-413 was planted in all agro-climatic zones found gall free. The most popular clones C3 and C7 and C10 were reported as infested with galls in all the agro-climatic zones of Tamil Nadu. In North eastern zone Clone C283 and in North western zone C271 were found most susceptible clones. This survey also concluded that *Leptocybe invasa* invaded through Cauvery delta zone and in western zone via North western zone. Clones C226, C274 and IFGTB clones were found gall free.

In North-Western India, Kumar *et al* (2015) evaluated 19 clones of *Eucalyptus* for tolerance against gall wasp at various stages. Gall wasp incidence was noticed on a whole plant basis at seedling stage. At age of 4 and 7 months, galls were counted from the upper, middle and lower crown. No clone escaped from gall damage. Clones C-526, P-23 and P-103 were the least affected than other clones. Incidence was higher in March than December.

Singh *et al* (2015) investigated the tolerance of 14 *Eucalyptus* clones and 2 *Eucalyptus* species of 15 months aged. Among all, only *E. torelliana* was found gall free (resistant). Five Clone viz ERK-4, P-411, P-413, P-526, P-2136 and one species *E. pellita* were found highly tolerant whereas four clones were found moderate tolerated viz B-2153,

JK-04, P-286, and SRO-16. Two clones JK-02 and JK-08 were moderately susceptible and three clones P-405, P-2049 and P-2155 and P-405 were found as highly susceptible. Gall wasp had significant and negative correlation with growth parameters such as tree height, volume and diameter at breast height. P-411 attained highest height 11.42 m was also high tolerance showed negative correlation. The highest average DBH (85.64 mm), average volume (22599.96 cm<sup>3</sup>) and average wet biomass (1129.99 kg) were obtained in P-526 (highly tolerant), which was at par and followed by P-411 (84.23 m, 22168.57 cm<sup>3</sup> and 1108.43 kg, respectively); P-413 (82.89 m, 20426.23 cm<sup>3</sup> and 1021.31 kg, respectively); SRO-16 (81.06 m, 18534.21 cm<sup>3</sup> and 926.71 kg, respectively); JK-08 (80.58 m, 18963.40 cm<sup>3</sup> and 948.17 kg, respectively) and B-2153 (76.89 m, 17926.87 cm<sup>3</sup> and 896.34 kg, respectively)

Roychoudhary *et al* (2016) concluded wider variation in the infestation caused *Leptocybe invasa* on *Eucalyptus* plantation at seedling stage and sapling stage in Madhya Pradesh based on survey of plantations and nurseries. 20 per cent gall infestation recorded on one month old seedlings and almost 100% infested plants at the age of one year seedlings whereas 80-100% gall infestation was noticed in 6 months old plantation and revealed that Gall induction with respect to seedling growth that there was an increase in gall induction irrespective of leaf and stems. Significant variation was observed for susceptibility among genotypes raised from seed and hybrid *Eucalyptus* against gall wasp.

In Punjab, Kumar and Dhillon (2016) worked out the genetic heritability and variability in *Eucalyptus* clones tolerance to gall wasp. The estimates of phenotypic and genotypic coefficients of variation clearly reflected large amount of variation for number of galls. Significant positive correlations were recorded for all characters except number of galls. Path analysis revealed that number of branches and number of galls had negative direct and indirect effect on volume index.

Yousuf *et al* (2017) carried a survey during 2010-14 in Uttar Pradesh, Haryana, Punjab and Uttarakhand. Gall infestation was reported in both young plantations and nurseries. Observations revealed that all *Eucalyptus* growing areas in North India is under the threat of attack of *Leptocybe invasa*. Biological control has been applied in Punjab during 2012-16, where infestation was regularly decreased and came below 10%.

In *Prosopis cineraria* L. four type of gall makers were reported and increase in the number of galls with vegetative growth of tree was also reported continuous increase in the gall population with vegetative growth of tree (Parihar 1994).

## 2.4 Leaf biochemical analysis

Tree possess several type of resistance mechanisms for protect itself against the attack of insect pest and disease. It includes cell wall lignins, sharp thorns, waxy layers on leaves, and secondary metabolites. These act as the first line of defence against insect pests. Nevertheless, insect damage to plants also leads to induction of various signalling pathways and responses such as defensive proteins, accumulation of secondary metabolites and oxidative burst (Gill *et al* 2010). Plants synthesize these secondary metabolites as they cannot depend upon physical mobility to escape their predators and therefore, have evolved a chemical defence against such predators (Lattanzio *et al* 2006). Plant biochemicals plays a decisive role in selection of the tree or plants by the insects for feeding, shelter and oviposition. Some biochemicals present in tree or plant provide nutrition to the insects, whereas some have antibiosis affect on the insect population.

The biochemical defence mechanism may consist of presence or absence of a particular chemical substance or a group of substances in a host plant which interferes with the pathogen attack or insect feeding. The biochemicals may be present before infestation or may be produced by the interaction of the host and pathogen attack or insect feeding (post infestation).

El-Akkad (2004) compared the protein content in galled, ungalled leaf samples and galled tissues of *Populus nigra* infested by galling aphids. The results showed that the protein content was different in the 3 different samples. Higher level of protein was recorded in the galled tissues followed by galled leaf and less in ungalled leaf. There was an expression of specific proteins (molecular weights of 65, 45, 37 and 25 k Da) in the protein pattern of the leaves with galls which were not found in the ungalled leaves. The presence of these specific polypeptides induced by galling in galled tissues suggests that some of these proteins may be stress proteins.

Khattab and Khattab (2005) noticed decrease in carbohydrates, total soluble protein contents in the gall infested leaves of *Eucalyptus* as compared to the un-galled healthy ones. Marmit and Sharma (2008) studied quantitative estimation of phenolics and related enzymes in insect-induced leaf galls of *Mangifera indica*. Total phenols, o-dihydroxy phenol and peroxidase were recorded higher in gall tissue while polyphenol oxidase activities were higher in normal counterparts compared to gall tissues.

Singh (2012) carried out the Biochemical analysis of two *Eucalyptus* species viz *E. pellita* and *E. torelliana* and 14 *Eucalyptus* clones for gall wasp in Gujarat, India. No definite pattern was recorded for protein and total soluble sugars for ungalled leaves while total phenol, chlorophyll 'a', chlorophyll 'b' and total chlorophyll were noticed highest in resistant *E. pellita*, higher in tolerant clones and less in susceptible. Carotenoid content was observed

more in highly susceptible clones than tolerant clones. In case of galled leaves, Total protein and total phenol content increased in all the *Eucalyptus* entries except *E. pellita* whereas total soluble sugar increased showed frequent increase in highly susceptible clones. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll decreased in all the entries indicating no distinct pattern in carotenoid content and in the content of Ca<sup>2+</sup> and K whereas, the decrease of total chlorophyll, chlorophyll 'a' and 'b' was higher in highly susceptible clones compared to other *Eucalyptus* entries and the decrease of Mg<sup>2+</sup> content was higher in susceptible clones compared to tolerant clones in galled leaves. SOD and PPO activities decreased in galled leaves.

Mukherjee *et al* (2016) revealed that the protein content was more in the galled leaf than ungalled leaves and protein content was also increased with the increase in the number of galls in *Terminalia arjuna* and *T. tomentosa*. The protein content was 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. The total soluble sugar in gall infested and healthy leaves and revealed that the steady increase in sugar content in the gall infested leaves initially which gradually decreased as the gall infestation proceeded. Comparatively a significant difference was recorded in non gall infested and gall infested leaves. The concentration of total sugars in galled leaves increased at the initial stage as the tissue disrupted and accumulates starch which is less in the non galled or healthy tissue.

Li *et al* (2017) clarifies the physiological response of *Eucalyptus* against *Leptocybe invasa* and measure the variation in content of total phenols, tannins, nitrogen, carbon, gibberellins, IAA and ABA in galled and ungalled leaf tissue of two *Eucalyptus* varieties DH201-2 and EA. Total phenols, nitrogen content gradually decreased but ABA and tannins increased during gall development in galled tissue.

Prasad *et al* (1997) observed gradual decrease in total starch, total sugar, reducing sugar and individual sugars in the leaflets of Khesari due to downy mildew disease. Non reducing sugar tended to increase the disease.

Kumar *et al* (2002) reported higher content of total sugars and reducing sugars in rust susceptible genotypes of pearl millet. After rust infection (caused by *Puccinia penniseti*), the level of total sugars and reducing sugars increased in resistant and moderately resistant genotypes, but decreased in the susceptible genotypes.

Coruh and Ercisli (2010) studied interactions between gall induction by *Diplolipis* spp. and leaf total phenolic content in *Rosa canina* genotypes. The results showed that seven

genotypes without insect damage had significantly greater total phenolics content in leaves compared to insect damaged *R. canina* plants. These results clearly indicated that total phenolic content in *R. canina* genotypes had no galling insect damage, which have an important part of chemical defence of *R. canina* plants against *Diplolepis* spp.

## CHAPTER III

### MATERIALS AND METHODS

The current investigation on “Studying variation among *Eucalyptus* clones for growth traits, flowering, and tolerance to gall wasp (*Leptocybe invasa*)” was carried out during 2018 to 2019. The details about the experimental sites, materials used, methodologies adopted and the observations recorded during the course of investigations are given in this chapter.

#### Experiment No. 3.1

##### I) Name of the experiment

Studying variation in the growth traits and flowering behaviour of *Eucalyptus* clones.

##### II) Location

The observations were recorded from the already established three year old *Eucalyptus* clonal trial. The experiment was carried out at Main Experimental Area, Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana. The experimental area is located at about 247 meter above the mean sea level and lies between 30° 90' N longitude and 75° 81' E latitude, which represents the central agro-climatic zone of the Punjab region

##### III) Methodology:

- Treatments : 14 clones
- Design : RBD
- Replications : 3
- Plot size : 5 trees

Fourteen clones were selected for estimating variation in growth traits and flowering behaviour of *Eucalyptus* clones. 5 trees of each clone planted at the spacing of 4 × 3 meter tree and planted in 3 replications.

**Table 3.1: Details of *Eucalyptus* clones developed by PAU used in the study**

S. No.	Name	Plus tree/ Progeny Number	Origin of Plus tree
1	PE-6	FC&RI, MTP/ET-5	TNAU, Coimbatore
2	PE-14	L-81/98	PAU, Ludhiana
3	PE-7	HS-6	HAU, Hisar
4	PE-5	OUAT,ET-10	Orissa University of Agriculture and Technology, Bhubaneswar
5	PE-11	13547/JD1043	Australia Seed Centre
6	PE-13	HS-3	HAU, Hisar
7	PE-8	16547/JD1572	Australia Seed Centre
8	PE-12	L-73/98	PAU, Ludhiana
9	PE-9	ET-10	Orissa University of Agriculture and Technology, Bhubaneswar

Nine clones were developed by Punjab Agricultural University, Ludhiana from the existing breeding programme from the selections in the provenance-cum-progeny trial. The details of plus tree/progeny are given in table 3.1. The plants were propagated under mist-chamber of the department of Forestry & Natural Resources. Five commercial clones used in the study are C-2135, F-316, C-413, C-72 and C-407.

#### IV) Observations

The following observations were recorded during this experimental study

##### 1. Tree height (m)

Height of the clonal *Eucalyptus* tree was measured from ground level to the tip of the leading shoot of canopy by using Ravi altimeter in meters and the height of observer was added.

##### 2. Diameter at breast height (cm)

Diameter at breast height of clonal *Eucalyptus* was measured by obtaining mean of two diameters measured along major and minor axis at the height of 1.37 m above the ground level by using tape. Diameter was measured in centimetres.

##### 3. Branch traits (Number, branch angle, branch diameter)

- a. Number of branches: The number of branches per tree were counted
- b. Branch angle : The branch angle of lower most five branches were recorded with the help of a geometric protactor.
- c. Branch diameter: The diameter of branches of lower most branches were measured with the help of vernier caliper at one cm from stem.

##### 4. Stem traits

- a. Straightness score (1 to 4)

Straightness was measured through manually or visual observations. Straightness was categorized into 4 categories as described by Hesheng *et al* (2012)

Straightness score	Categorization
1	Very crooked, serious bends in main stem
2	Slightly crooked, less bends in main stem
3	Mostly straight, 1-2 small bends
4	Complete straight, no obvious bends

- b. Clear Bole height (CBH):

Clear Bole Height was the height up to first major branch was recorded with the help of Ravi's Altimeter.

## **5. Time of flower bud initiation**

Flower bud initiation noticed the time (week of month) of the appearance of first floral bud on tree.

## **6. Flowering span**

Flowering span mean the time period (Days) of first opening of floral bud (Blossoming) to the opening of last floral bud

## **7. Intensity of flowering (low, average, high)**

Intensity of flowering means density or number of floral buds appeared on tree canopy. Intensity was categorized into 3 categories:

- a) Low = floral buds appear on less than 4 twigs
- b) Medium = floral buds appear on 4 to 8 twigs
- c) High = floral buds appear on more than 8 twigs

## **8. Floral insect visitor diversity**

Floral insect visitor diversity was recorded by manual counting of the number of insects that visits the blossomed or fully opened flowers of different clones of *Eucalyptus* (PE-7, PE-8, PE-9) for collecting nectar, pollen or other foraging activities. Further this, recording manually by counting the number of *Apis mellifera* in a period of 2 minutes on one tree of each clone of bloomed *Eucalyptus* at morning (10 AM), afternoon (1PM) and evening (4PM)

## **9. Gumnut characteristics**

The following observations were recorded for seed or gumnut morphological characteristics

- a. Gumnut length: Gumnut length was measured with the help of digital vernier calliper.
- b. Gumnut width: Gumnut width was measured with the help of digital vernier calliper.
- c. Peduncle length: the length of peduncle (floral part though which gumnut attached with branch or leaf) was measured with the help of digital vernier calliper.

## **V) Statistical analysis:**

The data were be subjected to statistically analyses and means were compared by LSD ( $p=0.05$ ) under Randomized Block Design (RBD) following the CPCS statistical software developed by Punjab Agricultural University, Ludhiana.

## Experiment No. 3.2

Screening of *Eucalyptus* clones against *Leptocybe invasa*.

### II) Location

Main Experimental Area, Department of Forestry & Natural Resources, Punjab Agricultural University, Ludhiana campus

### III) Methodology:

- Treatments : 14 clones
- Design : CRBD
- Replications : 3
- Observation period : Monthly interval

**Methodology:** Five plants of each *Eucalyptus* clone were marked for recording the gall wasp incidence. Three sets of fresh leaves samples were collected for studying various physiological and biochemical characters. The attack or incidence of *Leptocybe invasa* was recorded at monthly interval.

### IV) Observations:

#### 1. Gall wasp incidence

The incidence of gall wasp damage was recorded at monthly interval from March 2018 to February 2019. For this, 3 plants of every clone were selected in all 3 replications and further 3 branches were selected. The total number of galls induced on both leaf and petiole. This was later calibrated into percentage damage. Thereafter, clones were categorised on the basis of rating following Kumar *et al* (2015) as given below.

$$\text{Rating 0} = \bar{x}(A) < (\bar{X} - \sigma)$$

$$\text{Rating 1} = (\bar{X} - \sigma) < \bar{x}(A) < (\bar{X} - \sigma/2)$$

$$\text{Rating 2} = (\bar{X} - \sigma/2) < \bar{x}(A) < (\bar{X})$$

$$\text{Rating 3} = (\bar{X} + \sigma/2) > \bar{x}(A) > (\bar{X})$$

$$\text{Rating 4} = (\bar{X} + \sigma) > \bar{x}(A) > (\bar{X} + \sigma/2)$$

$$\text{Rating 5} = \bar{x}(A) > (\bar{X} + \sigma)$$

Where  $\bar{x}(A)$  is mean percentage of gall incidence for each clone,  $\bar{X}$  is the overall mean of gall incidence for all fourteen clones of *Eucalyptus*,  $\sigma$  is the standard deviation.

### V) Statistical analysis:

The data were subjected to ANOVA and means were compared by LSD ( $p < 0.05$ ) under Completely Randomized Block Design (CRBD) following the CPCS statistical software developed by Punjab Agricultural University, Ludhiana.

### Experiment 3

To study the biochemical characters of *Eucalyptus* in relation to incidence of gall wasp, *Leptocybe invasa*

#### II) Location

PG Laboratory of Department of Botany, College of Basic Science and Humanities, PAU

#### III) Methodology:

- Treatments : 10 clones
- Design : CRD
- Replications : 4

**Methodology:** For the biochemical analysis of clonal *Eucalyptus* leaves, 3 healthy and 3 infested fresh leaves of each clone were collected from the experimental plantation. The collected leaf samples were categorized into: (1) Healthy leaf (2) Infested leaf

Five plants of each *Eucalyptus* clone were marked for recording the gall wasp incidence. Three sets of fresh leaf were collected for studying various biochemical characters. Various biochemical parameters such as total soluble sugars (Dubois *et al* 1956), total soluble proteins (Lowry *et al* 1951) and total phenols (Swain and Hills 1959). The attack or incidence of *Leptocybe invasa* was recorded at monthly interval.

#### IV) Observations:

##### 1. Total soluble sugars

Total soluble sugars estimated from collected leaf samples according to 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:

1. 80% Ethanol
2. 5 % Phenol
3. Concentrated H<sub>2</sub>SO<sub>4</sub>

**Extraction:** Leaf sample of 0.1 g was crushed in pestle and mortar with 80% ethanol and then crushed material displaced in centrifugal tubes and centrifuged it at 2500 rpm for ten minutes in centrifugal machine. 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:** 0.1 ml of supernatant was taken in a test tube and then 0.9 ml distilled water was added in this test tube. After this, add 1 ml of 5% phenol and kept it for 10 to 15 minutes at room temperature. After this, add 5 ml of cold concentrated Sulphuric acid ( $H_2SO_4$ ) and again kept for 30 to 60 minutes at room temperature to cool down of tubes and then the absorbance readings was taken at 490 nm against the reagent blank. The concentration of the total soluble sugars/g was calculated from glucose standards (10-60  $\mu$ g) run simultaneously and total soluble sugar content was expressed as mg /g of dry weight

## 2. Total soluble proteins (mg/g fresh weight)

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

**Principle:** Protein (peptide bonding) in the sample react with the copper tartarate 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 2%  $Na_2CO_3$  was dissolved in 0.1N NaOH and made final volume of 100 ml.
  - 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 Ciocalteu'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.

## 3. Total phenols (mg/g dry weight)

The total phenol content were estimated according to the Swain and Hillis (1959).

### a) Extraction

0.1 g of fresh leaf tissue was dipped in the 10 ml of 80 per cent aqueous methanol, kept it overnight and then refluxed on hot plate to evaporate the methanolic content. Then the refluxed material was filtered and the volume was made up to 10 ml with adding distilled water. The extract thus obtained was used for the estimation of the total phenols.

### b) Reagents

- i) Folin & Ciocalteu's phenol reagent: The reagent was mixed in 1:1 v/v with distilled water just before use.
- ii) Saturated sodium carbonate solution (70.0 g/ 200 ml  $dH_2O$ )
- iii) Gallic acid (standard)

**c) Estimation :** 0.5 ml from the methanolic extract was taken in test tube and evaporated till

dryness. The residue was then dissolved in 6.5 ml of dH<sub>2</sub>O. Then, 0.5 ml of Folin & Ciocalteu's phenol reagent was added and thoroughly shaken. One ml of saturated sodium carbonate solution was then added after 5 minutes. The blue colour thus obtained was read at 760 nm against the blank after one hour in a spectrophotometer. The gallic acid in the range of 10–50 µg was used to prepare the standard curve.

#### V) Statistical analysis:

The data was be subjected to ANOVA and means were compared LSD (p=0.05) under Completely Randomized Block Design (CRBD) following the CPCS statistical software developed by Punjab Agricultural University, Ludhiana.

#### Index score analysis of *Eucalyptus* clones

Index scores were given to clones based on based on growth traits, stem quality, branch traits, gall wasp incidence scoring and flowering. The four class intervals of tree height, DBH, volume index, clear bole height, number of branches and straightness score were based on the range of each trait. The index score were also given on basis of gall wasp rating and flowering as described in table 3.2.

**Table 3.2: Class Intervals for index score for 14 *Eucalyptus* clones**

<b>Trait</b>	<b>1(2*)</b>	<b>2(4*)</b>	<b>3(6*)</b>	<b>4(8*)</b>
Tree height (m)	<12.58	12.58-13.90	13.91-15.22	>15.22
DBH (cm)	<12.04	12.04-13.31	13.32-14.58	>14.58
Volume (m <sup>3</sup> )	<0.206	206-0.278	0.279-0.350	>0.350
Clear Bole Height(m)	<7.40	7.40-9.13	9.14-10.86	>10.86
Number of Branches	>5.72	4.81-5.72	3.88-4.80	<3.88
Straightness score	<2.46	2.46-2.91	2.92-3.35	>3.35
Gall wasp rating	≥4	3	2	1
Flowering	Nil	Low	medium	High

\* Score of 2, 4, 6 and 8 were given for volume index only.

## CHAPTER IV

### RESULTS AND DISCUSSION

The results and discussion of the current study “Studying variation among *Eucalyptus* clones for growth traits, flowering and tolerance to gall wasp (*Leptocybe invasa*)” was carried out during 2018-19 are described in this chapter. For the sake of convenience, the results obtained are discussed under following headings in the light of appropriate reasoning and available literature. The study was divided into three experiments

#### 4.1 Studying variation in the growth traits and flowering behaviour of *Eucalyptus* clones.

- 4.1.1 Growth traits
- 4.1.2 Quality traits
- 4.1.3 Flowering behaviour
- 4.1.4 Diversity of floral visitors

#### 4.2 Screening of *Eucalyptus* clones against *Leptocybe invasa*.

#### 4.3 To study the biochemical characters of *Eucalyptus* in relation to incidence of gall wasp, *Leptocybe invasa*

### **4.1 Studying variation in the growth traits and flowering behaviour of *Eucalyptus* clones**

The results related to the variation in the growth traits, quality traits, flowering behaviour and diversity of floral visitors among fourteen clones are discussed under following sub-headings.

#### **4.1.1 Growth traits**

##### **4.1.1.1. Tree height (m)**

The data on tree height of fourteen clones of *Eucalyptus* during the study are presented in table 4.1. The tree height of clones varied from 11.26 m to 16.54 m. The difference among the clones were found to be significant ( $p < 0.05$ ). The highest tree height of 16.54 m was attained by clone PE-11 which was found statistically at par with five clones viz C-407 (16.53 m), C-413 (16.51 m), C-2135 (15.99 m), PE-6 (15.83 m) and C-72 (15.49 m). It was followed by F-316 > PE-5. PE On the other hand, minimum tree height was recorded in the clone PE-12 (11.26 m) which was also statistically at par with PE-13 (11.54 m) and PE-14 (11.93 m). As compared to the clonal check C-413 (16.51), two clones viz PE-11 (16.54) and C-407 were found having relatively higher height but were statistically at par. Eight clones recorded as the higher tree height than mean total height (14.23 m).

The significant difference in height among different clones may be due to its different and specific genetic makeup, as all the similar cultural practices were given to all *Eucalyptus* clones. Clone PE-11, C-407, and C-413 were promising ones.

Significant variation in the tree height of different clones of *Eucalyptus* were recorded in the current study are in conformity with the findings given by Oballa *et al* (2005) based on study at Kenya. Similarly Singh *et al* (2015) on the growth of different clones of *Eucalyptus* in Gujarat concluded the average height were obtained in the order of P-411 >P-413> P2136> P-526 > B-2153 > SRO-16.

Similar findings given by earlier workers such as Dutt and Tyagi (2011) in different *Eucalyptus* species, Huang *et al* (2000) among *E. urophylla* progenies, Dogra and Sharma (2005) among several *Eucalyptus* species and provenances, Sidhu *et al* (2005) in *E. tereticornis*, Dhillon and Singh (2010) among 83 progenies of *E. tereticornis*, and Reis *et al* (2011) in various *Eucalyptus* species. Likewise Xu *et al* (2003) found significant differences in *E. tereticornis* for height of tree and diameter at breast height.

**Table 4.1: Variation in total height among *Eucalyptus* clones**

Clone	Tree Height (m)	Rank
C-2135	15.99	4
F-316	14.35	7
C-413	16.51	3
PE-6	15.83	5
C-72	15.49	6
PE-14	11.93	12
PE-7	13.19	10
PE-5	14.32	8
PE-11	16.54	1
PE-13	11.54	13
PE-8	13.43	9
C-407	16.53	2
PE-12	11.26	14
PE-9	12.38	11
Mean±S.E.	14.23±0.31	
LSD 5%	1.10	

#### 4.1.1.2 Diameter at breast height (cm)

The results obtained for diameter at breast height gained by fourteen *Eucalyptus* clones at 4 years age are presented in table 4.2. Significant difference ( $p < 0.05$ ) were noticed among the clones. This table clearly showed that clone C-413 was ranked first out of the 14 clones with gaining the highest diameter at breast height of 15.85 cm. Clone C-413 was found

statistically at par with other four clones viz C-407 (15.29 cm) and PE-11 (15.04 cm), C-72 (14.90 cm) and F-316 (14.77 cm) from diameter point of view. Besides, eight clones had more diameter than average diameter (13.75 cm) and six clones had lesser diameter than average diameter. The minimum value of diameter at breast height was recorded in clone PE-14 (10.76 cm).

**Table 4.2: Variation in Diameter at breast height among 14 *Eucalyptus* clones**

Clone	Diameter at breast height (cm)	Rank
C-2135	14.42	7
F-316	14.77	5
C-413	15.85	1
PE-6	14.30	8
C-72	14.90	4
PE-14	10.76	14
PE-7	12.96	11
PE-5	14.53	6
PE-11	15.04	3
PE-13	13.07	9
PE-8	12.97	10
C-407	15.29	2
PE-12	10.86	13
PE-9	12.81	12
Mean±S.E.	13.75±0.25	
LSD 5%	1.22	

The result showed the effect of clones was significant in diameter, which may be attributed to their varying genetic potential. Similar kind of findings were reported by Oballa (1989) while studying variations in the diameter at breast height among the progenies of *E. grandis*, Huang *et al* (2000) among the progenies of *E. urophylla* progenies and Ginwal *et al* (2004) among the progenies of *E. tereticornis*, Dogra and Sharma (2005) compared the growth provenances *Eucalyptus*. Sidhu *et al* (2005) in *E. tereticornis*, Dhillon and Singh (2010) among the 83 progenies of *E. tereticornis*, Dutt and Tyagi (2011) in different *Eucalyptus* species and Reis *et al* (2011) in different *Eucalyptus* species were in accordance with the results. Similarly in Kenya, Balozzi *et al* (2010) found hybrid had higher diameter at breast height than *E. tereticornis*.

#### 4.1.1.3 Tree Volume Index

The tree volume Index is the indicator of the wood quantity of a tree and is generally estimated for the valuation of economic value and commercially utilization potential of any tree. The results related to volume index obtained from the current investigation are tabulated in table 4.3 at the age of 4 years. This table clearly indicates that the mean volume gained by fourteen *Eucalyptus* clones was ranged from 0.134 cubic meter to 0.419 cubic meter. Clone C-413 was ranked first in this volume index by gaining volume of 0.419 m<sup>3</sup>, which was found to be statistically at par with clone C-407 (0.391 m<sup>3</sup>) and PE-11 (0.374 m<sup>3</sup>). On the other hand, out of 14 clones, the clone PE-14 had gained least value (0.139 cubic meter) The average volume calculated from 14 clones was 0.280 cubic meter. Eight clones had more volume than average volume and six clones had less volume than average volume.

**Table 4.3: Variation in volume index among *Eucalyptus* clones**

Clone	Volume Index (D <sup>2</sup> H, m <sup>3</sup> )	Rank
C-2135	0.334	5
F-316	0.314	7
C-413	0.419	1
PE-6	0.323	6
C-72	0.344	4
PE-14	0.139	13
PE-7	0.222	10
PE-5	0.302	8
PE-11	0.374	3
PE-13	0.197	12
PE-8	0.226	9
C-407	0.391	2
PE-12	0.134	14
PE-9	0.205	11
Mean±S.E.	0.280±0.015	
LSD 5%	0.069	

Like height and diameter, difference among the *Eucalyptus* clones were significant ( $p<0.05$ ) for volume Index also. Many studies from India and abroad had also reported significant variation among the *Eucalyptus* clones. Tree to tree variation for volume was recorded and used for selecting best clones for further multiplication (Vennila *et al* 2011, Gangwar *et al* 2015). In *E. nitens*, Neilson and Gerrand (1999) observed the increase in total volume with stocking while mean tree parameters, diameter (over bark) and tree volume, were reduced.

## 4.1.2 Quality traits

### 4.1.2.1 Branch traits

Quality characters govern the wood quality of the tree. The results obtained during study shows the variation in the number of branches per tree among the fourteen *Eucalyptus* clones are presented in the Table 4.4. Significant differences among the clones were noticed. The highest mean number of branches per tree was observed in clone PE-12 (6.64) which was statistically at par with clone PE-5 (6.22) and were followed by C-2135>F-316>PE-13. Out of 14 clones, five clones had more number of branches than the average number of branches per tree (4.37) and nine clones had fewer branches than average branches. The lowest number of branches was registered in clone C-413 (2.97). The current study displayed significant variation in the number of branches per tree among the clones.

The results of the current study are similar with the findings of Balaji (2000) in *E. tereticornis*, Kulkarni (2004) among different *Eucalyptus* clones. Veerendra and Sharma (1990) reported that growth of branches was correlated with trees height in *Santalum album*.

**Table 4.4: Variation in *Eucalyptus* clones for various branch traits**

Clone	Number of branches	Rank	Branch angle (°)	Rank	Branch diameter (cm)	Rank
C-2135	5.14	12	43.75	9	5.89	11
F-316	5.11	11	39.58	3	6.67	14
C-413	2.97	1	43.75	9	5.92	12
PE-6	4.36	9	41.28	5	3.94	2
C-72	4.22	8	46.94	12	4.67	6
PE-14	3.53	3	43.61	8	4.33	3
PE-7	3.61	4	34.44	1	4.89	7
PE-5	6.22	13	50.42	14	5.29	10
PE-11	3.83	6	44.08	11	4.89	7
PE-13	4.64	10	39.75	4	4.44	4
PE-8	3.64	5	41.42	6	3.33	1
C-407	4.08	7	42.50	7	6.22	13
PE-12	6.64	14	47.22	13	4.50	5
PE-9	3.20	2	36.97	2	4.90	9
Mean±S.E.	4.37±0.19		42.55±0.70		4.99±0.17	
C.D. 5%	1.18		4.26		1.03	

Out of 14 clones, the average branch angle was noticed 42.55 and six clones had more branch angle than the average branch angle and smaller branch angle was found in eight clones as compared to average branch angle. Further the maximum branch angle was observed 50.42 in clone PE-5 which was found statistically at par with clone PE-12 (47.22) and C-72 (46.94). The minimum branch angle was observed in clone PE-7 (34.44).

The maximum branch diameter (thicker branches) of tree was recorded on clone F-316 (6.67 cm) which was statistically at par with three clones viz C-407 (6.22 cm), C-413 (5.92 cm) and C-2135 (5.89 cm). The thinner branches i.e. branches have lesser diameter were found on clone PE-8 (3.33 cm). Five clones were registered as having more branch diameter than overall mean branch diameter (4.99 cm) of 14 clones.

#### 4.1.2.2 Stem traits

Data related to the variation in 14 *Eucalyptus* clones for stem traits i.e. clear bole height (CBH) and stem straightness is tabulated in the table 4.5.

**Table 4.5: Variation in *Eucalyptus* clones for stem traits**

Clone	Straightness score	Rank	Clear bole height (m)	Rank
C-2135	2.61	8	9.83	2
F-316	2.17	10	5.67	14
C-413	3.54	2	9.17	4
PE-6	2.39	9	7.67	11
C-72	3.38	3	8.17	9
PE-14	2.11	12	6.67	13
PE-7	3.11	6	9.00	5
PE-5	2.00	14	8.50	8
PE-11	3.81	1	12.56	1
PE-13	2.11	12	6.83	12
PE-8	2.97	7	9.50	3
C-407	3.27	4	8.00	10
PE-12	2.16	11	8.83	6
PE-9	3.17	5	8.58	7
Mean±S.E.	2.77±0.10		8.50±0.30	
C.D. 5%	0.37		1.54	

Clone PE-11 registered the top rank with straightness score of 3.81 and was statistically superior to all other clones except three clones viz C-413 (3.54), C-72 (3.38) and C-407 (3.27). The lowest value was in case of PE-5 (2.00) which was statistically at par with four clones i.e. PE-13 (2.11), PE-14 (2.11), PE-12 (2.16) and F-316 (2.17).

The clear bole height was found to be the highest in clone PE-11 (12.56 m) which was statistically superior to all other clones. It was followed by clone C-2135 (9.83 m), PE-8 (9.50 m) C-413 (9.17 m), PE-7 (9.00 m) and PE-12 (8.83 m). On the other hand, the bottom ranking was found in clone F-316 (5.67 m).

Straightness and clear bole height are important quality traits which affect the amount of merchantable timber from a tree. The present study found significant variation among the clones. Clone PE-11, C-413, C-72 and C-407 were found to be promising for straightness score, whereas clone PE-11 outscored other than for clear bole height.

Similar kind of results given by Kumar (2013) in clonal *Eucalyptus* in Punjab conditions, CABI (2000) in *E. grandis*. In Pakistan by Mahmood *et al* (2003) in *E. camaldulensis*, Arnold *et al* (2004) in *E. dunnii* in Australia and Hesheng *et al* (2012) in *E. saligna*.

### **4.1.3 Flowering behaviour**

#### **4.1.3.1 Time of flower bud commencement**

Significant variation was observed in the flowering behaviour of different clones of *Eucalyptus*. Table 4.6 represent the data related to bud initiation (first floral bud appearance) and month of blossoming (opening) of floral buds. No emergence of floral buds was recorded in three clones i.e. C-72, PE-14 and PE-12. Bud appearance was firstly recorded on clone C-2135 in the 2<sup>nd</sup> week of June, followed on the clone PE-8 and PE-9 in the last week of June, further bud appearance was followed on clone PE-7 in the 1<sup>st</sup> week of July then floral buds emerge on two clones PE-5 and PE-13 in 2<sup>nd</sup> week of July. After this, the floral buds commenced on clone F-316, PE-11 and C-407 and later on clone C-413 in the 3<sup>rd</sup> week of August. These buds were bloomed within three to four months but no blooming of floral buds was recorded in two clones viz C-413 and C-407.

The maximum bloomed buds were recorded on three clones viz PE-7, PE-8 and PE-9 commenced from last week of October (C-2135) to February. The results of variation in the flowering commenced are accordance with reports of Ashton (1975) that the floral buds were protected by a capsular sheath for 4 to 6 months, the period of flowering was about three months. Hodgeson (1976) reported that floral buds reached anthesis stage after three to four months. Keatley *et al* (1999) found high variation in the flowering behaviour within the various species of *Eucalyptus*. Griffin (1980) reported variation in the flowering behaviour between two different years. Cremer *et al* (1978) reported the flowering time of *Eucalyptus* species from few days to two months. Similarly in *Grewia optiva*, Pant *et al* (1997) reported floral buds appearance during March-April to end June. Friedel *et al* (1993) reported that flowering time was associated with seasonal patterns of rainfall and temperature and intensity of solar radiation.

**Table 4.6: Variation in the flowering period among different *Eucalyptus* clones**

Clone	Bud initiation	Blossoming initiation
C-2135	2 <sup>nd</sup> week of June	4 <sup>th</sup> week October
F-316	3 <sup>rd</sup> week of July	2 <sup>nd</sup> week of December
C-413	3 <sup>rd</sup> week of August	No blossoming
PE-6	2 <sup>nd</sup> week of July	2 <sup>nd</sup> week of December
C-72	NIL	NIL
PE-14	NIL	NIL
PE-7	1 <sup>st</sup> week of July	4 <sup>th</sup> week November
PE-5	2 <sup>nd</sup> week of July	3 <sup>rd</sup> week December
PE-11	3 <sup>rd</sup> week of July	1 <sup>st</sup> week November
PE-13	2 <sup>nd</sup> week of July	3 <sup>rd</sup> week December
PE-8	4 <sup>th</sup> week of June	4 <sup>th</sup> week November
C-407	3 <sup>rd</sup> week of July	No blossoming
PE-12	NIL	NIL
PE-9	4 <sup>th</sup> week of June	3 <sup>rd</sup> week November

**4.1.3.2 Intensity of flowering**

Data related to the variation in the intensity of flowering are presented in the table 4.7 which showed that the profuse amount of flowering was recorded on three clones viz PE-7, PE-8 and PE-9. Further, the medium intensity of flowering was observed on two clones PE-5 and PE-6. No flowering was recorded on clone C-72, PE-12 and PE-14. Few categorized floral buds registered on clone C-407 and C-413.

**Table 4.7: Variation in the intensity of flowering among *Eucalyptus* clones**

Sr. No.	Clone	Intensity of Flowering
1	C-2135	Medium
2	F-316	Low
3	C-413	Low
4	PE-6	Medium
5	C-72	NIL
6	PE-14	NIL
7	PE-7	High
8	PE-5	Medium
9	PE-11	Low
10	PE-13	Low
11	PE-8	High
12	C-407	Low
13	PE-12	NIL
14	PE-9	High

The results of present study are accordance with the findings of intensity of flowering by Ford (1983) determined through opercula count and Keatley *et al* (1999) opercula traps were used to examine the timing and intensity of flowering.

### 4.1.3.3 Morphological features of Gumnuts

The morphological features were studied in eight *Eucalyptus* clones which bears flowering out of fourteen clones. As we early discuss, the floral buds were appeared from the month of July to October end in different clones and generally remain in two to three months in the bud stage and then it these were converted to anthesis (buds opens and flower stage). All the buds were opened in two to three months and converted into gumnut (fruit stage). We studied the morphological features of these gumnuts of seven clones. The data related to gumnut diameter, gumnut length and peduncle length are tabulated in table 4.8 and clearly indicates about the diameter of gumnut, length of gumnut and peduncle length of gumnut (length of peduncle through gumnut is attached with branch). The Average diameter of gumnut was found to be 6.61 mm, gumnut length was 6.00 mm and peduncle length was 3.01 mm. The Largest diameter of gumnut with the value of 7.15 mm was found in clone PE-5 which was found significantly different from other clones and followed by clone PE-9 (6.78 mm) which was statistically at par with other four clones viz PE-7 (6.67 mm), C-2135 (6.66 mm), PE-6 (6.55 mm) and PE-13 (6.48mm). The minimum diameter of gumnut was measured in clone PE-11 (6.05 mm).

The maximum length of gumnut was observed in the clone PE-6 (6.90 mm) which was statistically at par with the three clones viz PE-11 (6.62 mm), PE-5 (6.43 mm) and PE-13 (6.41 mm). The shortest length of gumnut was recorded in the clone PE-7 (5.98 mm).

**Table 4.8: Morphological features of fruits (gumnut) of various *Eucalyptus* clones**

Clone	Gumnut Diameter (mm)	Gumnut Length (mm)	Peduncle length (mm)
PE-5	7.15	6.43	2.15
PE-6	6.55	6.90	3.80
PE-7	6.67	5.98	3.03
PE-8	6.57	6.33	2.57
PE-9	6.78	6.00	2.78
PE-11	6.05	6.62	1.89
PE-13	6.48	6.41	3.29
C-2135	6.66	6.00	3.01
Mean±SE	6.61±0.069	6.34±0.079	2.82±0.121
LSD (p<0.05)	<b>0.35</b>	<b>0.50</b>	<b>0.14</b>

The clone PE-6 (3.80 mm) was ranked first in the peduncle length of gumnut. PE-6 was also found statistically different from other clones and followed by clone PE-13 (3.29 mm). On the other hand, if discussing about minimum peduncle length was measured in the clone PE-11 (1.89 mm).

Similar kind of the investigations carried out by Mangalore (2016) in Kiwi fruit (*Actinidia deliciosa*) found the significant variation in the average diameter of flower bud ranges from (0.28 cm to 5.08 cm), average length of flower bud (0.2 cm to 1.48 cm) and mean pedicle length (0.18 cm to 1.94 cm) of kiwi flowers. Significant variation among fruit traits was reported earlier in *Dalbergia sissoo* by Singh and Pokhriyal (2001), *Jatropha curcas* (Ashraf *et al* 2007 and Dhillon *et al* 2008) and in *Acacia nilotica* (Dhillon and Khajuria 1995), *Prosopis juliflora* (Sharma *et al* 1994), *Terminalia chebula* (Thakur *et al* 2008) and *Pongamia pinnata* (Jaishankar *et al* 2014).

#### 4.1.3.4 Floral visitors on *Eucalyptus* flowers

Data related to the floral visitors on flowers of clonal *Eucalyptus* in the month of November 2018 to February 2019 is given in table 4.9. It was observed that all the species of honey bee of Punjab were noticed as visitors on the fully bloomed flowers of clonal *Eucalyptus*.

**Table 4.9: Diversity of floral visitors**

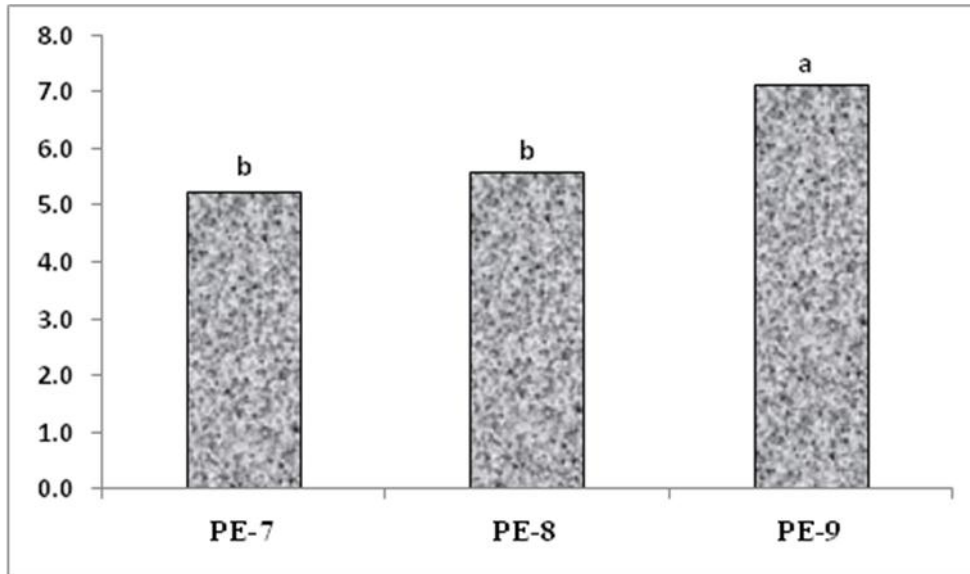
Sr. No.	Insect	Order
1	<i>Apis mellifera</i> (Italian honey bee)	Hymenoptera
2	<i>Apis cerana indica</i> (Asian honey bee)	Hymenoptera
3	<i>Apis dorsata</i> (Doomna or large honey bee)	Hymenoptera
4	<i>Apis florea</i> (Small honey bee)	Hymenoptera
5	Yellow wasp	Hymenoptera
6	Potter wasp	Hymenoptera

These insects may visit for nectar and pollen collection on *Eucalyptus* bloomed flowers and helps in the pollination mechanism of *Eucalyptus*. On the other hand, in turn *Eucalyptus* rewards these pollinators by providing nectar for its feeding requirement.

Similar kind of reports given by Punjab Agricultural University, Ludhiana in publications of commercial bee keeping regarding *Eucalyptus* is the major floral source i.e. both nectar and pollen for honey bee foraging in Punjab. *Eucalyptus* is considered in the floral calendar i.e. April end to 1<sup>st</sup> mid of July of foraging sources in Punjab conditions. Similar reports were given by Pryor (1976) revealed pollen remained available to pollinating agents, Hodgeson (1976) pollination was carried out by insect pollinators, Hingston (1998) reports seven bird and seventy one insect species repeatedly visits on *Eucalyptus* flowers House (1997) and Chambers (1997) reports flowering precocity was highly heritable but the Jones *et al* (2011) flowering trait was controlled by both genetic and environmental factors.

#### 4.1.3.5 *Apis mellifera* visitation on different clones

In figure 4.1, data are given related to the mean number of *Apis mellifera* visitation in the whole day recorded at three different timings per day on three different clones (PE -7, PE-8 and PE-9 bears profuse flowering). The maximum number of honey bee visitation was recorded on clone PE-9 (7.1) and followed by clone PE-8 (5.6). The minimum number of visitation was recorded on clone PE-7 (5.2). It indicates that clone PE-9 is best for bee keeping point of view.

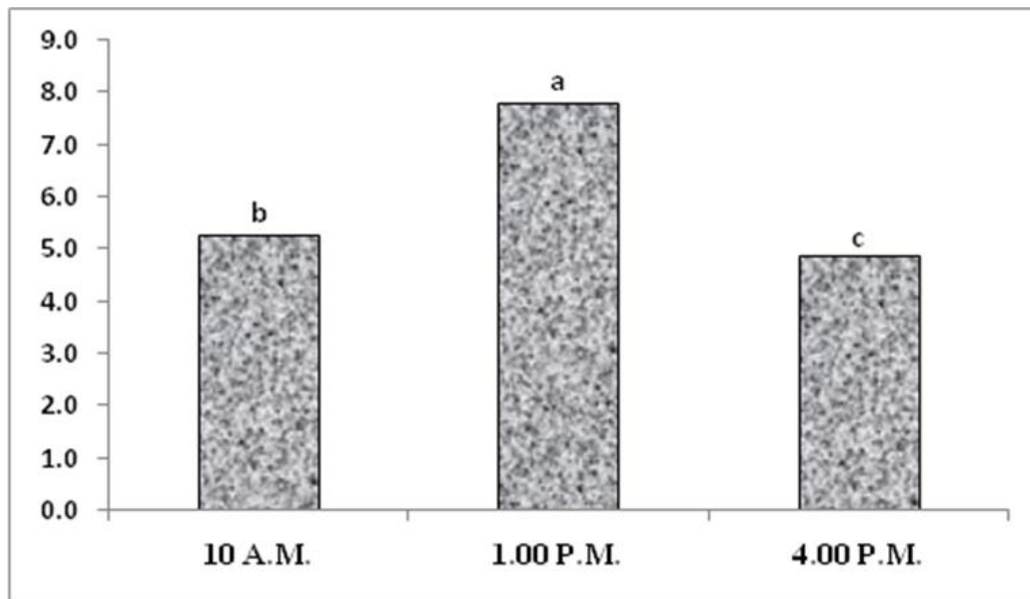


**Fig. 4.1: Frequency of honey bee visitation (number of visit per two minute) on three *Eucalyptus* clones.**

Clone PE-9 found as the most attractable to honey bee (*Apis mellifera*) in the study which may be due to its floral structure or may provide more reward to visitors. Similar kind of findings presented by Seeley and Levien (1987) that the density of floral visitor insects on bloomed flowers related with the weather conditions, nectar potential, the floral structure of the tree and intensity of flowering of plant.

#### 4.1.3.6 Diurnal variation in frequency of Honeybee visitation on *Eucalyptus* clones

*Eucalyptus* is a cross pollinated tree which is pollinated through various pollinating agents such as insects, wind and birds etc. Data related to the number of honey bee (*Apis mellifera*) visitation (per two minute) on fully bloomed *Eucalyptus* flowers was recorded from the month of December 2018 to February 2019 on 3 clones i.e. PE-7, PE-8 and PE-9 at morning (10:00 am), then afternoon (1:00 pm) and evening (4:00 pm) are illustrated in figure 4.2. The highest number of visitation of *Apis mellifera* was found maximum at afternoon time (5.5 honey bee visits per 2 minute) and followed by morning time (3.7 honey bee visits in two minute) and in last the less number of visitation was recorded at evening time (3.2 per two minute).



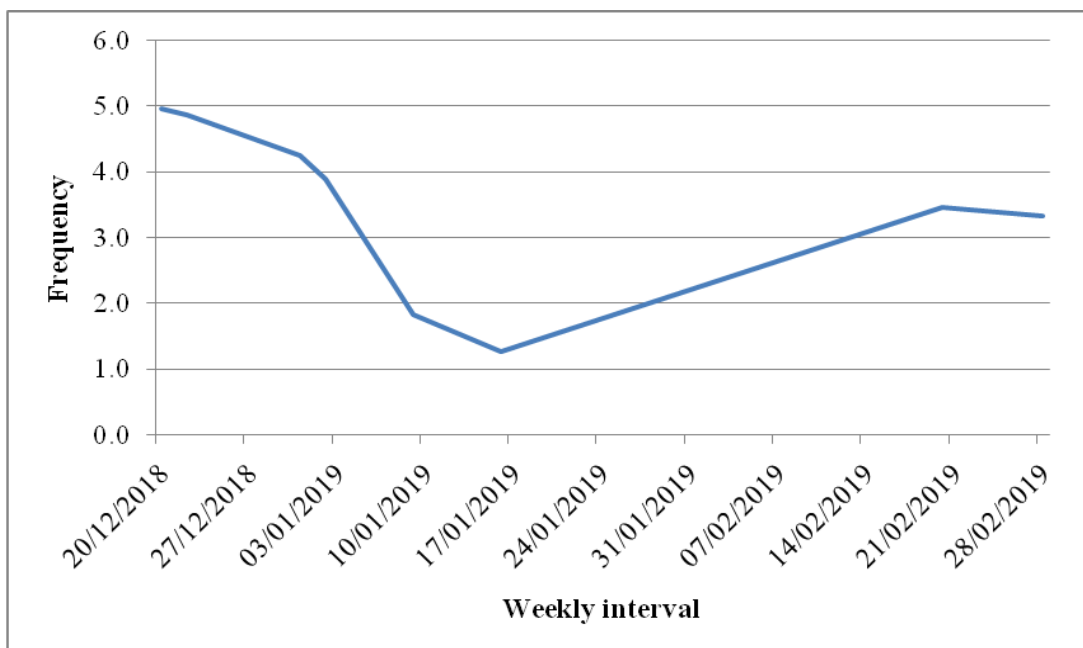
**Fig. 4.2: Diurnal variation in Frequency of honey bee visits (Number per 2 min) on *Eucalyptus* flowers**

The higher honey bee visitation found at afternoon time in the study may be due to increase in the temperature, because the foraging activities of honey bee also increased with increase in the temperature. Similar kind of the investigations carried out by the Free (1993) that the metabolic activities of insect increases with the increase in the temperature and visit more flowers at higher temperature. Similar kind of findings presented by Seeley and Levien (1987) that the density of floral visitor insects on bloomed flowers related with the weather conditions, the floral structure of the tree and density of flowering.

#### **4.1.3.7 Temporal variation in honeybee visits**

Data regarding the mean number of honey bee visitation on *Eucalyptus* clones are presented in Fig. 4.3. This figure clearly shows that the visitation rate of honey bee on bloomed flowers was on peak (5.00) during 4<sup>th</sup> week of December 2018. After that the gradual decrease in the number of visitation was recorded till 2<sup>nd</sup> week of January 2019 (1.30) and again the visitation was increased till 3<sup>rd</sup> week of February (3.50) and remains almost similar in the February end (3.33).

The abundance of *Apis mellifera* decreases with increase in chilling winter in January and again in February, the visitation was increased as the temperature starts rising after this. In Brazil, Hilgert-Moreira *et al* (2014) reports the availability of flowering *Eucalyptus* spp. throughout the year allowed bees to use pollen and nectar.



**Fig. 4.3: Temporal variation in honeybee visits (Number per 2 min) on *Eucalyptus* flowers**

#### **Experiment 4.2 Screening of *Eucalyptus* clones against *Leptocybe invasa*.**

This experiment was conducted to study the variation among the different *Eucalyptus* clones for tolerance to the attack of gall wasp *Leptocybe invasa*

##### **4.2.1 Incidence of *Leptocybe invasa***

This study on seasonal incidence (percentage of the leaf infested with galls) of *Leptocybe invasa* was recorded from January 2018 to December 2018 on all 14 clones at monthly interval. Results obtained from observations recorded for gall wasp incidence are presented in table 4.10 and graphically in figure 4.4. We found that the clone F -316 was maximum infested with galls in all the twelve months. Moreover, Maximum and minimum gall infestation were recorded on this clone in the month of April (72.33 %) and December (16.67%) respectively.

In the month of January, overall average incidence of *Leptocybe invasa* was 9.40 per cent. The highly gall infested clone was found F-316 in this month which had galls on 21.67 per cent leaves. Clone F-316 was found statistically at par with clone PE-12 (17.67 per cent). Clone C-12 was escaped from the attack of *L. invasa* and no infestation was found in clone PE-8. Six clones registered as more infested than overall mean infestation.

**Table 4.10: Incidence of *Eucalyptus* gall wasp (*Leptocybe invasa*) on various clones during different months of the year**

Clone	January	February	March	April	May	June	July	August	September	October	November	December
C-2135	13.67	16.00	25.67	44.33	35.67	36.00	32.33	22.67	22.33	14.00	8.33	5.00
F-316	21.67	30.67	45.33	72.33	62.33	60.67	55.67	41.67	36.67	21.67	18.33	16.67
C-413	4.33	6.33	22.67	29.00	22.67	20.33	17.00	14.67	9.00	0.00	0.00	2.33
PE-6	12.33	11.67	25.67	21.67	28.33	25.00	25.00	30.00	17.00	8.00	6.67	8.67
C-72	6.33	9.00	27.33	24.00	22.67	21.33	18.67	16.67	7.67	5.33	4.33	4.00
PE-14	4.67	8.00	23.33	27.67	18.00	25.67	17.00	15.00	7.67	2.33	0.00	3.00
PE-7	12.33	14.00	23.33	46.67	36.67	32.67	33.33	23.33	17.00	13.67	3.33	5.00
PE-5	7.67	9.33	34.33	29.00	28.33	21.00	17.67	20.00	12.33	9.33	14.00	12.67
PE-11	4.33	8.33	22.67	30.33	22.33	16.67	18.67	18.33	11.67	1.67	0.00	2.33
PE-13	10.67	14.33	30.67	28.33	27.33	25.00	17.67	18.67	13.67	9.67	8.33	8.33
PE-8	0.00	8.33	22.33	32.33	26.67	18.67	18.33	16.67	11.00	6.67	17.00	15.33
C-407	7.33	9.67	24.00	29.67	21.67	26.00	21.67	18.00	13.33	3.33	5.00	5.00
PE-12	17.67	22.33	34.00	56.67	52.33	38.67	41.33	30.00	25.67	16.00	12.67	8.67
PE-9	8.67	10.00	26.67	31.67	27.67	19.00	19.00	17.67	11.00	6.67	7.67	8.00
Mean± SE	9.40± 0.92	12.71± 1.05	27.71± 1.14	35.98± 2.22	30.90± 1.91	27.62± 1.80	25.24± 1.78	21.67± 1.22	15.43± 1.30	8.45± 1.01	7.55± 1.10	7.50± 0.88
CD 5%	4.21	3.95	7.87	8.20	4.80	5.46	5.05	6.07	6.35	2.33	1.40	2.03

The maximum infestation was recorded in clone F-316 (30.67 per cent) during February and followed by PE-12 (22.33 per cent). Nine clones recorded as lesser infestation than overall average incidence of 12.71 per cent. The minimum percentage of galls on leaf was found on clone C-413. During the month of March, the maximum gall infestation was reported again on clone F-316 (45.33 %) which was statistically higher than other clones and was followed by clone PE-5 (34.33), PE-12 (34.00) and PE-13. These three clones were found statistically at par with each other. Clone PE-8 was found least infested (22.33) in this month and it is statistically at par with clone C-413, PE-11, PE-7 and PE-14.

In April, the maximum gall infested leaves were found on clone F-316 with value of 72.33 per cent which was significantly higher than other clones. The minimum gall infestation was found in clone PE-14 (21.67%). It was followed by clone PE-12, PE-9 and C-2135.

In May month, the average gall infestation was 30.90 per cent. Among the 14 clones, the minimum leaf gall infestation was found on PE-14 (18.00 %) and maximum gall infestation found in clone F-316 (62.33 per cent) which was statistically higher than other clones followed by PE-12 (52.33 per cent).

During the month of June, the maximum leaf gall infestation was shown by clone F-316 (60.67 per cent) which was significantly higher infestation than other clones. F-316 was followed by clone PE-12 (38.67) which was statistically at par with clone C-2135. On the other hand, the minimum gall infestation we observed on the clone PE-11 (16.67 per cent).

The minimum per cent of leaf infestation on two clones C-413 and PE-14 in the month of July. The infestation was recorded on the both clones was 17.00 per cent. On the other hand, significantly higher gall infestation was found on clone F-316 (55.67 per cent) and infestation was followed by clone PE-12. During August, significantly higher per cent of leaf damage was found on clone F-316 (41.67 per cent) whereas the significantly lowest gall infestation was recorded in clone C-413(14.67 per cent).

In September, significantly higher gall incidence was again recorded on the clone F-316 (36.67 per cent) and followed on clone PE-12 (25.67 per cent) and clone C-2135 (22.33 per cent). These two clones were also found statistically at par with each other. On the other hand, the minimum leaf gall infestation was found on clone C-72 (7.67 per cent) and PE-14 (7.67 per cent).

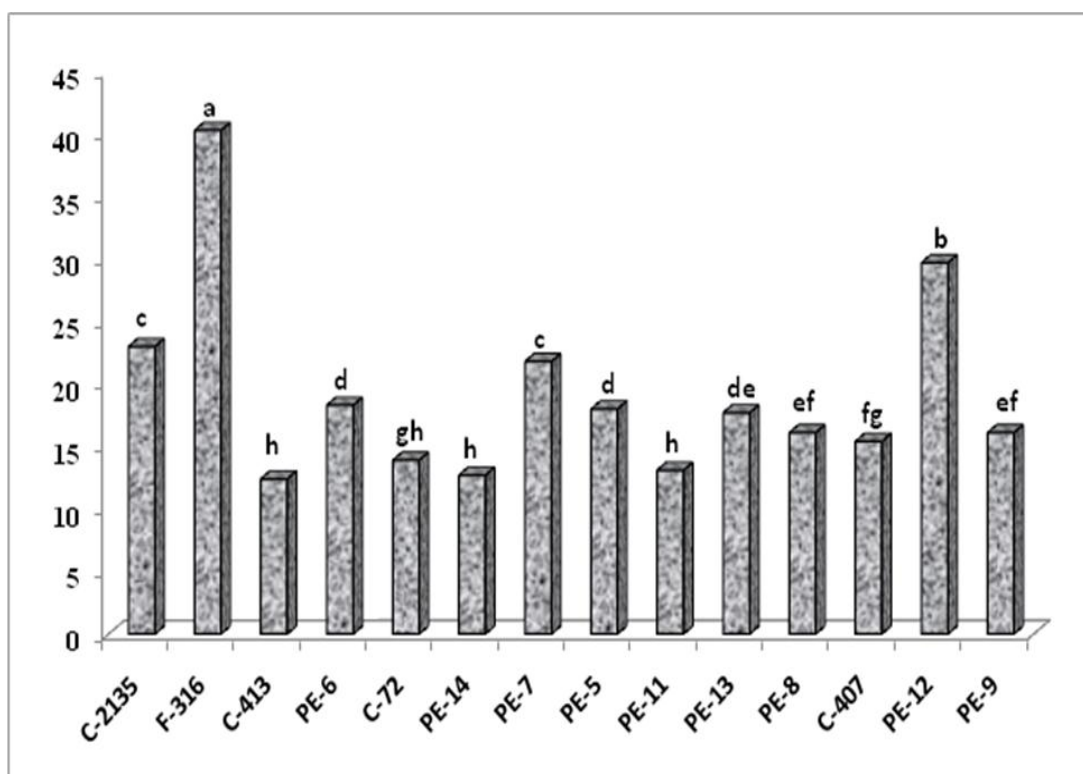
In the next month October, significantly higher mean gall infestation was noted on clone F-316 (21.67 per cent) and was found to be statistically different than other clones. Incidence after F-316 followed by clone PE-12 (16.00) which was statistically at par with clone C-2135 (14.00) and PE-7 (13.67) whereas no incidence was showed on clone C-413.

Clone C-413, PE-11 and PE-14 were escaped from the infestation of *L. invasa* in the month of November and the significantly higher gall infestation was found on clone F-316 (18.33 per cent) which was followed by clone PE-8 had 17.00 gall infestation. During December, the minimum number of galls were on clone C-413 and PE-11(both the clones had 2.33 per cent gall infestation) whereas the significantly higher gall infestation was found on clone F-316 (16.67 per cent). The overall mean infestation was observed 7.50 per cent.

The gall wasp incidence was also reported from Portugal (Branco *et al* 2005), Vietnam (Thu *et al* 2009), Tanzania (Petro *et al* 2015), Kenya (Nyeko *et al* 2010) and China (Fang-li *et al* 2012).

#### 4.2.2 Variation in the incidence of *L. invasa* among different *Eucalyptus* clones

The incidence of gall wasp was observed as the percentage of the leaf gall infestation on monthly interval for the period of twelve months on the 14 *Eucalyptus* clones. On the basis of pooled data over the year, the significant variation was ( $p < 0.05$ ) was noticed among the clones. During the study, three clones of *Eucalyptus* were found as highly susceptible (gall infestation more than on 20 per cent leaves) viz clone F-316 (40 per cent), PE-12 (29.7 per cent) and C-2135 (23 per cent) against *Leptocybe invasa*.



**Fig. 4.4: Incidence of *Eucalyptus* gall wasp on different clones recorded from field trial at Ludhiana (Age 3 years)**

The maximum gall infestation was recorded on Clone F-316 which was found statistically different from other clones

Further, six clones were found as moderate infested (gall infestation on 15 to 20 per cent leaves) with galls viz clone PE-13 (17.7 per cent), PE-8 (16.1 PER cent), PE-9 (16.1 per cent) and C-407 (15.4 per cent).

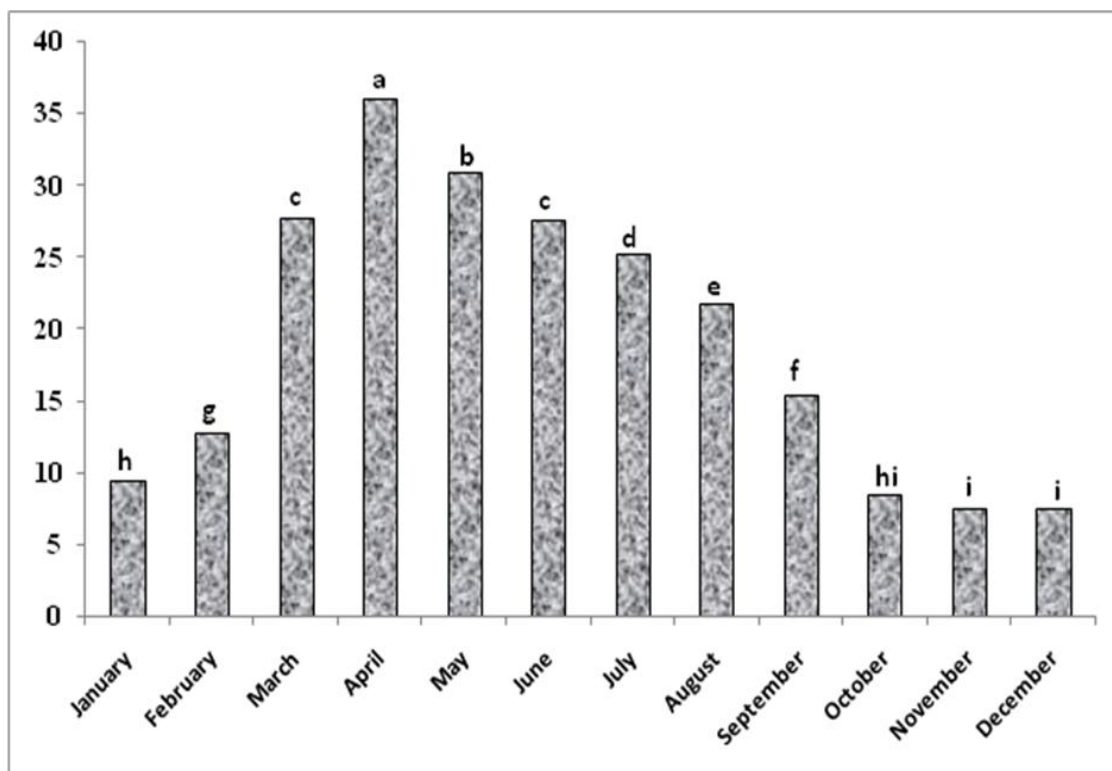
In this study, out of 14 clones, only four clones observed as the tolerant (10 to 15 per cent leaf infested with galls) clones viz C-72 (13.9 per cent), PE-11 (13.1 per cent), PE-14 (12.7 per cent) and C-413 (12.4 per cent). The minimum gall infested clone was found clone C-413 but statistically similar to the clones PE-14, PE-11 and C-72.

No clone was found to be escaped from the attack of *Leptocybe invasa* and the gall infestation was varied from 12.4 per cent on clone C-413 to 40.3 per cent on clone F-316. The results of current study are accordance with the findings of Rameshbhai (2010) and Singh (2012). The current study documents substantial differences in *L. invasa* infestation and damage among clones of *Eucalyptus* spp. These differences may be due to factors including differences in host physical and nutritional characteristics, and to differences in host biochemical defences (Bentur and Kaslode 1996).

#### **4.2.3 Variation in the monthly incidence of *Leptocybe invasa***

The incidence of gall wasp was observed for twelve months and infestation according to different months was categorised into three categories ie abundant infested months (overall field infested more than 10 per cent), moderate infested (overall field infested from 10 to 20 per cent) and minimum infested months (overall field infested less than 10 per cent or pooled gall infestation less than 10 per cent). Six months were reported as higher gall infestation on the overall field of 14 *Eucalyptus* clones ie March (27.7 per cent), April (36 per cent), May (30.9 per cent), June (27.6 per cent), July (25.2 per cent) and August (21.7 per cent). The highest gall infestation was reported in the month of April which was statistically different than other eleven months. Gall infestation in April month was followed by May which was also significantly different than other months. After this the infestation was followed in May, June, July and August which were statistically different from each other.

The month of September and February were reported as a moderate infested month with the pooled average of gall infestation was recorded 15.4 per cent and 12.7 per cent respectively. The gall infestation in September and February was significantly different from each other.



**Fig. 4.5: Monthly incidence of *Leptocybe invasa***

The minimum gall infestation was noticed in the months of November (7.5 per cent), December (7.5 per cent), October (8.5 per cent), and January (9.4 per cent). The month of November and December was registered as the minimum infested months with infestation of 7.5 per cent in both months. Gall infestation in the month of January was found statistically at par with October further which was also found statistically similar with the month of November and December.

Gall infestation starts increasing from the month of January and reaches on peak in the April then it starts decreasing continuously from May to the November and remains similar in December.

Similar kind of findings reported by Rameshbhai (2010) in Gujarat that the incidence was increased from April and at the peak in June. Similarly Singh (2012) reported that the incidence begins increased from February to May. Similarly Kulkarni (2010) reports that gall intensity was higher in April than December, similarly, gall attack was higher from March to October and at a minimum during November to February because of very low temperature in Northern India. However, the trend was opposite to the trend in Central and Southern India here the attack was higher during September to April. Similarly, leaf damage in *Eucalyptus* clones was higher during May to June in *E. camaldulensis* and *E. citrodora* (Rajpoot 2012).

**Table 4.11: Categorization of *Eucalyptus* clones based on infestation scores**

Gall score	Number of clones	Clones
0	0	NIL
1	5	C-2135, C-413, C-72, PE-13 and PE-12
2	5	PE-6, PE-14, PE-11, PE-8 and PE-9
3	1	PE-5
4	1	PE-7
5	2	F-316 and C-407

**Experiment No. 4.3 To study the biochemical characters of *Eucalyptus* in relation to incidence of gall wasp *Leptocybe invasa***

The variation in the different biochemical traits i.e. total soluble sugars, total soluble proteins and total phenols in the leaves of clonal *Eucalyptus* due to the attack of *Leptocybe* are discussed as following headings :

**4.3.1 Total soluble sugars (mg/g)**

Data related to the content of total soluble sugars in leaves of clonal *Eucalyptus* is tabulated in the table 4.12. The content of total soluble sugars was noticed remarkably higher in the infested leaves as compared to the healthy leaves of all clones of *Eucalyptus*. The percentage increase over healthy in the content of total soluble sugars in infested leaves as compared to healthy leaves varied from 8.57 per cent (PE-5) to 106.66 per cent (PE-6). The highest total soluble sugar content i.e. 84.99 mg/g was recorded in clone F-316 (gall infested) which was highly susceptible to *Leptocybe invasa* and followed by in infested clone PE-13 (95.16 mg/g). On the other hand, the minimum content i.e. 3.59 mg/g of total soluble sugar was recorded in clone C-2135 (healthy).

The increase in the amount of total soluble sugar in the leaves after infestation may act as attractant or stimulant for *Leptocybe invasa*. Total Soluble Sugars had found positive correlation with the susceptibility of gall wasp. The higher sugar content in leaves was reported to be beneficial for the insect feeding. (Hartlag 1998, Yang *et al* 2003).

**Table 4.12: Variation in the total soluble sugars concentration (mg /g fresh weight) in healthy and infested leaves of clonal *Eucalyptus***

Clone	Healthy	Infested	Percentage increase over healthy
C-2135	3.59	6.79	89.14
F-316	45.01	84.99	88.82
C-413	5.68	8.52	50.00
PE-6	23.12	47.78	106.66
PE-14	17.48	20.45	16.99
PE-5	20.88	22.67	8.57
PE-11	23.83	28.67	20.31
PE-1 3	31.23	60.95	95.16
PE-8	17.38	20.15	15.94
C-407	36.5	40.02	9.64
PE-12	24.65	27.4	11.16
PE-9	33.14	58.92	77.79
Mean±S.E.	23.54±2.41	35.61±4.71	
LSD (p<0.05)	3.35	3.62	

TSS is an important dietary constituent for insect development and acts as the feeding stimulant. Similar kind of investigations reported by the early workers such as Machado *et al* (2015) in maize where the content of total soluble sugars increased in the susceptible cultivars indicates that it imparts susceptibility to *Atherigona naqvii*. TSS was a vital dietary constituent and acts as the feeding stimulant. In Ranchi Mukherjee *et al* (2016) reports the level of total soluble sugars increases with the increase in the number of galls on leaves of *Terminalia tomentosa* and *Terminalia arjuna*. Similarly Singh (2012) reports the increase of sugar content in gall infested leaves of highly susceptible entries of *Eucalyptus*.

#### **4.3.2 Total soluble protein (mg/g)**

Data related to the level of total soluble Protein in leaves of clonal *Eucalyptus* is tabulated in the table 4.13. The content of total soluble protein was noticed remarkably lesser in infested leaves as compared to the healthy leaves of all clones of *Eucalyptus*. The percentage decrease over healthy in the content of total soluble protein in infested leaves as compared to healthy leaves varied from 3.65 per cent (PE-9) to 15.74 per cent (PE-8). The highest total soluble protein content i.e. 12.64 mg/g fresh weight was recorded in clone PE-8

(healthy) and followed by in healthy clone PE-11 (12.63 mg/g fresh weight). On the other hand, the minimum content i.e. 3.24 mg/g fresh weight of total soluble protein was recorded in clone F-316 (Infested) which was highly susceptible to *Leptocybe invasa*.

**Table 4.13: Total soluble protein concentration (mg /g fresh weight) in healthy and infested leaves of clonal *Eucalyptus***

Clone	Healthy	Infested	Percentage Decrease over healthy
C-2135	7.82	7.12	8.95
F-316	3.67	3.24	11.72
C-413	5.36	5.13	4.29
PE-6	7.06	6.77	4.11
PE-14	10.37	9.78	5.69
PE-5	7.96	7.05	11.43
PE-11	12.63	11.78	6.73
PE-13	6.69	5.76	13.90
PE-8	12.64	10.65	15.74
C-407	8.04	7.07	12.06
PE-12	8.3	7.72	6.99
PE-9	9.59	9.24	3.65
Mean±S.E.	8.34±0.53	7.61±0.50	
LSD 5%	0.72	1.69	

In healthy leaves of clonal *Eucalyptus*, the least value of total soluble protein was found in the clone F-316 (3.67 mg/g fresh weight). The decrease in the amount of total soluble protein in the leaves after infection may act as attractant or stimulant for *Leptocybe invasa*. Total Soluble protein had found negative correlation with the incidence of gall wasp.

Similar kind of findings given by Khattab and Khattab (2005) that the decrease in the content of total soluble protein content in the gall infested leaves of *Eucalyptus*. Higher amount of proteins has been reported in the infested sorghum genotypes against shoot fly (Bhise *et al* 1996, Patil and Bagde 2017, Bhoge *et al* 2017). Similarly Chhabra (2019) reports the level of protein was decreased in aromatic rice varieties after the post infection of Bakanae (*Fusarium moniliforme*) disease in rice *oryza sativa*. However, Padamaja *et al* (2014) reported higher levels of total proteins content in resistant sorghum cultivars as

compared to susceptible ones. However, higher protein content was recorded in the infested leaf of *Terminalia tomentosa* and *T. arjuna* by Mukherjee *et al* (2016)

### 4.3.3 Total phenol (mg/g)

Data related to the level of total phenol in leaves of clonal *Eucalyptus* is tabulated in the table 4.14. The content of total phenol was noticed remarkably higher in the infested leaves as compared to the healthy leaves of all clones of *Eucalyptus*. The percentage increase over healthy in the content of total phenol in infested leaves as compared to healthy leaves varied from 9.47 per cent (PE-13) to 69.30 per cent (PE-11). The highest total phenol content i.e. 12.58 mg/g fresh weight was recorded in clone PE-14 (infested) and followed by in infested clone C-413 (10.73 mg/g fresh weight). On the other hand, the minimum content i.e. 4.33 mg/g fresh weight of total phenol was recorded in clone C-2135 (healthy).

**Table 4.14: Variation in the total phenol concentration (mg/g) in healthy and infested leaves of clonal *Eucalyptus***

Clone	Healthy	Infested	Percentage increase over healthy
C-2135	4.33	4.75	9.70
F-316	5.19	5.92	14.07
C-413	8.59	10.73	24.91
PE-6	6.39	7.87	23.16
PE-14	10.34	12.58	21.66
PE-5	4.85	6.29	29.69
PE-11	5.96	10.09	69.30
PE-13	6.55	7.17	9.47
PE-8	8.57	9.52	11.09
C-407	5.24	6.37	21.56
PE-12	5.57	6.56	17.77
PE-9	5.01	6.14	22.55
Mean±S.E.	6.38±0.38	7.83±0.48	
LSD 5%	1.10	1.22	

Similar kind of finding given by Chhabra (2019) reports the level of phenol was increased in non-aromatic rice varieties after the post infection of Bakanae disease in rice *oryza sativa*. In Jharkhand, Mukherjee *et al* (2016) reported the level of total phenol was increases with the increase in the gall severity on leaves of *Terminalia tomentosa* and

*Terminalia arjuna*. Similarly in Gujarat, Singh (2012) reports the increase of total phenol content in gall infested leaves of highly susceptible entries of *Eucalyptus*. However Li *et al* (2017) reports gradual decrease in the phenolic content after the gall formation.

#### 4.4 Evaluation of *Eucalyptus* clones based on growth, quality, flowering and gall wasp incidence

Classification of superior trees on the basis of index scores analysis is presented in Table 4.15. Index scores ranged from 12 to 32 among the clones. Highest index score of 32 was observed in clone C-413 and PE-11 which indicated its overall superiority in growth, quality as well as tolerance to gall wasp attack. These were followed by clone C-72. Minimum index score of 12 was found in clone PE-12 which was followed by PE-14.

**Table 4.15: Index scores for different characters recorded in 14 clones of *Eucalyptus* spp.**

Clone	Tree height	DBH	Volume	No. of branches	Clear bole height	Straightness score	flowering	Gall wasp incidence	Total
C-2135	4	3	6	2	2	3	2	4	26
F-316	3	4	6	2	1	1	1	1	19
C-413	4	4	8	4	4	3	1	4	32
PE-6	4	3	6	3	1	2	2	3	24
C-72	4	4	6	3	4	2	0	4	27
PE-14	1	1	2	4	1	1	0	3	13
PE-7	2	2	4	4	3	2	3	1	21
PE-5	3	4	6	1	1	2	2	2	21
PE-11	4	4	8	4	4	4	1	3	32
PE-13	1	2	2	3	1	1	1	4	15
PE-8	2	2	4	4	3	3	3	3	24
C-407	4	4	8	3	3	2	1	1	26
PE-12	1	1	2	1	1	2	0	4	12
PE-9	1	2	2	4	3	2	3	3	20

## CHAPTER V

### SUMMARY

*Eucalyptus* is one of the most widely planted hardwood genus and belongs to family Myrtaceae. There are more than 800 species of *Eucalyptus* belongs to two genera *Eucalyptus* & *Corymbia*.

In India, *Eucalyptus* was first planted around 1790 at Nandi hills near Banglore by Tippu Sultan and commercial plantations of *Eucalyptus tereticornis* (Mysore gum) started from 1952. The clonal plantation culture in India was initiated from 1992. Gall forming insect gall wasp *Leptocybe invasa* caused heavy damage to *Eucalyptus* plantations throughout the world. In Punjab, the gall wasp was noticed first time in 2009. The pest causes severe injury to the young plantations by producing galls on the leaf midrib, petiole and young turgid shoots of seedlings, sapling and coppiced shoots.

With the adoption of clonal *Eucalyptus*, the farmers in Punjab and adjoining states shifted to the monoculture of few fast growing clones. Incidentally these clones have negligible flowering and Punjab State Horticulture Department had given feedback that trees of clonal *Eucalyptus* is adversely affecting apiculture in the state. Keeping in view the concerns of beekeepers and the threat of large scale spread of gall wasp, the present study is planned to study variation for tolerance to gall wasp and to study flowering behaviour in the existing germplasm in hand. Therefore the current study entitled “Studying variation among *Eucalyptus* clones for growth traits, flowering and tolerance to gall wasp *Leptocybe invasa*”

- i. To study the variation among *Eucalyptus* clones for growth and flowering behaviour to explore potential for Apiculture.
- ii. To study variation among *Eucalyptus* clones for tolerance/resistance to gall wasp.

To meet the above objectives, the present study was conducted at Main Experimental Area, Department of Forestry and Natural Resources, Punjab Agricultural University, Ludhiana during 2018-19. Five commercial and nine clones developed by PAU, Ludhiana from the existing germplasm were used in the study. The whole study was categorized into three experiments.

- The first experiment was studying variation in the growth traits and flowering behaviour of *Eucalyptus* clones. Various growth traits (Tree height, DBH and volume), stem traits (straightness and clear bole height) and branch traits (number of branches, branch diameter and branch angle). The maximum mean total height was recorded in the clone PE-11 (16.54 m) which was found statistically at par with which was found statistically at par with five clones viz C-407 (16.53 m), C-413 (16.51 m), C-2135 (15.99 m), PE-6

(15.83 m) and C-72 (15.49 m). The maximum value of Diameter at Breast Height and volume index was found in clone C-413. The maximum branch diameter was noticed in clone F-316. The clear bole height was found maximum in clone PE-11 (12.56 m).

- Floral buds appeared from the month of June and continued up to July. Profused flowering recorded on three clones i.e. PE-7, PE-8 and PE-9. On the other hand, no floral buds were noticed on C-72, PE-12 and PE-14. The diameter of gumnut was found maximum in clone PE-5. Both the maximum gumnut length and peduncle length was observed in clone.
- The diversity of insect visitors was observed on fully bloomed flowers of three clonal *Eucalyptus* i.e. PE-7, PE-8 and PE-9. All the four species of honey bee i.e. *Apis mellifera*, *A. dorsata*, *A. indica* (Indian honey bee) and *A. florea* (small honey bee) which are naturally present in the Punjab conditions were observed to visit on clonal *Eucalyptus*. The honey bee visitation was recorded maximum in the afternoon session and higher visitation was recorded on clone PE-9.
- The second experiment was screening of clonal *Eucalyptus* against *Leptocybe invasa* (gall wasp). It was found that the maximum percentage of leaf gall infestation was recorded on clone F-316 in all the twelve months of year and clone C-413, PE-14, PE-11 and C-72 were recorded as less infested. Clone C-413 was found least infested which was statistically at par with the PE-11, PE-14 and C-72. Gall incidence was recorded highest in the month of April and starts to decrease from May to December and gradually increased January to March and reaches at peak in the month of April.
- The third experiment was to study the biochemical characters of clonal *Eucalyptus* in relation to the incidence of gall wasp (*Leptocybe invasa*). It was found that the content of total soluble protein was lesser in the leaves after the gall formation whereas the content of total soluble sugar and total phenol was higher in the gall infested leaves. Highest content of total soluble sugar was found in highly gall infested clone F-316 and phenol was found in PE-14. The lowest content of total soluble protein was recorded in the clone F-316.

## **Conclusion**

On the basis of overall evaluation of *Eucalyptus* clones based on growth, quality, flowering and tolerance to gall wasp incidence, clone C-413 and PE-11 were found with higher score of 32 and followed by clone C-72.

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## VITA

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