# Floral biology and reproductive effort of *Aesculus indica* (Wall. ex Camb.) Hook: An andromonoecious species in temperate region of Garhwal Himalaya, India.

## Thesis

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# CERTIFICATE

This is to certify that the thesis entitled **"Floral biology and reproductive effort of *Aesculus indica* (Wall. ex Camb.) Hook: An andromonoecious species in temperate region of Garhwal Himalaya, India"** submitted in partial fulfilment of the requirements for the degree of **Master of Science in Forestry** with major in **Tree Improvement** of the College of Post-Graduate Studies, VCSG Uttarakhand University of Horticulture & Forestry, College of Forestry, Ranichauri, is a record of ***bonafide*** research carried out by **Priya Pradhan, Id.No:UUHF/15341,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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

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We, the undersigned, members of the Advisory Committee of **Priya Pradhan, Id.No: UUHF/15341,** a candidate for the degree of **Master of Science in Forestry** with major in **Tree Improvement**, agree that the thesis entitled **"Floral biology and reproductive effort of *Aesculus indica* (Wall. ex Camb.) Hook: An andromonoecious species in temperate region of Garhwal Himalaya, India"** may be submitted in partial fulfilment of the requirements for the degree.

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## GENERAL ABBREVIATIONS

| | |
|---|---|
| % | Percent |
| $^{o}C$ | Degree Celsius |
| ANOVA | Analysis of Variance |
| cm | Centimeter |
| CV | Coefficient of Variation |
| df | Degree of freedom |
| Dev. Style | Developed style |
| *et al.* | *et alia* |
| etc. | Etcetera |
| Fig. | Figure |
| g | Gram |
| Hrs. | Hours |
| *i.e.* | That is |
| LC | Lower Canopy |
| m | meter |
| Max. | Maximum |
| Min. | Minimum |
| MC | Middle Canopy |
| mm | millimeter |
| RH | Relative Humidity |
| SD | Standard deviation |
| Sec. | Second |
| UDev. Style | Underdeveloped Style |
| UC | Upper Canopy |
| Wt. | Weight |

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# CHAPTER - 1
# INTRODUCTION

# 1. INTRODUCTION

The genus *Aesculus* was classified by Linnaeus in 1737 in the order Sapindales. This genus, of the family Hippocastanaceae, comprises of 13-19 species, numerous botanic varieties, cultivars and natural hybrids spread across the Northern hemisphere, mainly in temperate climates (Hardin, 1957; 1960). According to Hardin's scheme (1957; 1960), the species are organized into 5 sections: Aesculus, Calothyrsus, Macrothyrsus, Parryana and Pavia. Species were grouped into these sections primarily based upon variability in bud, flower and fruit morphology. The current distribution of *Aesculus* is spread across the Northern hemisphere with seven species found in North America, one species in Central Europe and at least five and potentially up to thirteen species in China and spread across Asia (Fang, 1981). The inflorescences of all *Aesculus* are erect terminal panicles. They vary slightly in shape from pyramidal to cylindrical, and in flower density from tightly compressed to free (Bertin, 1980).

*Aesculus indica* (Indian horse chestnut) is a large, rounded tree and falls under the section Calothyrsus, which is common along the Himalayan lowlands in the elevations of 1000-3000m, in the forests and shady valleys across Northern Afganistan, Pakistan, Kashmir, North India and Nepal (Troup, 1921). It is found in Wet temperate forests and shady ravines, Zone 7-9 designations on the USDA Hardiness Zone Map (Gilman and Watson, 1993), [-10 to 35$^0$C], it prefers moist well-drained soil. It grows in either full sun or light partial shade. The range of *A. indica* is limited by its sensitivity to temperatures in excess of 35$^0$C and its need for a steady supply of moisture. This makes *A. indica* the most climatically sensitive species of all *Aesculus*. It has the widest acceptable pH range of 6.1 to 7.5 (Sternberg and Wilson, 1995). The height of the tree ranges from 10-30m. The leaves are palmately compound, composed of seven leaflets which are obovate in shape. It produces long erect panicles of white flowers.

The tree has higher food value as leaves are used as cattle fodder in parts of Northern India. Its seeds are dried and ground into bitter flour, called Tattawakher. The bitterness is caused by saponins, which are rinsed out by thoroughly washing the flour during its preparation. The flour is often mixed with wheat flour to make chapatis and also to make a halwa (porridge) and sometimes is served as a dalia (a type of porridge or gruel) during fasting periods (Rajasekaran and Singh, 2009). It is used in traditional Indian medicine, for the treatment of some skin diseases, narcotic, acrid and in the relief

of headaches. The seeds are rich in saponin and also contain oil, which is used in rheumatism and also applied to wounds. The seeds contain aescin that has been used in dermatology for the treatment of venous stasis and its most serious complication, cellulitis (Cristoni and diPierro, 1998), where it increased capillary blood flow and reduced edema. The bark of *A. indica* has been used as a tonic because of its astringent properties (Hooker, 1859). The wood is used to make utensils and pots and is also used for making water-troughs, packing-cases, tea boxes and decoration articles (Rajasekaran and Singh, 2009). It is also recommended for buffer strips around parking lots or for median strip plantings in the highway and shade tree. These trees are commonly planted in parks, arboreta, college campuses, and home landscapes (Dirr, 1990).

The genus *Aesculus* has an unusual reproductive strategy including a large portion of functionally staminate flowers, and a high microspore to mature seed ratio. Hardin (1956) concluded that the term andromonecious (having perfect and staminate flowers on the same plant) best characterized the flowering biology of the genus since both functionally staminate flowers and at least a few complete flowers could be found within individual plants. The functionally staminate flowers have a vestigial gynoecium with an undeveloped ovary and stunted style. Complete flowers have fully developed androecium and gynoecium (Hardin, 1956). Pistillate inflorescences were found infrequently in *A. hippocastanum* and in *A. pavia*, where two plants out of many hundreds had only functionally female flowers (Bertin, 1980). Proportions of female-fertile flowers are as low as 0.1% in *Aesculus* (Sedgley and Griffin, 1989). Uneven distribution of complete flower in some species of *Aesculus* has been reported by authors Bertin, 1980, Suo *et al*., 1995, Benseler, 1968, Coker and Totten, 1937. Suo *et al*. (1995), using *A. turbinata*, reported that panicles from the upper crown (presumably receiving optimum photosynthetically active radiation) were longer and had a greater number of flowers per panicle and a higher sex ratio than those from lower portions of the crown. Reproductive biology is important to combine phenology, pollinator's activity and incompatibility systems as a whole approach in studying reproductive ecology (Burques *et al*., 1987). Floral biology or reproductive biology is vital for the survival and perpetuation of the plant species and yet, the information on the basic aspects of reproduction is scarce in wild tree species especially the non-economic species (Chan *et al.*, 2011). The knowledge of reproductive biology is essential for the effective protection of endangered plants, especially for species with small populations

(Spira, 2001; Evans *et al*., 2004; Xiao and Xu, 2006) and to improve desired traits or to develop new varieties. A closer understanding of reproductive biology is absolutely essential for developing management strategies (Sohrabi *et al*., 2014).

To understand floral biology it is a necessity to know about anthesis. It is an active process based on the physiological and ecological behavior of a plant. The flowering time of each species is genetically fixed, which is highly variable with the environmental factors, mainly precipitation and temperature. Climatic conditions partly influence the time of anthesis, anther dehiscence and density of flower that can ultimately affect the quantity of pollen produced by an individual (Stanley and Linskens, 1974). The pollen production of an individual tree is the function of the number of pollen grains per anther, number of anthers per flower, number of flower per inflorescence, number of inflorescence per tree and size of the individual. In *A. indica* Khanduri and Sharma (2007) had noted, different range of pollen grains per anther in two consecutive years. The number of inflorescences, flowers, anthers and pollen grains per anther varied considerably from individual to individual and one year to another (Khanduri and Sharma, 2007). The estimation of total pollen production per plant is useful not only from an aerobiological but also from an agronomical standpoint, as the production of seeds often depends on the production of pollen (Campbell and Halama, 1993). This study has also focused on providing some information on variability of pollen production in *A. indica.*

Pollen fertility is an important aspect that can help in determining the successful adaptation of a plant species (Qureshi *et al.*, 2002). In any breeding programme, pollen germination studies are essential for estimation of the quantity of pollen required for controlled pollination studies (Chauhan and Katiyar, 1996) and artificial pollen grain germination is the surest test of pollen fertility. In *A. chinensis*, the highest percentage of pollen germination and greatest pollen tube length was observed on medium containing 20% sucrose over both fresh and stored pollen samples (Ostrolucka and Bencat, 1987). In *A. turbinata*, where the optimal temperature for fresh pollen germination and pollen tube growth was 20°C maximum pollen tube length at 8 hrs after plating was observed (Suo *et al*., 1995).

It is evident that fruit production in *Aesculus* is very low when compared to the abundance of flowers commonly produced. Low fruit and flower ratios are typical of many hermaphroditic plant species (Sutherland, 1986). It is common for xenogamous,

hermaphroditic flowering plant to produce more flowers and ovules than fruits and seeds (Bawa and Weeb, 1984). In *A. parviflora* low seed-set is reported compared to flowers. The final number of seeds within a fruit is the result of a sequential process that starts with an initial number of ovules in the ovary and ends with the successful development of fertilized ovules. In plants in which predation is not a critical factor for seed production, the upper limit for seed number per fruit is set by the number of ovules in the ovary, and the final seed: ovule ratio is mainly determined by fertilization success and seed abortion. Seed abortion, which occurs at different developmental stages within the fruit, has been shown to be non-random and based on the genotype of the embryo (Korbecka *et al.*, 2002); however, lower seed: ovule ratios can also result from the abortion of unfertilized ovules within the fruit (hereafter, ovule abortion). Once fertilization has occurred, fruit and seeds start to mature. However, not all will mature. There are several factors responsible for the selective abortion of ovules and seeds at different stages of development (Charleswhorth, 1989). The seed/ ovule and the fruit/ flower ratios are the main parameters for evaluating species fecundity and can be used to measure the degree of reproductive efficiency of a population (Cruden, 1972). Flower, fruit and seed predation is also a highly significant limiting factor for reproductive success and has a direct influence on population recruitment (Cardoso and Lomônaco, 2003). Reproductive success is when an individual is more successful at surviving and reproducing respective to other individuals of the species (Bergstrom and Dugatkin, 2012). Reproductive success in plants results from a number of processes. Fruit production (the number of seeds that the tree produces initially) is the starting point for measuring reproductive potential. Fruit production in individuals can be affected by factors such as the amount of available resources (e.g., light, water, and nutrients) and competition by the surrounding neighborhood of plants for those resources (Lee and Bazzaz, 1982; Graham *et al.*, 2003). Fruit development depends strongly on photosynthesis and light availability (Brady, 1987; Raven *et al*., 1992; Taiz and Zeiger, 1998). Leaves in the upper part of trees shade those below, one would expect a vertical stratification of light within the tree. Consequently, upper leaves are expected to produce more photosynthates than lower, shaded leaves. Zones of the canopy that receive more sunlight are expected to distribute more photosynthates locally, from the leaf to the adjacent fruit: local translocation of resources hypothesis (Lynch and Gonzalez, 1993; Proietti *et al.*, 2000; Raven *et al*., 1992; Taiz and Zeiger, 1998).

However, the major causes of low fruit production are uncertain, in part because many aspects of the reproductive biology of *Aesculus* are unknown or incomplete. The purpose of the present study is to supply some of this missing information by reporting on *Aesculus* flower structure and development during the prefertilization period, concentrating on the time of receptivity, development of the stigma, insect visitations, pollen-ovule ratio. Such studies may help to identify the factors affecting the reproduction of individual plants and the subsequent maintenance or regeneration of populations (Xiaohong *et al.*, 2013). Yet, limited studies have been performed to explore the reproductive effort of genus *Aesculus*, hence this will be the first communication report regarding floral biology and reproductive effort of the species *A. indica*. Taking into the consideration of all above facts in mind, this study has been designed to understand the floral biology and reproductive effort in depth. These studies are of great value for theoretical model building as well as to formulating effective conservations measures, which is widely applicable in many fields of tree breeding, silviculture, and plant growth rate assessment.

The specific objectives of the study were to:

1) Examination of floral developmental stages and floral architecture along with anthesis and stigma receptivity in relation to the time of the day and associated weather conditions.
2) Estimation of variability in pollen production, pollen viability, and pollen germinability.
3) Determination of reproductive success and reproductive potential in terms of fruit and seed set percentage in different crown layers.
4) Documentation of major pollen vectors of *Aesculus indica*.

# CHAPTER - 2
# REVIEW OF LITERATURE

# 2. REVIEW OF LITERATURE

The reproductive biology of a species involves more than just knowing the reproductive cycle. Each species has a reproductive potential (RP) determined by the number of flowers per tree and seeds per fruit that can be produced at a particular age and size of the tree. The tree also has a reproductive success (RS) determined by the proportion of flowers and seeds that survive to maturity. Floral biology or reproductive biology is vitally necessary for the survival and perpetuation of the plant species it is also important for the effective protection of endangered plants, especially for species with small populations and to improve desired traits or to develop new varieties. A closer understanding of reproductive biology is absolutely essential for developing management strategies. A critical review of existing literature on reproductive biology, floral morphology, structure and development, anthesis, pollen production, pollen ovule ratio, stigma receptivity, pollen viability, breeding system, pollen germination, and reproductive success in different crown layers of species *Aesculus indica* has been summarized given below.

## 2.1 *Reproductive biology*

*Aesculus spp.* has an unusual reproductive strategy including a large portion of functionally staminate flowers, and a high microspore to mature seed ratio. Hardin (1956) concluded that the term andromonecious best characterized the flowering biology of the genus since both functionally staminate flowers and at least a few complete flowers could be found within individual plants. Bertin (1980) also claimed that the distribution of complete flowers was uneven. *A. parviflora* and *A. pavia* had sex ratios of approximately 5.5%, and similar were reported for other *Aesculus* species [*A. turbinata*, 1.3 to 9.3% (Suo *et al*., 1995); *A. pavia*, 1.1% (Bertin, 1980); *A. californica*, <5% (Benseler, 1968); and *A.* sylvatica, 7.0% (Coker and Totten, 1937)]. Suo *et al*. (1995), in *Aesculus turbinata*, reported that panicles from the upper crown (presumably receiving optimum photosynthetically active radiation) were longer and had a greater number of flowers per panicle and a higher sex ratio than those from lower portions of the crown.

Floral biology study has helped in effective breeding programmes that could be undertaken in future for better *in-situ* and *ex-situ* conservation and cultivation for commercial purposes of the species (Nautiyal *et al.*, 2009) and examining floral

phenology, including phases of micro and mega gametophyte development and viability, could help to identify pollen-related obstacles that prevent higher success in setting fruit (Simpson, 2010). As such, to conserve and utilize forest genetic resources a knowledge of ecology, reproductive biology and factors influencing pollination, seed set and seed sterility of indigenous species are necessary (Bangarwa, 1993). The knowledge of the reproductive biology of flowering plants is important for determining barriers to seed and fruit set, for conservation, and for understanding pollinations. Pollination, pollen germination, and stigma receptivity must be analyzed critically on a species by species basis, as it reflects the basic criteria for breeding programmes (Bhattacharya and Mandal, 2004).

Floral biology or reproductive biology is vital for the survival and perpetuation of the plant species. The study of floral biology allows us to understand the viability of a population, and its gene flow which is important in the conservation of wild species, as they are usually very restricted and habitat specific (Lee *et al.*, 2006). The information on pollination biology is not only required for the comprehensive understanding of the efficiency of the breeding system of a species and its evolutionary success but also for effective optimization of yield, conservation and rational genetic improvement (Shivanna and Mohan, 1993).

### 2.2 *Floral morphology, structure, and development*

The morphology of flowers plays a significant role in assessing relationships and in arriving at taxonomic conclusions. The difference in number, shape and position of floral parts, color pattern and various phonological traits are all features that are important in floral morphology (Pandey *et al.*, 2006). The shape and size of the flower can influence reproductive output by affecting the behavior of pollinator and rates of visitation (Galen and Newport, 1987).

Bertin (1980) reported that the distribution of complete flowers was uneven in *Aesculus spp.* More complete flowers were clustered toward the central axis of the rachis and on the outward facing side of the panicle rather than toward the trunk of the tree. He suggested that the complete flowers in this position would be more likely to receive pollen from another plant based on the feeding habits of bumble bees. Also, large and heavy fruits would receive greater support in a basal position on the inflorescence. Differences in gynoecium development were apparent only in buds

that were a week or less away from blooming (Benseler, 1968; Bertin, 1980). Coker and Totten (1937) reported mean panicle lengths of *A. pavia* is 12-15 cm. Structural, physiological and cytochemical features of the stigma are of prime importance in the biology of sexual reproduction and seed formation (Bawa, 1976).

### 2.3 *Anthesis*

The anthesis is an active process based on the physiological and ecological behavior of a plant. The work on anthesis was first done by De Cugnac and Obaton in 1935. Knowledge of anthesis and pollen production is essential to the study of pollination, developing a functional model for forecasting pollen concentrations and to understand more about the ecological background of pollen dispersal.

In the current scenario of global warming, flowering time is considered as the best way for judging the climatic changes change at local and global scale (Khanduri *et al.*, 2003). The flowering time of each species is genetically fixed, which is highly variable with the environmental factors, mainly precipitation and temperature. Climate change has affected many aspects of the biology of trees and its effect on plant's first flowering dates would be of great significance (Khanduri *et al.*, 2008). Therefore, reproductive phonological analysis of trees in terms of anthesis and anther dehiscence provides a potential tool to unravel critical questions related to monitoring and modeling of climate change.

### 2.4 *Pollen production*

Pollen is produced by plant stamen, the male reproductive organ of flower, and is very important for the sexual reproduction of flowering plants (Hu, 2005). There are many factors affecting pollen quantity per anther. Pollen quantity in the anther is influenced by the size of pollen sacs, the number of microspores mother cells, climate and nutrient conditions, and level of cultivation and management (Ren *et al.*, 2007). Liu *et al*. (2003) and Tan *et al*. (2009) stated that pollen quantity in anthers also depends on whether anthers develop normally. In *A. indica* Khanduri and Sharma (2007) had noted, the production of pollen grains per anther ranged from 3485.30 ± 89.78 to 3802.00 ± 79.56 in 1998 and 3342.28 ± 102.13 to 3904.79 ± 98.73 in 1999. The number of inflorescences, flowers, anthers and pollen grains per anther varied considerably from individual to individual and one year to another (Khanduri and Sharma, 2007). Plants total pollen production also varies from lower altitude to upper

altitude and from one year to the next year (Khanduri and Sharma, 2002). The initial condition generally favoring pollen grains production are warmth, dryness and sunshine during formation of flower primordia on the previous year, favorable precipitation during the vegetative growing season, lack of winter killing and sunshine prior to pollination. The environmental factors influencing the pollen production of a variety of deciduous trees and spruce and pine were reported by Scamoni, 1955. Pollen production per anther for several angiosperm species and contended that the levels of pollen production in a particular species is a function of its genotype and the amount of pollen grains per anther might be genetically fixed (Joppa *et al.*,1968).

The estimation of total pollen production per plant is useful not only from an aerobiological but also from an agronomical stand point, as the production of seeds often depends on the production of pollen (Campbell and Halama, 1993). Pollen quantity is correlated with fruit production for two reasons: (i) a higher quantity of pollen can favor enhanced fertilization and competition among male gametophytes; (ii) the higher number of pollen grains demonstrates a higher quantity of total inflorescences (Reale *et al.*, 2006). The amount of pollen produced by a flower reflects the probability that a sufficient number of pollen grains will reach the stigma.

### 2.5 *Pollen ovule ratio*

The pollen–ovule ratio is an important floral trait that reflects the mating system of a plant (Cruden, 1977; Preston, 1986; Jürgens *et al*., 2002). Self-incompatible species have significantly higher pollen-ovule ratios than self-compatible species, resulting both from fewer pollen grains and more ovules of the latter (Mione and Anderson, 1992). Cruden (1977) was the first to observe that outbreeding plants tend to have higher pollen–ovule ratios. Cruden (1977) wrote that "P/O's reflect the likelihood of sufficient pollen grains reaching each stigma to result in maximum seed set. The more efficient the transfer of pollen, the lower the P/O should be." The pollen-ovule ratio of the dioecious species is among the highest of all, resulting only from fewer ovules.

Mione and Anderson (1992) pointed out that the positive relationship between seed size and the pollen–ovule ratio could be a spurious correlation. Given negative correlations between seed size and ovule number and between the pollen–ovule ratio

and ovule number, an increase in the pollen–ovule ratio with increasing seed size must follow. That plants with large seeds tend to produce few seeds and plants with light seeds produce many seeds are known as the 'seed size/seed number trade-off' (Shipley and Dion, 1992; Jakobsson and Eriksson, 2000). Several studies (cited in Cruden, 1977) show that xenogamous species produce more pollen grains, without systematic variation of ovule quantity than related SC and/or autogamous species (*i.e.*, the lower P/Os of the SC and/or autogamous species resulted from lower pollen quantity).

Cruden (2000) created a model to examine and describe the interrelation of floral traits of animal-pollinated plants, including pollen number, pollen size, P/O ratio, stigma area and depth, stigma receptivity, and the pollen-bearing area of the pollinator. Cruden (2000) stated that the P/ Os of wind-pollinated plants are higher than those of animal-pollinated plants, and there is little difference in the pollination efficiency of the various animal vectors. Cruden (2000) also found that the P/Os of plants that provide only pollen as a reward to animal pollinators were higher than those that provide nectar as a reward to animal pollinators. On the basis of the pollen-ovule ratio of some flowering plants, Cruden (1977) divided the breeding systems into five classes: 1) Xenogamy with P/O's 5859.20 $\pm$ 936.5, 2) Facultative xenogamy with P/O's 796.60 $\pm$ 87.7, 3) Facultative autogamy with P/O's 168.80 $\pm$ 22.1, 4) Obligate autogamy with P/O's 27.70 $\pm$ 3.1 and 5) Cleistogamy with P/O's 4.70 $\pm$ 0.7.

### 2.6 *Stigma receptivity*

For successful fertilization, it is desirable that the pollen is transferred to the receptive stigma of another flower. In many cases, fertilization can occur when the pollen grain is deposited before the receptive period as long as it remains viable long enough to be able to germinate as soon as the flower becomes receptive (Thomson and Barret, 1981). The stigmatic secretions reportedly contain lipids, amino acids and perhaps sugars (Baker, 1973) and are thought to prevent drying of the stigmatic surface and to provide a suitable medium for pollen germination (Baker *et al.*, 1973). It has been suggested that stigma and transmitting tissue secretions have many functions, such as attraction and nourishment for floral visitors (Lord and Webster, 1979), pollen adhesion to pollinators (Kandasamy and Kristen, 1987).

The positive correlation between stigmatic morphological traits and higher stigma receptivity has been reported in many plants, such as straight style, enlarged stigma and turgid stigmatic papillae in teak (Tangmitcharoen and Owens, 1997), widely-opened stigmatic grooves, taller stigmatic papillae and abundant secretion in silky oak (Kalinganire *et al*., 2000), and elongation of stigmatic papillae and increased amounts of stigmatic exudate in almond (Yi *et al*., 2006). These reports indicated that we could primarily judge the stigma receptivity by the stigmatic morphological traits, which would provide some reference for the judgment of the effective pollination period (EPP) (Williams, 1965; Galen and Plowright, 1987).

### 2.7 *Pollen viability*

Pollen viability and the efficiency of pollen transfer partially determine the reproductive success of a species (Abdelgadir, *et al.*, 2012). Pollen viability exerts a direct control on pollen stigma interactions (Heslop-Harrison *et al*., 1984; Heslop-Harrison and Heslop-Harrison, 1992), fruit set and gene flow (Dafni and Firmage, 2000) apart from programmes of practical plant breeding and crop improvement (Stanley and Linskens, 1974; Shivanna and Rangaswamy, 1992; Seyrek *et al*., 2016). Pollen viability and longevity have been reported to control successful cross-pollination in Hordeum genotypes (Parzies *et al*., 2005).

Pollen fertility is an important aspect that can help in determining the successful adaptation of a plant species. It is necessary to identify the factors influencing pollen germination and elongation of the tube (Char *et al.*, 1973; Quereshi *et al.*, 2002). Pollen germination studies are essential for the estimation of the quantity of the pollen required for controlled pollination. Artificial germination of the pollen grains is the surest test of pollen fertility (Nautiyal *et al.*, 2009; Khurana and Khosla, 1979; Chauhan and Katiyar, 1996). There is generally a time lag between pollen shedding and its transfer to the stigma. During this period pollen grains are exposed to a wide range of environmental stresses, particularly of temperature and humidity, which affect their competitive ability to sire vigorous progeny (Raina *et al.*, 2003). Any success in breeding experiments or artificial pollination procedures should be accompanied by tests on pollen viability and timing and duration of the stigma receptivity (Lavithis and Bhalla, 1995; Stone *et al*., 1995; Nautiyal *et al*., 2009).

## 2.8 *Breeding system*

Breeding system is the mode of transmission of gene from one generation to the next through sexual reproduction. Breeding system is grouped into two broad categories: inbreeding and outbreeding (Dwivedi *et al.*, 2016). The former is strongly promoted by autogamy or habitual self pollination whereas later refers to cross pollination. An incompatibility system promotes outbreeding. The breeding system determines the spread of genetic variability in the population (Sindhu and Padmanabha, 1996). The knowledge of the breeding system is also fundamental for correctly interpreting the pattern of genetic variations within and among populations (Nybom, 2004; Glemin *et al.*, 2006). The effective pollen transfer to the stigma and control of its germination and growth are most important for the reproductive success of each species (Dwivedi *et al.*, 2016).

## 2.9 *Pollen germination*

Germination is the first most critical morphological event in the pollen to fulfill its ultimate purpose of discharging the male gametes in the Embryo sac. It is significant to understand the physiology and biochemistry of pollen germination. Our knowledge of the physiology and biochemistry of pollen germination and the tube growth comes largely from *in-vitro* studies (Patel and Mankad, 2015). In many species, *in-vitro* pollen germination is dependent on the addition of key substrates such as calcium nitrate to the germination media (Steer and Steer, 1989).

As a major component of pollen tube reserves carbohydrates has a vital role to play in its germination and growth. Sucrose is the best carbohydrate source for pollen germination and tube growth for most of the systems investigated (Patel and Mankad, 2015). Pollen grains are rich in proteins, carbohydrates, minerals and vitamins (especially B-complex with low values of fats, sodium and fat-soluble vitamins like D, K, and E). A nutrient medium (in vitro) is supplemented with some essential compounds necessary for pollen germination and tube growth. Sucrose is the carbohydrate source for pollen germination and tube growth (Tupy, 1960). Sugar in the culture medium serves functions like (a) maintaining osmotic pressure of the medium and (b) as a substrate for metabolism of pollen. 15% sucrose in water chestnut (Hoque and Arima, 2000), 12% sucrose in the media is good for *Brassica oleracea* (Ferrari and Wallace, 1975) was found good.

Pollen tube growth can affect the outcome of self- versus cross-pollination, as pollen tubes from self-pollen may grow slower or have higher rates of attrition than those from cross pollen (Aizen *et al*., 1990). In *Aesculus chinensis*, the highest percentage of pollen germination and greatest pollen tube length was observed on medium containing 20% sucrose over both fresh and stored pollen samples (Ostrolucka and Bencat, 1987). In *A. turbinata*, where the optimal temperature for fresh pollen germination and pollen tube growth was $20^0$ C maximum pollen tube length at 8 hrs after plating was observed (Suo *et al*., 1995).

**2.10 *Reproductive success in different crown layer***

The reproductive success of a species is directly linked to its population dynamics since species stability in a given environment is dependent mainly on the amount and on the quality of their offspring, which allows them to be stable in their environment (Wiens, 1984; Wiens *et al.*, 1987). Pollen grains are reduced male gametophytes which, upon pollination, produce pollen tubes that grow through the pistil to effect fertilization and seed set. Most estimates of the reproductive success of hermaphroditic plants are based solely on the haploid contribution through the female function, ovule and seed production (David *et al*., 2012).

Sindu and Padmanabha (1996) stated that good percentage of fruit initiation indicated better success in fertilization rate, but the lower percentage of the mature fruits indicated presence of genotypic barriers to embryo development, hence it can be inferred that self-incompatibility is floating in the population. There are a number of specific mechanisms that can lead to a positive relationship between female reproductive success and floral density. Some observational studies have found positive correlations between floral density and pollinator visits, pollen loads, fruit set and seed set (Feinsinger *et al*., 1986; Klinkhamer and de Jong, 1990; House, 1993; Steven *et al*., 2003). Individuals occurring at low densities may suffer from insufficient pollen quantity if they attract fewer pollinators and/or receive fewer conspecific pollen grains per pollinator visit (Feinsinger *et al.*, 1986; Klinkhamer and de Jong, 1990). They may suffer from insufficient pollen quality if they are surrounded with incompatible individuals, which become more likely as the number of individuals in their neighborhood decreases (Ramsey and Vaughton, 2000). Plants that are dioecious, self-incompatible and heterostylous may be particularly vulnerable to pollen limitation when floral density decreases (House, 1993). It should be noted,

however, that there are also mechanisms that can lead to a negative relationship between female reproductive success and floral density, such as increased competition among plants for resources and pollinators (Fritz and Nilsson, 1994; Larson and Barrett, 1999; Baker *et al.*, 2000). Flower, fruit and seed predation is also a highly significant limiting factor for reproductive success and has a direct influence on population recruitment (Wenny, 2000; Cunnigham, 2000; Silva *et al.*, 2002; Mahoro, 2003; Cardoso and Lomônaco, 2003).

Floral visitors can differ in their ability to transfer pollen; changes in the pollinator assemblages following fragmentation may affect plant reproductive success (Aizen and Feinsinger, 1994b). Seed set per fruit is pollen- limited or pollinator-limited when small amounts of pollen are deposited on stigmas (Bierzychudek, 1981). Shaanker and Ganeshaiah (1990) found that pollen deposition patterns regulate the seed number per fruit in multi-ovulated species.

The impact of pollinators on the mating system of plants (*i.e.* the level of outcrossing) and their reproductive success is dependent, in part, on the level of self-compatibility exhibited by the plant. Indeed levels of self-compatibility can vary dramatically within (Young and Brown, 1998; McIntosh, 2002) and among plant populations (Vaughton, 1989; Medrano *et al*., 1999; Eckert, 2002).

Fruit development depends strongly on photosynthesis and light availability (Brady, 1987; Raven *et al*., 1992; Taiz and Zeiger, 1998). Variation in light availability can affect photosynthetic rate, photosynthate production, dry matter allocation, and various leaf phenotypic traits and physiology including leaf conductance, chloroplast size, and mean stomatal densities (Lynch and Gonzalez, 1993; Mehrotra *et al*., 1998; Proietti *et al.*, 2000; Raven *et al.*, 1992; Sellin and Kupper, 2005; Taiz and Zeiger, 1998). Because leaves in the upper part of trees shade those below, one would expect a vertical stratification of light within the tree. Consequently, upper leaves are expected to produce more photosynthates than lower, shaded leaves. Zones of the canopy that receive more sunlight are expected to distribute more photosynthates locally, from the leaf to the adjacent fruit: local translocation of resources hypothesis (Lynch and Gonzalez, 1993; Proietti *et al.*, 2000; Raven *et al*., 1992; Taiz and Zeiger, 1998). It is possible that the putative intra tree vertical stratification of fruit varies as a function of light availability or other factors (Houle *et al*., 2007).

### 2.11 *Seed abortion*

The final number of seeds within a fruit is the result of a sequential process that starts with an initial number of ovules in the ovary and ends with the successful development of fertilized ovules. In plants in which predation is not a critical factor for seed production, the upper limit for seed number per fruit is set by the number of ovules in the ovary, and the final seed: ovule ratio is mainly determined by fertilization success and seed abortion. Pollen limitation usually constrains the number of ovules that develop into seeds, reducing fertilization success (Calvino, 2014). However, even after fertilization has been achieved, abortion of developing embryos can substantially reduce the number of seeds. Seed abortion, which occurs at different developmental stages within the fruit, has been shown to be non-random and based on the genotype of the embryo (Korbecka *et al.*, 2002); however, lower seed: ovule ratios can also result from the abortion of unfertilized ovules within the fruit (hereafter, ovule abortion). Causes of ovule abortion are diverse and are well known in crop plants that frequently exhibit abortion of older ovules.

Independently of whether seed: ovule ratios are influenced by ovule or seed abortion, differences in seed number per fruit alter the cost of the fruit for the mother plant. Hence, plants invest fewer resources per seed in multi-seeded fruits than in few-seeded fruits (Obeso, 2004). Accordingly, as a strategy to conserve resources, mother plants avoid the development of fruits with few seeds (Obeso, 2004). However, plants can still exhibit positively skewed frequency distributions for seed number per fruit, so that many fruits are usually few-seeded in several species (Shaanker *et al.*, 1988). Because ovule abortion occurs at an earlier developmental stage than does seed abortion (i.e., before fertilization), the former is expected to reduce fruit costs to a greater extent than does the latter, allowing the plant to redirect the conserved resources at an earlier stage. In a seminal paper, Lloyd (1980) indicated that abortion of floral organs “should be made as early as possible to minimize the wastage in unrewarded expenditure”.

# CHAPTER - 3
# MATERIALS AND METHODS

# 3. MATERIALS AND METHODS

The study entitled "Floral biology and reproductive effort of *Aesculus indica* (Wall. ex Camb.) Hook: an andromonoecious species in temperate region of Garhwal Himalaya, India" was carried out in the field area of College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand since April 2016 to November 2016, in order to analyze the mentioned objectives. The experiment details relating to site description, materials used and procedures followed during the course of study are described under following heads:

## 3.1 *Study area*

The study site is maintained by College of Forestry and it lies between $30^0$ 18" North latitude and $78^0$ 24" East longitudes with an altitude of 1600-2192m above sea level (amsl) (Fig. 1). Soil is silty clay loam, brownish black in color coming under the series of orthents (Entisol) and pH of soil ranges between 5.3-5.6. The nearby vegetation comprises of temperate, coniferous forest, and dominated by broad leaved tree species of *Myrica esculenta, Pyrus pashia, Plantanus orientalis, Quercus leucotricoflora.*

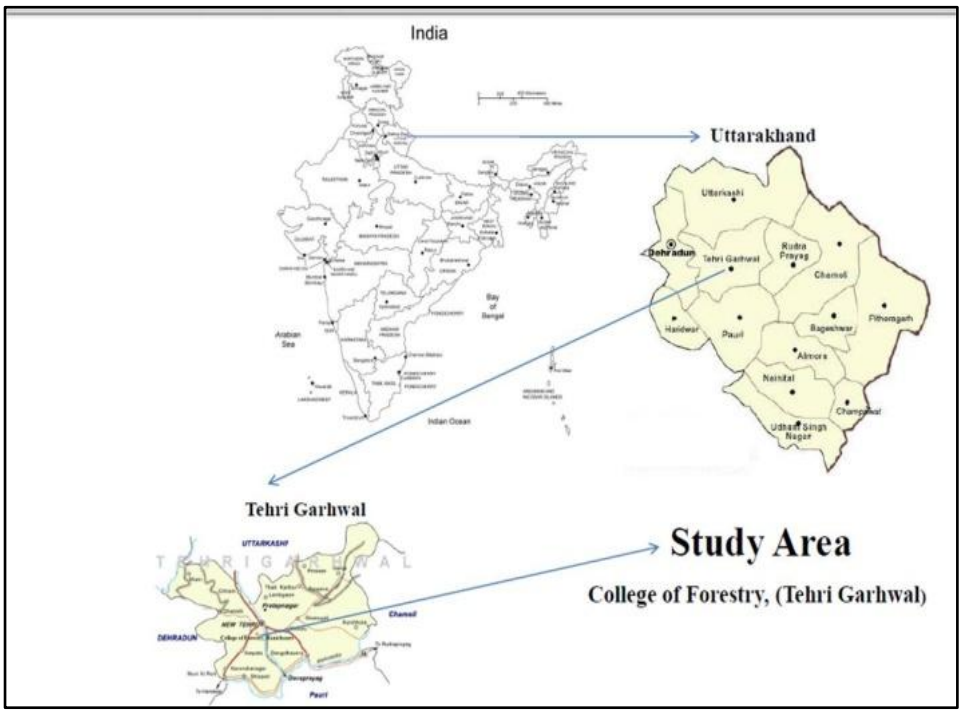



**Fig 1:** Location map of the study area.

### 3.1.1 *Climate*

With the increasing or decreasing altitude the climate of a region varies to a greater extent. The study area typically experiences moist temperate type of climate with chilled winters. The mean monthly maximum and minimum temperature during the study period (March-November, 2016) varies between $7.0^0$C to $29.9^0$C respectively. The highest rainfall was received in the month of July 2016 *i.e.* 415 mm and lowest in November 2016 *i.e.* 0.0 mm.

Three trees of *Aesculus indica* were selected which were situated under varying microclimatic conditions. The trees were chosen on the basis of accessibility, convenience and ensured selected trees were exposed to abundant sunlight. Pollen germination, pollen grain estimation, pollen viability were conducted in laboratory conditions. The investigations designed to find out the following information regarding floral biology and reproductive effort of *Aesculus indica*:

3.2 Floral morphology, structure and development
3.3 Anthesis
3.4 Pollen production
3.5 Pollen ovule ratio
3.6 Stigma receptivity
3.7 Pollen viability
3.8 Pollen germination
3.9 Breeding system
3.10 Flower visitors
3.11 Reproductive Success in different crown layer
3.12 Statistical analysis

## 3.2 *Floral morphology, structure, and development*

The studies pertaining to floral, structure and development started from the beginning of floral bud initiation. Twenty flowers were selected and marked on the three selected sample trees for observation. Flowers were tagged by using tags prior to the initiation of flower buds and observations have been recorded from 18th March till 19th June. Basic stages of flower development were defined by a detailed examination of randomly selected 20 flowers from three sample trees. The developmental stages of flowers were conducted separately for the developed style flowers and under

developed style flowers. Flowers were photographed at various stages of development. The morphological parameters such as type of inflorescence, inflorescence length, number of racemes/ inflorescence, raceme length, number of flowers/ inflorescence, number of flowers with developed style and under developed style / inflorescence, number of petals/ flower, petal length, number of sepals, number of stamens/ flower, stamen length and pistil length of both developed and under developed style were measured and recorded from fifteen randomly selected flower.

Twenty inflorescences each was selected randomly from three sample trees. The number of flowers per inflorescence was determined by counting it manually and the average number of flowers per inflorescence was estimated. The color change on the petals was closely observed and the time duration from anthesis to senescence was recorded. Flowers were observed at a different time within a day to appraise the development of stigma. To elucidate the anther structure and morphological changes of the stigma, more number of sample flowers were collected at 2 days interval and observed through the microscope.

Observations on phenophases were also recorded which includes: leaf initiation and drop; flowering and anthesis and fruit setting and dispersal. Phenological changes were observed every day during the period of a flowering season until fruit maturity and seed dispersal.

### 3.3 *Anthesis*

Ten inflorescences were randomly tagged from five different twigs of three sample trees. The inflorescence in the trees was selected based on the accessibility for taking the frequent observations. Inflorescences were monitored at every two hours (0600h, 0800h, 1000h, 1200h, 1400h and 1600h) interval to determine the anthesis. Daily rates of anthesis were recorded from the tagged inflorescence for successive days. Opened flowers were counted and removed to avoid error due to duplication and over counting. Information regarding the mean temperature and mean humidity of the particular site were collected from Agro-meteorology department of College of Forestry, Ranichauri (Appendix 1 and 1.1). The recorded data of a number of opened flowers/ inflorescence among trees were correlated with temperature, humidity and time to get the influence of these factors in anthesis. The effect of time (0600h, 0800h, 1000h, 1200h, 1400h and 1600h), air temperature, RH of the day and tree variation on

the number of opened flowers were examined using TWO WAY ANOVA, with time as fixed effects- independent variables.

### 3.4 *Pollen production*

The total pollen production in *Aesculus indica* was conducted on buds that were near anthesis, so pollen was mature but anthers had not dehisced. Pollen estimation was done separately for flower with developed style and flower with under developed style. It was done on a series of four steps that is-

i. Estimation of the number of pollen grains within an anther was done by noon loop method (Khanduri, 2012). Using three anthers from different flowers from a tree and was repeated on the rest of the trees. From a solution of 5% glycerol, five drops have been taken and transferred to a test tube. The anthers were obtained from closed flower just prior to anthesis, were laid into the test tube containing glycerol 5% and smacked and pollen grains were suspended in the medium. From this concentrate, a small drop was taken by using micropipette and pollen grains were counted under the microscope. This was repeated on five drops and the number of pollen grains was estimated by using the formula;

   $P = (\sum Tp/N) \times n$

   Where; $\sum Tp$ is the total number of counted pollen grains in the five drops, N is the total number of sample (slide) used for counting and n is the total number of drops.

ii. Estimation of pollen production per flower; the inflorescence was collected from the selected trees and flowers were repeatedly observed to find out the number of anthers on each flower. Production of pollen grains per flower was estimated by multiplying the number of pollen grains per anther by the number of anthers per flower.

iii. Estimation of pollen production per inflorescence has been done by collecting twenty inflorescences from each tree and an average number of flowers per inflorescence were determined by counting manually. Pollen production per inflorescence was obtained by multiplying the average number of flowers in the inflorescence with average number of pollen grains per flower.

iv. Estimation of pollen production within a tree was done by the divisional method. The entire tree was divided into number of sections such as the number of primary branches arising from the main trunk, followed by the count

of the number of secondary branches arising from each primary branch and this procedure repeated up to lateral shots on which the inflorescence has been developing. The probable number of inflorescence per lateral shoot was determined manually and multiplies its value with the total number of lateral shoot per tree, which has given the total number of inflorescence within a tree. Ultimately the estimated total number of inflorescence was multiplied by the average number of pollen grains per inflorescence to get the value of the average pollen productions within a tree. To ameliorate the accuracy the result derived from divisional method was correlated with the total number of inflorescence counted manually within a tree and eventually made a regression equation related to total number of inflorescence production and the number of lateral shoots. The formula derived to calculate the pollen production within *Aesculus indica* is mentioned below;

$$\sum TP = \sum N \times F \times A \times P$$

$\sum TP$: Total pollen grains/ tree

$\sum N$: Number of inflorescences/ tree

F: Average number of flowers/ inflorescence

A: Average number of anthers/ flower

P: Average number of pollen grains/ anther

### 3.5 *Pollen ovule ratio*

Pollen-ovule ratios were determined by dividing the estimate of the number of pollen grains per flower by the number of ovules per flower (Cruden, 1977). The pollen estimation method was followed as mentioned earlier and for estimating the number of ovules per flower dissection method was opted. Cross section of ovary was taken and ovule number was counted. Twenty flowers were used to obtain the ovule number per flower. The ratio was derived from the mean pollen quantity and the mean ovule quantity.

### 3.6 *Stigma receptivity*

The receptive period of stigma was examined for both developed and under developed style flowers. The receptivity period was known through the application of hydrogen peroxide. Stigma receptivity was determined by immersing stigma of flowers in three percentage aqua's hydrogen peroxide; bubbling indicating peroxide

activity and stigma receptivity (Carrington *et al.*, 2003). Flowers from different inflorescence were selected and (immersed) tested regularly at two hours time interval up to thirty-eight hours to determine the average duration of stigma receptivity. Bubbling was observed using 10x hand lens at different stages of flowers. The presence of bubbling was recorded and the procedure repeated in remaining trees to predict the average duration of stigma receptivity.

### 3.7 *Pollen viability*

Pollen viability in *in-vitro* was assessed by using acetocarmine staining methods (Alexander, 1969). Five anthers were selected from developed style flower and five anthers from under developed style flower of five inflorescences from each sample tree. The anthers were placed on a small petridish and smacked with a glass rod to disperse pollen grains. Pollens were dusted on a clean microscopic slide with a hair brush in which a drop of 0.2% acetocarmine solution was added and it was kept for 10-15 minutes to allow the pollen to absorb the stain. The slide was then examined under the microscope. Deeply stained normal looking pollen grains within the microscopic slide were counted as viable pollen, shrivelled and weakly stained was recorded as non-viable. From the data average number of viable and non-viable pollen grains per flower in a tree was calculated and expressed in percentage. The same procedure was repeated on inflorescences collected from the rest of the sample trees. With the help of column chart, the viability was shown in percentage of viability of pollen grains among tree.

### 3.8 *Pollen germination*

Pollen germination test *in vitro* on *Aesculus indica* was done by taking pollen grains separately from the developed style flowers and under developed style flowers and were germinated to optimize media composition. The media used to check the pollen germination were sucrose (10%, 20% and 30%). Pollen was collected from undehised anthers of flowers of different inflorescences. The bulk of pollen was distributed onto germination media (20 ml) each. The germinated pollen grains were observed under the microscope from each solution at an interval of (1h, 2h, 3h, 5h, 8h, and 24h) in order to check the percentage increment in germination with varying time under different solutions. Pollen grains were considered germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra and Wedel,

2000). The percentage increment in pollen germination at different time intervals under different solutions were recorded and tabulated in order to assess the effect of different concentrations of growth regulators on germinations.

### 3.9 *Breeding system*

Assessment of breeding system have done by applying different treatments on randomly selected ten inflorescences with developed style flower each on three different experimental trees, which had already been selected and each treatment was performed on every tree in order to minimize error due to the genetic variations. The following treatments were performed

(1) Open pollination- it was assessed by tagging twenty flowers randomly from each sample trees. This was conducted to check flowers under natural condition; the flowers were left without any intervention and observed for fruit set.

(2) Autogamous self-pollination- it was assessed by bagging twenty buds prior to anthesis in each sample trees, the flowers were left bagged throughout the flowering period and observed for fruit set.

(3) Open cross pollination- it was assessed by removing the anthers from the flowers prior to anthesis in twenty flowers of each sample tree and was observed for fruit set.

(4) Apomixis- it was assessed by removing both the anther and the stigma while in bud stage. It was conducted to examine the fruit formation without fertilization.

### 3.10 *Flower visitors*

Determination of pollinators and their visitation rates was done by counting the visiting insect species on all the selected trees. Several inflorescences were observed simultaneously and the number of insects visiting the open flower was recorded. Insect visiting the inflorescence were observed for their foraging behavior, the number of individuals and duration of visit. Each tree was observed over the course of the whole day from 0600 to 1600h in every 2 hr interval for 8 days in May. The insects were captured using insect trapping net (Sweep net) and polythene bags. Insect specimens were dried and placed into separate rectangular paper and pasted on it by using ball pins. For very small insects' double mounting method was opted. Unidentified insects

were identified with the help of pathology and entomology department of College of Forestry, Ranichauri. The frequency of visiting with relative time was recorded.

### 3.11 *Reproductive Success in different crown layers*

The crown length (vertical distance from tip of the leading shoot to crown point) of the selected sample trees was measured using Haga altimeter. The total crown length was then divided into three equal portions namely upper canopy layer, middle canopy layer and lower canopy layer. The total number of inflorescence in different canopy layer was counted manually and was multiplied by the average number of flowers per inflorescence to get the total number of flowers. Twenty inflorescences each in upper, middle and lower canopy were tagged in each sample tree and were observed for the fruit formation per inflorescence. The fruit formed in the tagged inflorescence were counted. The average fruit set in each canopy layer was calculated by multiplying the number of fruit set per inflorescence by the total number of inflorescence in each canopy layer.

After the fruit formation, the total number of fruit set per inflorescence was recorded manually for different crown layers and correlated it with the total flowers within a tree and then the percentage of fruit set was estimated by dividing the total number of matured fruits with total number of counted flowers within a tree and expressed as percentage. This value was calculated separately for the upper canopy, middle canopy and lower canopy. The same procedure was applied for all the three sample trees. Each fruit consists of only one seed so the number of fruit produced is equal to the number of seeds produced per tree. Various fruit parameters were also recorded, the development of fruits was observed regularly and the final dimensions were recorded after full ripening of the fruits. The length and diameter of the fruits were measured with the help of Vernier caliper. The average weight and size of fruits were calculated on the basis of twenty representative fruits from each of the three trees.

To estimate female reproductive success we calculated total seed production per plant by multiplying the mean number of fruits per plant by the mean number of seeds per fruit (Dafni, 1992).

### 3.12 *Statistical analysis*

Student's t- test (test for difference in mean) were performed for assessing the variations in total flower production and total fruit set on the tree, seeds produced in

different canopy layer and difference in pollen production of flowers with developed and under developed style. The effect of time period (06:00, 08:00, 10:00, 12:00, 14:00 and 16:00), air temperature and RH of the day on the number of flowers opened was examined using TWO- WAY ANOVA, with the time, temperature and RH as fixed effects- independent variables. TWO- WAY ANOVA was used for analyzing the effect of time interval and the sucrose concentrations on pollen germination, time, and concentration of solutions as independent variables and also for analyzing the seed set in different canopy layers between the sample trees.

# CHAPTER - 4

# RESULTS

# 4. RESULTS

**4.1 *Floral morphology, structure and development:*** The *Aesculus indica* tree sex expression is seen as andromonoecious in nature. The inflorescence bear two types of flowers one with flowers having developed style (perfect) and other with under developed style both having androecium (male) (Plate 3), where the flowers with under developed style do not produce fruit. The inflorescence type of *A. indica* is compound raceme (Tyrsus) bearing terminal panicles, broad at the base forming a pyramidal shape. The flowers are pedicellate and are zygomorphic in nature. Each flower has crinkled edge. It is polypetalous (corolla composed of many separate petals) and has four petals clawed which are narrow at the base and broad at the top (unequal in breadth) the place of 5th usually vacant. The sepals are fused (gamosepalous), tubular, tomentose and have five lobes. The flower has seven stamens (occasionally 6, 8 and 9) which are filiform, tomentose, narrow and oblong. It is curved upward and longer than the petals. Pollen dehiscence start after 5-6 hrs of anthesis and it continues for 3-4 hrs in one anther. In one flower there are seven anthers, the pollen dehiscence is not simultaneous, it takes 2 days for all the anthers to complete the dehiscence within the flower. It is clear that the maturation of all anthers within the flower is not at the same time. The ovary is superior, the flower is hypogynous. The flower has one pistil having simple and slender style. One of the unique feature of this species noticed that it bears two types of flowers one with the developed style and other with under developed style (Plate 3). The production of flowers with developed and under developed style within an inflorescence is significant with t= 10.603 at p= 0.05 (df= 18) (Fig. 3-5). It has the sessile ovary with three cells having two ovules in each cell total counting to six. Flowers open with a slit longitudinally that widened gradually along the margins, forming a clawed structure. It takes one day for complete blooming for a flower. Floral Display, Pollination display and Fruit display of *A. indica* are shown in Table 2.

Reproductive phenological studies in *Aesculus indica* shows that the flower bud initiation takes place in the month of March and the first fruit developed in the last week of April fully ripened in October to November and took an average duration of 7.5 months for the completion of one reproductive cycle. One of the peculiar feature observed in this species that the commencement of new leaves and the new inflorescence together.

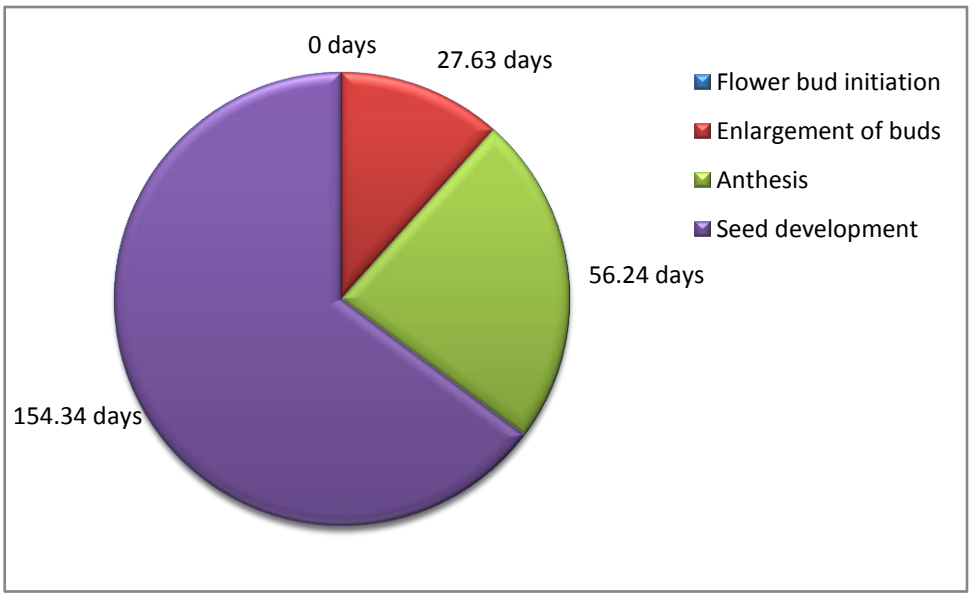



**Fig.** 2. Reproductive cycle of *Aesculus indica*

Regular close observations took from randomly selected inflorescence in three sample trees shows that the inflorescence commenced in March (18 March 2016) and it required around 27.63 ± 2.18 (13 April 2016) days for enlargement of buds (Fig. 2).

The flowers having developed style had the style protruding out when the flower was still immature but it was not observed in the case of flowers having under developed style (Plate 2). The average time taken for complete blooming of one inflorescence was 56.24 ± 1.85 days. After fertilization, the flowers having developed style turned into a bulged ovary but not all the bulged ovary set into the fruit, an immature fruit falls was witnessed in the month of June. The changes in the reproductive development of androecium and gynoecium of flowers of *Aesculus indica* has been illustrated in Table 1. The flowers having under developed style did not participate in fertilization (no fruit formation was observed) and were dried and shrunk after 5$^{th}$ day of anthesis and finally abscission took place at the 8$^{th}$ day of anthesis. The pictorial representation of floral developmental stages from bud initiation till the full blooming has been shown in Plate 1. The mean number of days for fruit development from anthesis was 154.34 ± 5.2 days (27$^{th}$ April to 10$^{th}$ October). During September the fruits become brown and by October the seeds started to disperse.

**Table 1**. Reproductive developmental changes in the Androecium and gynoecium of *Aesculus indica* flowers.

| | |
|---|---|
| 1st day (28th May) | Pistil develops. Yellowish green colour of petal, anther dehisce after 3-4 hrs of anthesis, stigma is pink in colour. |
| 2nd day (29th May) | Anther dehiscence continues the petal colour changes to yellowish orange. |
| 3rd day (30th May) | Anther dehiscence over. Anther starts drying, petal colour changes to pink. Style starts bending. |
| 4th day (31st May) | Ovary starts developing |
| 5th day (1st June) | Petal and filament starts drying |
| 6th day (2nd June) | Petals and filament starts falling |
| 7th day (3rd June) | Petals and filament continues falling. Stigma turns brown in colour. |
| 8th day (4th June) | Ovary develops and style starts to dry. |

**Table 2**. Floral Display, Pollination display and Fruit display of *Aesculus indica*

| **Floral Display** | |
|---|---|
| Inflorescence type | Compound raceme (Tyrsus, thyrsoid cyme) |
| Length of inflorescence | 29.56 ± 3.4 |
| Flower type | Flowers have developed style and under developed style with stamens on both type. Zygomorphic. Pedicellate |
| Sepals | **5** lobes, Gamosepalous, tubular, tomentose |
| Petals | 4 clawed, unequal breadth (Narrow at base and broad at top), Polypetalous. Whitish, yellowish green when young, turns orange then pink while maturation |
| Odour | Absent |
| Average length of petals | 2.35 ± 0.28 cm (flower with developed pistil)<br>2.21 ± 0.23 cm (flower with under developed pistil) |
| Average width of petals | 0.92 ± 0.27 cm (flower with developed pistil)<br>0.93 ± 0.24 cm (flower with under developed pistil) |
| No. of pistil/ flower | 1 |
| Pistil length | 3.19 ± 0.23 cm (flower with developed pistil)<br>1.13 ± 0.08 cm (flower with under developed pistil) |
| Style | Simple, slender |
| Stigma | Dark pink colour when receptive |
| Ovary type | Sessile, 3 celled |
| Number of ovules/ flower | 6 |
| Androecium | Polyandrous, 7 anthers per flower |
| Time of anther dehiscence | 5-6 hrs after anthesis |
| Mode of anther dehiscence | Longitudinal |
| Length of panicle | 29.56 ± 3.4 cm |
| No. of spikes | 31.01 ± 7.96 |
| Total no. of flowers/ inflorescence | 341.9 ± 65.67 to 441.4 ± 74.62 |
| No. of flowers with developed pistil/ inflorescence | 13.3 ± 3.19 to 20.15 ± 6.68 |

Table 2 continued: Floral Display, Pollination display and Fruit display of *Aesculus indica*

| | |
|---|---|
| **Pollination display** | |
| Mode of pollination | Entomophilous (Melittophily) |
| Type of dichogamy | Protogyny (stigma become receptive before anthesis) |
| Major pollinators | Bumble bee |
| **Fruit display** | |
| Number of fruits per inflorescence | 2.6 ± 1.61 to 3.15 ± 2.05 |
| Number of seeds per fruit | 1 |
| Weight of fruit | 29.93 ± 2.96 grm |
| Weight of seed | 17.85 ± 2.09 grm |
| Length of seed | 25.71 ± 1.09 mm |
| Diameter of seed | 35.72 ± 1.66 mm |

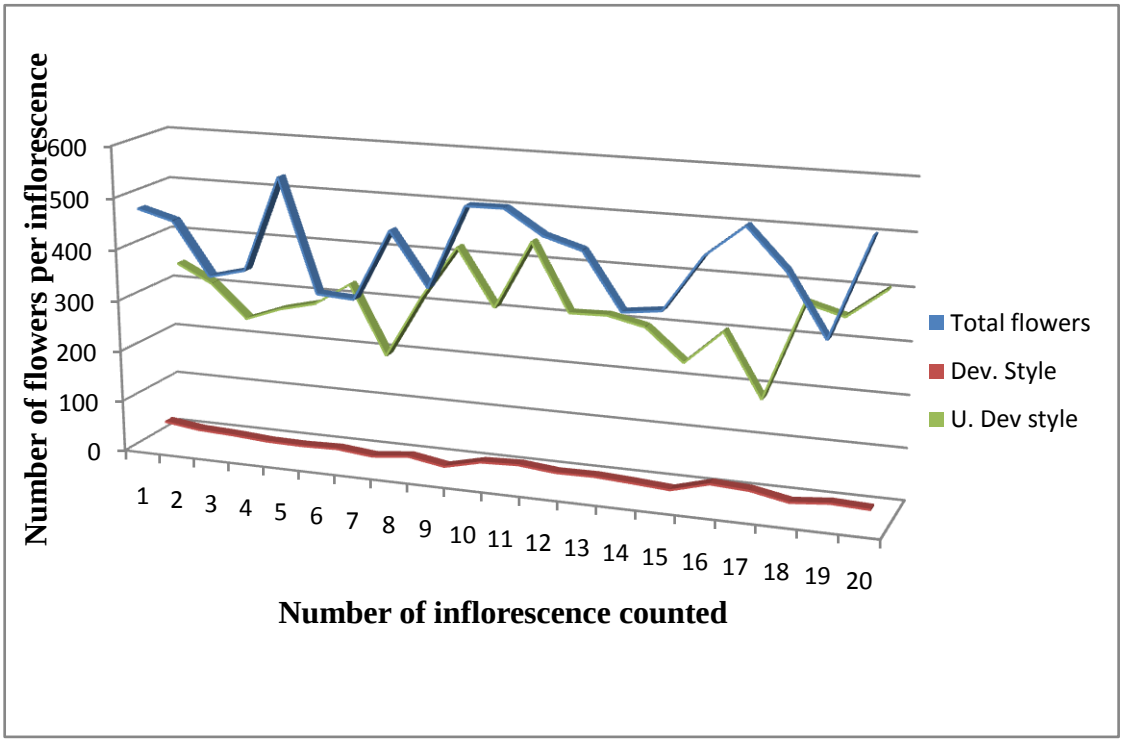



**Fig. 3.** Flower count per inflorescence in sample tree 1.

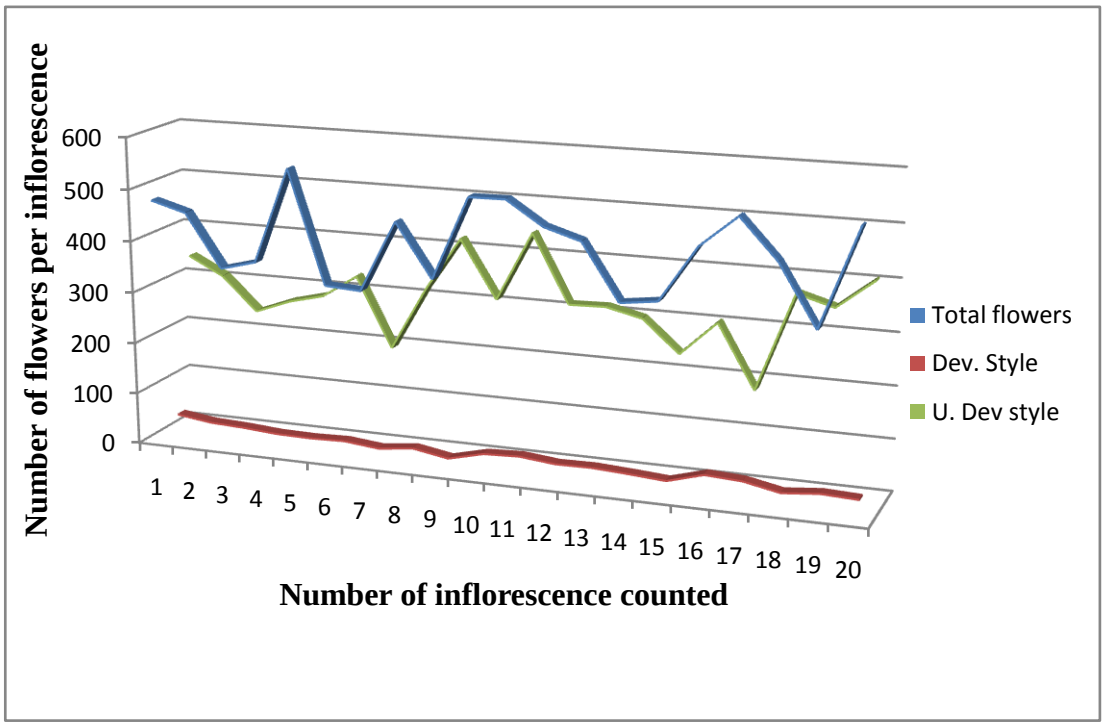



**Fig. 4.** Flower count per inflorescence in sample tree 2

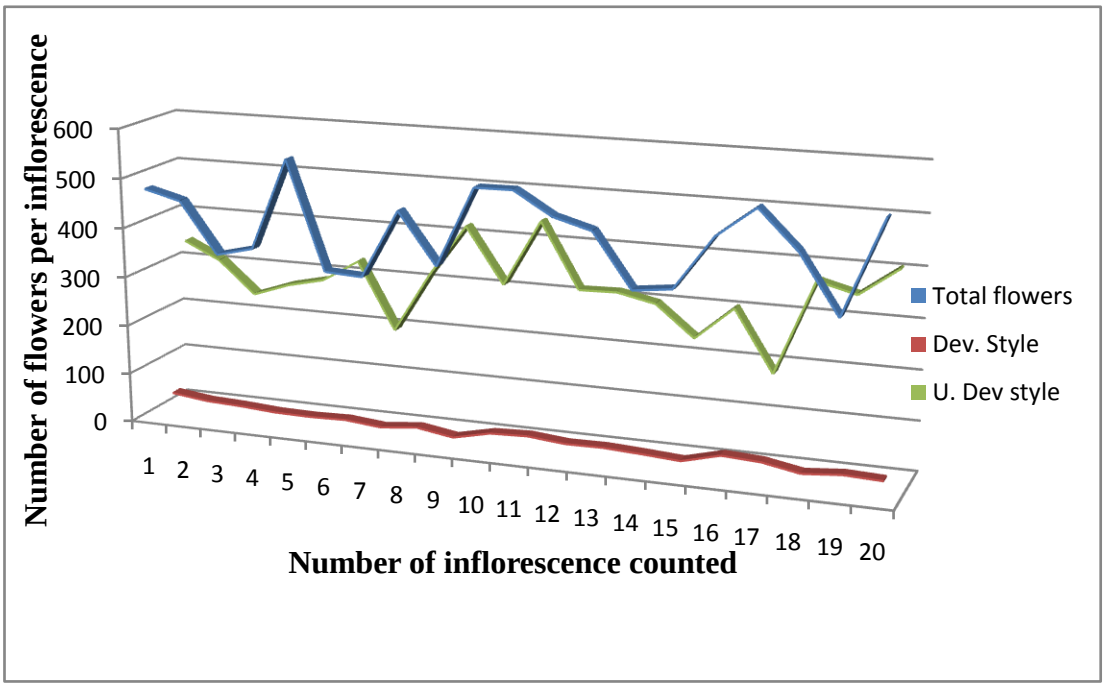



**Fig. 5.** Flower count per inflorescence in sample tree 3

### 4.2 *Anthesis*

Flower opening starts from the second week of April to the second week of June. The anthesis of flower starts with a slit longitudinally that widened gradually along the margins, forming a clawed structure; it often took one day for complete opening

however, it may slightly varied in accordance with the associated weather conditions. Within a given cincinnus (a tight, modified helicoids cyme in which the pedicles are very short), the flower bud closest to the rachis was the first to mature and subsequent development proceeded outward. The acropetal blooming pattern was observed. The flowering pattern of *A. indica* was asynchronous *i.e.* buds and flowers are at different stages of development even on the same tree. The average time taken for complete blooming of one inflorescence was 56.24 ± 1.85 days.

Anthesis occurred between 06:00 to 16:00 hr of the day. The peak period of blooming was recorded between morning hours 06:00 to 08:00 of the day at all the selected trees for which the anthesis percent ranged from 49.7% to 52.87%. It was also noticed that the minimum percentage of anthesis occurred at the 16:00h of the day ranging from 4.95% to 5.82%. It was found that the rate of flower opening maximum at 8-6 hrs of the morning and decreases steadily with the increase in time of the day (Table 3-5).

**Table 3**. Anthesis in *A. indica* in relation with the time of the day in sample tree 1.

| Date<br>Time (hrs) | Number of flowers opened | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 20-30 April | 1-10 May | 11-20 May | 21-30 May | 31-9 June | 10-19 June | Total flowers opened | Anthesis% |
| 6:00 | 187 | 289 | 384 | 325 | 191 | 68 | 1444 | 52.17 |
| 8:00 | 65 | 73 | 94 | 68 | 64 | 17 | 381 | 13.76 |
| 10:00 | 31 | 48 | 53 | 55 | 37 | 11 | 235 | 8.49 |
| 12:00 | 49 | 66 | 74 | 69 | 50 | 16 | 324 | 11.7 |
| 14:00 | 30 | 43 | 68 | 57 | 36 | 13 | 247 | 8.92 |
| 16:00 | 17 | 39 | 24 | 24 | 24 | 9 | 137 | 4.95 |
| **Total** | 379 | 558 | 697 | 598 | 402 | 134 | 2768 | _ |

**Table 4.** Anthesis in *A. indica* in relation with the time of the day in sample tree 2.

| Date<br>Time (hrs) | Number of flowers opened | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 20-30 April | 1-10 May | 11-20 May | 21-30 May | 31-10 June | 11-20 June | Total flowers opened | Anthesis% |
| 6:00 | 266 | 359 | 378 | 326 | 155 | 71 | 1555 | 52.87 |
| 8:00 | 72 | 84 | 88 | 74 | 53 | 21 | 392 | 13.33 |
| 10:00 | 41 | 46 | 54 | 52 | 28 | 15 | 236 | 8.02 |
| 12:00 | 68 | 68 | 75 | 68 | 46 | 12 | 337 | 11.46 |
| 14:00 | 49 | 51 | 53 | 57 | 34 | 17 | 261 | 8.87 |
| 16:00 | 37 | 38 | 27 | 31 | 19 | 8 | 160 | 5.44 |
| **Total** | 533 | 646 | 675 | 608 | 335 | 144 | 2941 | _ |

**Table 5**: Anthesis in *A. indica* in relation with the time of the day in sample tree 3.

| Date<br>Time (hrs) | Number of flowers opened | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 20-30 April | 1-10 May | 11-20 May | 21-30 May | 31-9 June | 11-20 June | Total flowers opened | Anthesis% |
| 6:00 | 263 | 328 | 397 | 268 | 164 | 75 | 1495 | 49.7 |
| 8:00 | 85 | 88 | 103 | 76 | 64 | 17 | 433 | 14.39 |
| 10:00 | 46 | 72 | 74 | 62 | 48 | 14 | 316 | 10.5 |
| 12:00 | 68 | 71 | 61 | 69 | 56 | 13 | 338 | 11.24 |
| 14:00 | 41 | 48 | 53 | 55 | 42 | 12 | 251 | 8.34 |
| 16:00 | 35 | 39 | 24 | 37 | 31 | 9 | 175 | 5.82 |
| **Total** | 538 | 646 | 712 | 567 | 405 | 140 | 3008 | _ |

The two way ANOVA results for anthesis in trees by the effect of tree to tree variation and fixed variable time has shown that there was no significant effect of tree to tree difference on anthesis with F value of 3.26 which is less than the statistical value at 5% (P= 0.05) level of significance and 2 degree of freedom. The calculated F-value of effect due to variation in time is 968.55 which is much higher than the computed statistical value at 5% (P= 0.05%) level of significance and 5 degree of freedom.

Analysis of the number of opened flowers per day revealed high significance at the time of the day (Table 6).

**Table 6**. Two way ANOVA of the effect of time and tree differences on anthesis in *Aesculus indica*

| Source of variation | Degrees of freedom | Sum of squares | Mean sum of squares | F Value (p= 0.05%) |
|---|---|---|---|---|
| Variation due to time | 5 | 105580.17 | 21116 | 968.55** |
| Variation due to trees | 2 | 141.99654 | 70.9983 | 3.26NS |
| Error | 10 | 218.01766 | 21.8018 | |
| Total | 17 | 105940.18 | | |

*Significance at P<0.05, **Significance at P<0.01, NS= Non Significant

### 4.3 *Pollen production*

The number of inflorescences, flowers and pollen grains per tree varied considerably from one individual tree to another individual. The total number of inflorescence production per tree varied between 55 and 394 and the total flower production per inflorescence ranged between 341.9 ± 65.6 to 441.4 ± 74.6 whereas the flowers with developed style varied between 13.3 ± 3.2 to 20.2 ± 6.7. The total flower production among the three selected trees oscillated between 18804 ± 3611 to 151906 ± 24179. An average production of pollen grains ranged from 7597± 91 to 7739.6 ± 169 per anther in flowers with under developed style and 7590 ± 56 to 7781 ± 170 per anther in flowers with developed style. The variation of pollen production in between flowers with developed style and flowers with under developed style was not significant, with the computed t value of t= 0.261, p= 0.05% and df= 4. An average pollen production per flower ranged between 53183.69 ± 643 to 54177 ± 1189 and 18363790± 476673 to 23914032 ± 524987 per inflorescence and 1010008499 ± 26217045 to 8078958841 ± 97679046 per tree. Analyses of pollen production per flower, per inflorescence and per tree, are given in Table 7.

**Table7**. Total pollen production in *Aesculus indica*

| | Sample tree 1 N=3 | Sample tree 2 N=3 | Sample tree 3 N=3 |
|---|---|---|---|
| Total number of flowers | 18804 ± 3611 | 151906 ± 24179 | 134627 ± 22759 |
| No. of pollen/ anther | 7673 ± 199 | 7597 ± 91 | 7739 ± 169 |
| No. of pollen/flower | 53711 ± 1394 | 53183± 643 | 54177 ± 1189 |
| No. of pollen/ inflorescence | 18363790 ± 476673 | 20504971 ± 247916 | 23914032 ± 524987 |
| No. of pollen/ tree | 1010008499 ± 26217045 | 8078958841 ± 97679046 | 7293779871 ± 160121314 |

N= Number of flowers taken for pollen estimation.

### 4.4 *Pollen ovule ratio*

The flowers had 7 anthers and thus were estimated to have a total of 53130 ± 392 to 54471.69 ± 1190 pollen grains in flowers with developed style and 53183 ± 643 to 54177 ± 1189 pollen grains in the flowers with under developed style. There are six ovules per flower (Plate 7); hence the pollen ovule ratio varied from 8855 ± 65 to 9078 ± 198 pollen grains per ovule in flowers with developed style and 8863 ± 107 to 9029 ± 198 pollen grains per ovule in the flowers with under developed style for *Aesculus indica.* The difference in pollen production between flowers with developed style and flowers with under developed style was not significant. The numbers of ovules within the flower are observed constant (6) infers, there is not much variation in the pollen ovule ratio. Cruden (1997) divided the breeding systems into five classes: (i) Xenogamy (P/O's 5859.20 ± 936.5), (ii) Facultative xenogamy (P/O's 796.60 ± 87.7), (iii) Facultative autogamy (P/O's 168.80 ± 22.1), (iv) Obligate autogamy (P/O's 27.70 ± 3.1) and (v) Cleistogamy (P/O's 4.70 ± 0.7). Thus, the pollen ovule ratio of *Aesculus indica* falls within the reported range of Xenogamy.

### 4.5 *Stigma receptivity*

The Hydregen peroxide ($H_2O_2$) test of stigma receptivity has shown that the stigmas of both flowers with developed style and flower with under developed style are

receptive. Flowers were shown active bubbling when immersed in hydrogen peroxide solution. The stigmas are receptive before the opening of bud. This implies that it possesses protogyny (dichogamy). The bubbling has been retained for a period of five to six days. The peak period of stigma receptivity is on the first day of flower opening (Plate 5). The receptivity constantly reduces from the first day to the sixth day. The stigma looked fresh and pink in colour throughout its receptive phase. Eventually, stigma becomes dry, shrink and brown in colour which made clear the termination of the receptive phase.

### 4.6 *Pollen viability*

The viability of pollen grains was measured by the acetocarmine test. The percentage of viable pollen per slide ranged between 87.54 ± 4.02% to 90.04 ± 0.82% in flowers with developed style (Fig. 6; Table 8) and 85.34 ± 3.23% to 89.57 ± 3.65% in flowers with under developed style (Fig. 7; Table 9). The viable pollen grains were deeply stained, round and normal looking (Plate 6). The percentage of shriveled weakly stained nonviable pollen was recorded from 9.94 ± 0.83% to 12.45 ± 4.02% in flowers with developed style and 12.26 ± 2.46% to 14.65 ± 3.23% in flowers with under developed style.

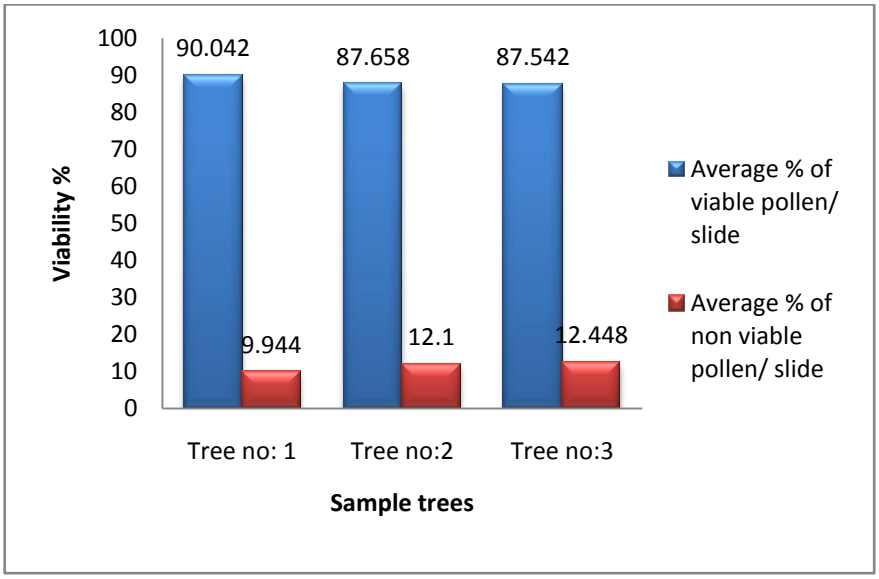



**Fig. 6**. Pollen viability in flowers of *Aesculus indica* having developed style

**Table 8**. Pollen viability in flowers of *Aesculus indica* having developed style

| N=5 | Tree no: 1 | Tree no: 2 | Tree no:3 |
|---|---|---|---|
| Average no. of stained pollen grains/slide | 268 ± 37.8 | 190.4 ± 76.1 | 174.8 ± 89.0 |
| Average no. of weakly stained pollen/ slide | 29.6 ± 4.5 | 23.8 ± 4.5 | 22.4 ± 6.8 |
| Average no. of total pollen grains/ slide | 297.6 ± 41.6 | 214.2 ± 80.6 | 197.2 ± 93.5 |
| Average % of viable pollen/ slide | 90.04 ± 0.8 | 87.66 ± 2.4 | 87.54 ± 4.0 |
| Average % of non viable pollen/ slide | 9.94 ± 0.8 | 12.1 ± 2.6 | 12.45 ± 4.0 |

N= Number of anthers examined for pollen viability

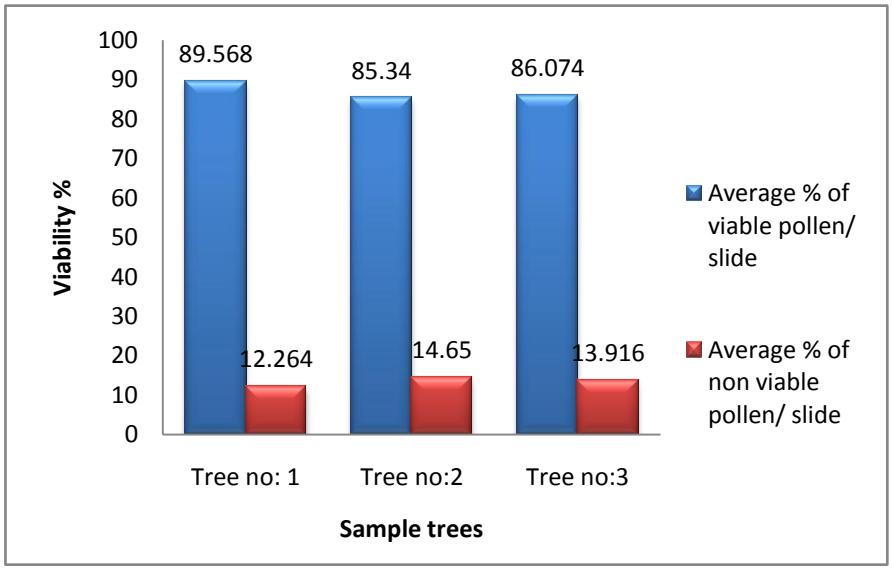



**Fig. 7.** Pollen viability in flowers of *Aesculus indica* having under developed style

**Table 9**. Pollen viability in flowers of *Aesculus indica* having under developed style

| N=5 | Tree no: 1 | Tree no: 2 | Tree no:3 |
|---|---|---|---|
| Average no. of stained pollen grains/slide | 194 ± 77.9 | 131 ± 25.91 | 115.6 ± 39.05 |
| Average no. of weakly stained pollen/ slide | 22.4 ± 4.49 | 22.8 ± 6.4 | 18.2 ± 4.31 |
| Average no. of total pollen grains/ slide | 213.8 ± 78.8 | 154.6 ± 29.9 | 133.8 ± 43.0 |
| Average % of viable pollen/ slide | 89.5 ± 3.6 | 85.34 ± 3.2 | 86.0 ± 1.4 |
| Average % of non viable pollen/ slide | 12.2 ± 2.4 | 14.65 ± 3.2 | 13.9 ± 1.4 |

N= Number of anthers examined for pollen viability

### 4.7 *Pollen germination*

The effect of sucrose indifferent concentrations for pollen germination is displayed in the figure 7 and 8. Among the sucrose concentration, maximum germination of 87.57% in flowers with developed style and 87.18 % in flowers with under developed style was observed in 20% sucrose concentration followed by 82.67% in flowers with developed style and 81.86% in flowers with under developed style was observed in 10% sucrose.

The pollen germination is increasing with increase in time till 5 hours after which there is a decline in the germination rate. The maximum percent of germination was observed in 5 hours after immersing in the media in both the flower types (Fig. 8 and Fig. 9).

**Fig. 8**. Effect of sucrose concentrations on pollen germination of flowers with developed styles in *Aesculus indica.*

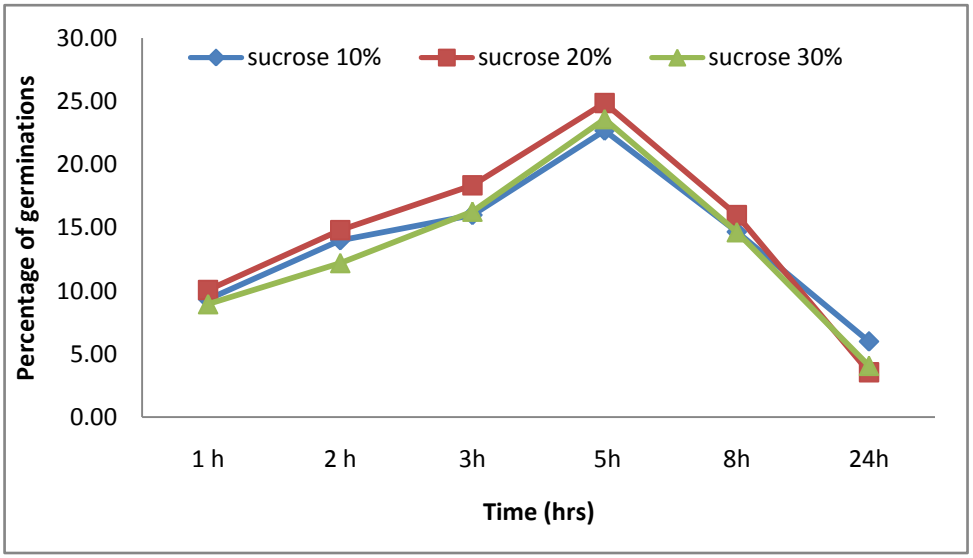
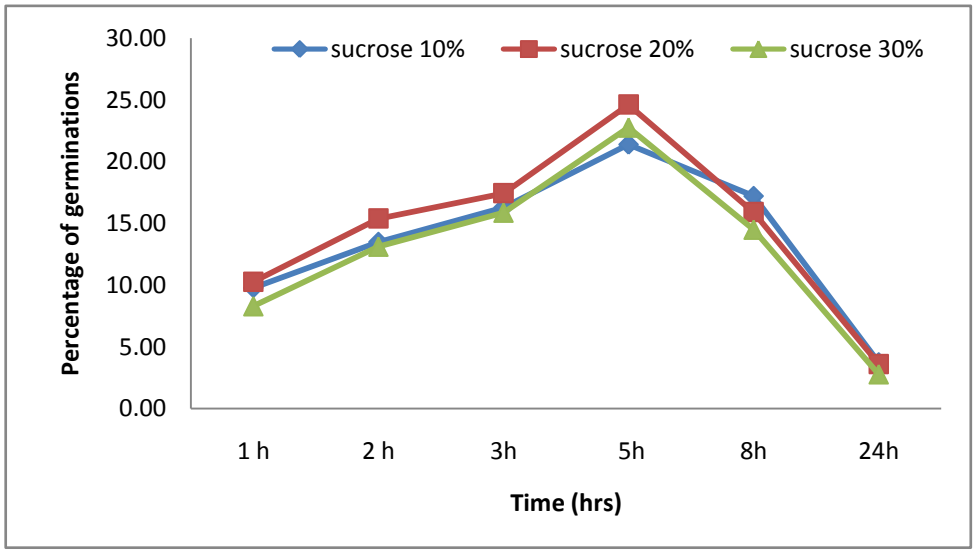



**Fig. 9.** Effect of sucrose concentrations on pollen germination of flowers with under developed styles in *Aesculus indica*




The two way ANOVA results for pollen germination with factors sucrose concentration (10%, 20% and 30%) and time interval (1h, 2h, 3h, 5h, 8h and 24h) has shown that there was significant effect of both time interval and sucrose concentration. The calculated F value of effect due to time interval is 67.847 in case of flowers with developed style (Table 10) and 48.734 in case of flowers with under developed style (Table 11) which is much higher than the statistical value at 5% (P= 0.05) level of significance and 5 degree of freedom. The calculated F-value of effect due to sucrose concentration is 31.130 in case of flowers with developed style and 18.108 in case of

flowers with under developed style which is more than the computed statistical value at 5% (P= 0.05%) level of significance and 2 degree of freedom.

**Table 10.** ANOVA of the effect of time and sucrose concentrations on pollen germination of flowers with developed style.

| Source of variation | Degrees of freedom | Sum of squares | Mean sum of squares | F value P=0.05% |
|---|---|---|---|---|
| Time interval | 5 | 1402.4444 | 280.489 | 48.734** |
| Sucrose concentration | 2 | 208.44444 | 104.222 | 18.108** |
| ERROR | 10 | 57.555556 | 5.75556 | |
| Total | 17 | 1668.4444 | | |

**Table 11.** ANOVA of the effect of time and sucrose concentration on pollen germination of flowers with under developed style.

| Source of variation | Degrees of freedom | Sum of squares | Mean sum of squares | F value P=0.05% |
|---|---|---|---|---|
| Time interval | 5 | 2269.1111 | 453.822 | 67.847** |
| Sucrose concentration | 2 | 416.44444 | 208.222 | 31.130** |
| ERROR | 10 | 66.888889 | 6.68889 | |
| Total | 17 | 2752.4444 | | |

*Significance at P<0.05, **Significance at P<0.01, NS= Non Significant

### 4.8 *Breeding system*

The result from the natural and controlled pollination treatments is summarized in Table 12. There is not much difference on fruit set between the open pollination and the open cross pollination. The reproductive success (Number of fruits/ Number of flowers) is higher in open cross pollination 0.367 and 0.267 in case of and open pollination. Breeding experiments demonstrated that flowers of *Aesculus indica* possessing self-incompatibility as no fruit set were observe in autogamous self-pollinations. Reproductive potential (Seed/ Ovule) is same for both Open Cross pollination and Open pollination (0.167). As maximum seed set per fruit is one in both the pollination treatments, which implies that out of six ovules only one is developed into seed and the

rest five ovules are aborted (Plate 9). All the flowers that fertilized do not convert into mature fruit as pre mature fruit fall has been witnessed.

**Table 12**. Effect of pollination treatments in fruit and seed set in *Aesculus indica.*

| Pollination treatment | FL | FR | FR/FL | FR. Wt. (g) | A. Ov | S | S/Ov |
|---|---|---|---|---|---|---|---|
| Apomixis | 60 | - | - | - | - | - | - |
| Open Cross pollination | 60 | 22 | 0.367 | 29.61 ± 2.99 | 6 | 1 | 0.167 |
| Open pollination | 60 | 16 | 0.267 | 29.16 ± 2.33 | 6 | 1 | 0.167 |
| Self pollination | 60 | - | - | - | - | - | - |

FL= Flower, FR= Fruit, FR. Wt.= Fruit Weight, A. Ov= Average number of ovules and S= Average seed set per fruit

### 4.9 *Flower visitors*

Five insect species from three orders Hymenoptera, Thysanoptera and Lepidoptera were observed, collected, and identified from the flowers of *Aesculus indica.* Among the pollinators, the most frequent visitors in flowers were *Bombus spp.* and Thrips. Thrips were also observed crawling over the flowers. No differences between the visitation rates by insects to staminate (under developed) or complete (developed) flowers was observed. The inflorescence received most *Bombus spp.* in the morning hours from 06:00- 08:00 and almost absent during the noon. Bumble bees tended to visit the outward facing flowers and the flowers at the base of the panicle first. Most pollinators to visit the flowers were observed between 08:00-10:00 hours of the day. More particularly the insect *Tagiades menaka* was found more frequently during the evening hours from 16:00 - 18:00. The butterflies were observed feeding at the top of the inflorescence and move downward visiting both buds ready to open and open flowers. Flies were also found to be visiting the unopened flower buds. Humming bird moth was also observed visiting the flowers, they tended to visit the panicle apex first. The images of the pollinators visiting flowers are illustrated in Plate 4. Observations made on floral visitors have been shown in Table 13.

**Table 13.** Insect visitors observed on the flowers of *Aesculus indica*

| Time | Name of visitor | Scientific Name | Order | Number of individual | Duration of visit |
|---|---|---|---|---|---|
| 06:00 - 08.00 | Bumble bee | *Bombus spp.* | Hymenoptera | 22 | 13-15 sec |
| | Thrips | *Heterothrips aesculii* | Thysanoptera | Infinite | |
| 08:00 - 10:00 | Bumble bee | *Bombus spp.* | Hymenoptera | 12 | 8-13 sec |
| | Honey Bee | *Apis mellifera* | Hymenoptera | 7 | 4-8 sec |
| | Thrips | *Heterothrips aesculii* | Thysanoptera | Infinite | |
| | Himalayan Spotted Flat (Butterfly) | *Celaenorrhinus munda* | Lepidoptera | 5 | 7-13 sec |
| 10:00 - 12:00 | Bengal Spotted Snow flat (Butterfly) | *Tagiades menaka* | Lepidoptera | 4 | 6-16 sec |
| | Thrips | *Heterothrips aesculii* | Thysanoptera | Infinite | |
| | Honey Bee | *Apis mellifera* | Hymenoptera | 2 | 4-8 sec |
| 12:00 - 14:00 | Bengal Spotted Snow flat (Butterfly) | *Tagiades menaka* | Lepidoptera | 2 | 6-16 sec |
| | Honey Bee | *Apis mellifera* | Hymenoptera | 4 | 4-8 sec |
| | Thrips | *Heterothrips aesculii* | Thysanoptera | Infinite | |
| 14:00 - 16:00 | Bengal Spotted Snow flat (Butterfly) | *Tagiades menaka* | Lepidoptera | 3 | 6-16 sec |
| | Bumble bee | *Bombus spp.* | Hymenoptera | 9 | 8-13 sec |
| | Thrips | *Heterothrips aesculii* | Thysanoptera | Infinite | |
| | Honey Bee | *Apis mellifera* | Hymenoptera | 4 | 4-8 sec |
| | Humming bird moth | *Hemaris spp.* | Lepidoptera | 2 | 1-2 sec |
| 16:00 - 18:00 | Bengal Spotted Snow flat (Butterfly) | *Tagiades menaka* | Lepidoptera | 10 | 16sec |

### 4.10. *Reproductive success in different crown layer*

Regular observation on the flowers of *Aesculus indica* has shown that the fruit setting started after 15 to 16 days of anthesis with visible changes in the size of the ovary. As the number of inflorescence increases from lower canopy layer to the higher canopy layer the number of flowers also increases which leads to the increase in the fruit production in upper canopy layer when compared to lower canopy layer. Total number of fruit set per inflorescence in lower canopy layer ranges from 2 ± 0.63 to 2.45 ± 1.39, 2.33 ± 1.07 to 3.25 ± 1.95 in middle canopy layer and 3.47 ± 2.09 to 3.75 ± 2.64 in upper canopy layer. The seed set percentage of *Aesculus indica* ranges from 0.53% to 0.64% in the lower canopy, 0.68% to 0.84% in middle canopy and 0.82% to 1.01% in upper canopy layer. The result of t-test indicated that there is a significant variation in total number of counted flowers and total fruit set, with the computed t value of t= 16.26 at P=0.05% (Fig. 10-12). The seed set percent is similar to that of fruit set

percent, as number of seed formed per fruit is one (Plate 8). The reproductive success varied in between the crown layers.

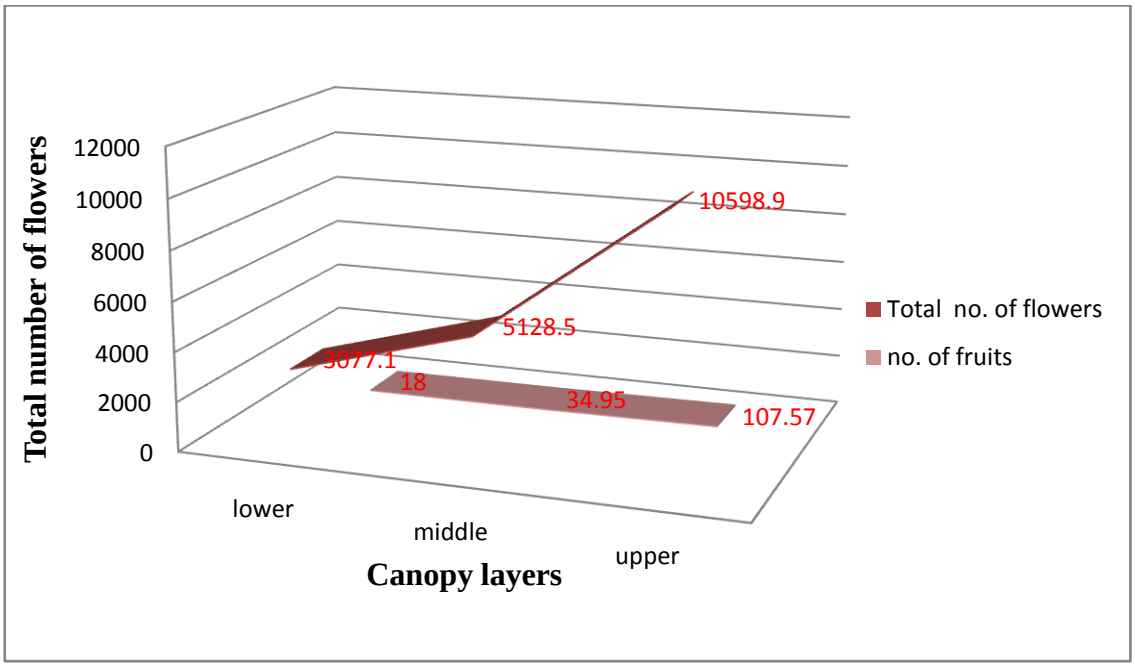



**Fig. 10.** Reproductive success in different crown layers of sample tree 1

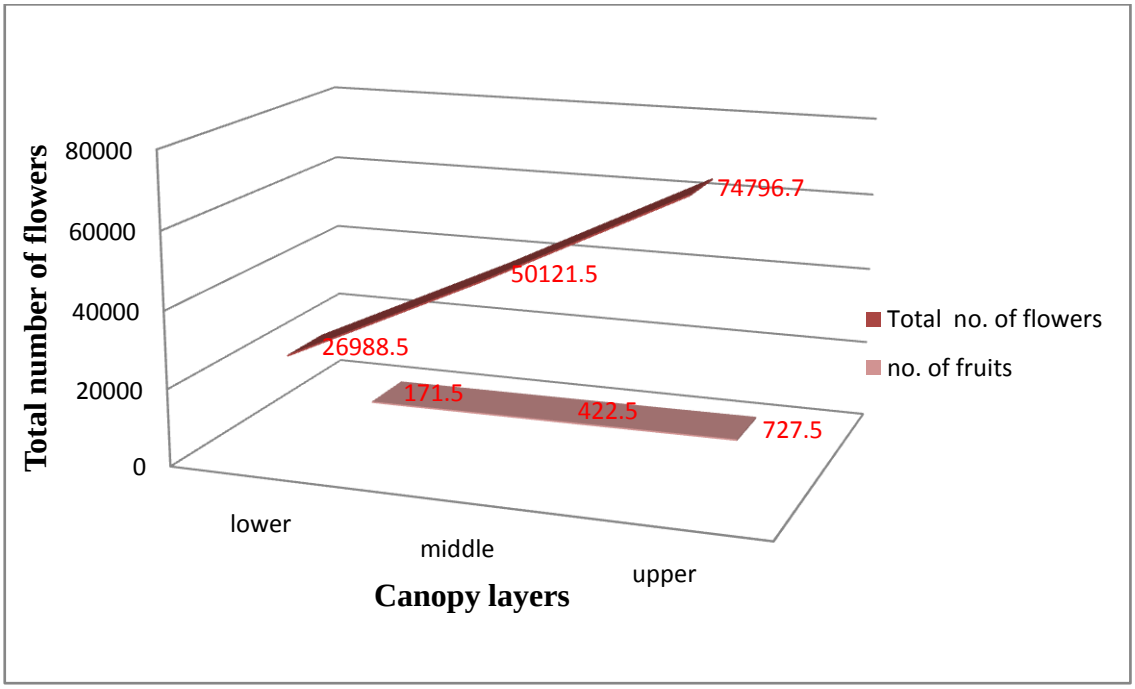



**Fig. 11.** Reproductive success in different crown layers of sample tree 2

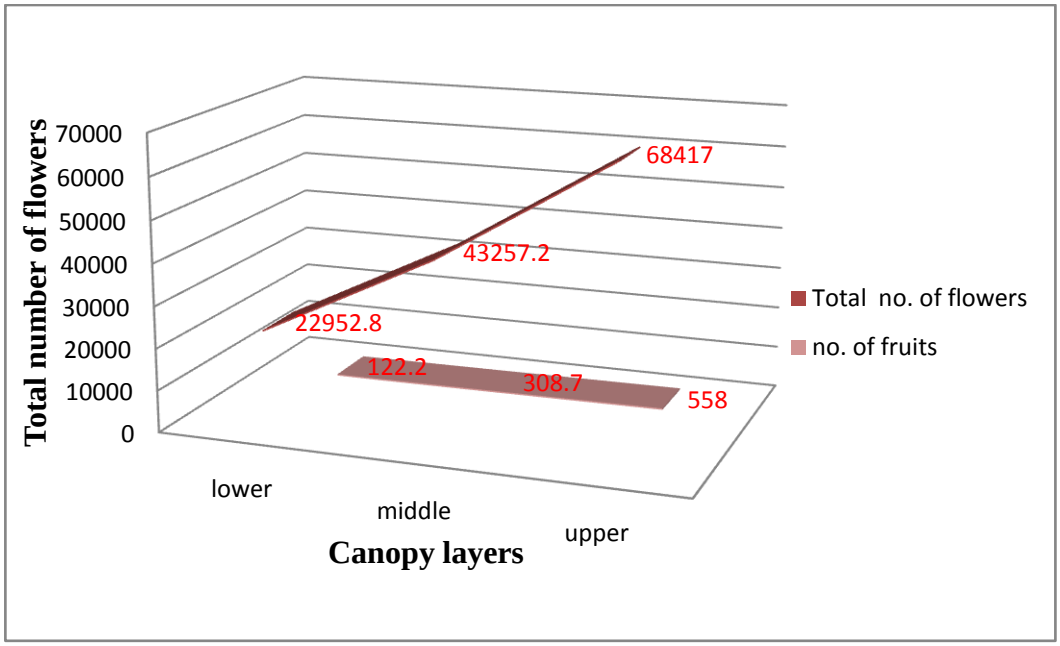



**Fig. 12.** Reproductive success in different crown layers of sample tree 3

Total no. of flowers= Total number of flowers, no. of fruits= number of fruits

The reproductive success of *Aesculus indica* in lower canopy layer ranged from 0.0053 to 0.0064, in middle canopy 0.0068 to 0.0084 and in upper canopy layer 0.0082 to 0.0101. The number of ovules per ovary was 6 and the number of ovules that converted into seed was one, aborting the rest five ovules results the reproductive potential of 0.167 in all the three canopy layers. Details of the observations are summarized in Table 14.

**Table 14.** Reproductive success in different crown layer

| Tree No. | 1 | | | 2 | | | 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | L. C. | M. C. | U. C. | L. C. | M. C. | U. C. | L. C. | M. C. | U. C. |
| Total no. of inflorescence | 9 | 15 | 31 | 70 | 130 | 194 | 52 | 98 | 155 |
| Total flower production | 3077.1 ± 591.08 | 5128.5 ± 985.13 | 10598.9 ± 2035.94 | 26988.5 ± 4296.23 | 50121.5 ± 7978.70 | 74796.7 ± 11906.68 | 22952.8 ± 3880.34 | 43257.2 ± 7312.95 | 68417 ± 11566.41 |
| Total no. of fruit/ inflorescence | 2 ± 0.63 | 2.33 ± 1.07 | 3.47 ± 2.09 | 2.45 ± 1.39 | 3.25 ± 1.95 | 3.75 ± 2.64 | 2.35 ± 1.39 | 3.15 ± 1.68 | 3.6 ± 2.37 |
| Total no. of fruit/ tree | 18 ± 5.67 | 34.95 ± 16.05 | 107.57 ± 64.79 | 171.5 ± 97.3 | 422.5 ± 253.5 | 727.5 ± 512.16 | 122.2 ± 72.28 | 308 ± 164.64 | 558 ± 367.35 |
| % of fruit set | 0.58% | .68% | 1.01% | 0.63% | 0.84% | 0.97% | 0.53% | 0.71% | 0.82% |
| Average weight of fruit (g) | 29.92 ± 2.96 | | | 28.71 ± 2.51 | | | 29.27 ± 2.79 | | |
| Total weight of fruit/ tree (g) | 538.56 ± 53.28 | 1045.7 ± 103.45 | 3218.49 ± 318.41 | 4923.77 ± 355.17 | 12129.98 ± 1060.48 | 20886.53 ± 1826.03 | 3576.79 ± 340.94 | 9015.16 ± 859.32 | 16332.66 ± 1556.82 |
| Average no. of ovules/ ovary | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Average no. of seed/ fruit | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Average no. of seed/ tree | 143 ± 88.55 | | | 1241.1 ± 807.7 | | | 924.15 ± 567.3 | | |
| Reproductive success | 0.0058 | 0.0068 | 0.0101 | 0.0064 | 0.0084 | 0.0097 | 0.0053 | 0.0071 | 0.0082 |
| Reproductive potential | 0.167 | 0.167 | 0.167 | 0.167 | 0.167 | 0.167 | 0.167 | 0.167 | 0.167 |

L.C.= Lower canopy, M.C,= Middle canopy and L.C.= Lower canopy

The two way ANOVA results for seed set in different canopy layers with variation due to sample tree has shown that there was significant effect of both crown layers and sample trees. The calculated F value of effect due to crown layer variation is 7.582 which is higher than the statistical value at 5% (p= 0.05%) level of significance and 2 degree of freedom. The calculated F-value of effect due to variation between sample trees is 8.327 which is more than the computed statistical value at 5% (p= 0.05%) level of significance and 2 degree of freedom (Table 15).

**Table 15**. ANOVA for seed set in different crown layers of *Aesculus indica*.

| Source of variation | Degrees of freedom | Sum of squares | Mean sum of squares | F cal (P= 0.05%) |
|---|---|---|---|---|
| Variation due to crown layer | 2 | 164638.95 | 82319.5 | 7.582* |
| Variation due to trees | 2 | 180808.75 | 90404.4 | 8.327* |
| Error | 4 | 43427.759 | 10856.9 | |
| Total | 8 | 388875.46 | | |

*Significance at P<0.05, **Significance at P<0.01, NS= Non Significant

# CHAPTER – 5
# DISCUSSION

# 5. DISCUSSION

Studies on reproductive biology and breeding systems are important basic tools to carry out any future tree breeding programme. The efficiency of reproductive system depends upon our understanding of factors affecting reproductive biology. Studies on variation in flowering, pollination, seed set; seed quality, seed viability and germination are of practical importance. It is desirable to collect all the information regarding the reproductive biology of the species to carry out any tree improvement programme. About 5000 species of trees and shrubs exist in Indian forest ecosystem (Gamble, 1902; Champion and Seth, 1968). However, little is known about the reproductive biology and breeding systems of these species. The present study was carried out to explore the floral biology and reproductive effort of *Aesculus indica*-An ecologically and economically important tree species of temperate Himalaya

## 5.1 *Floral morphology*

In *Aesculus indica* two types of flowers have been observed one with the developed style other with the underdeveloped style with androeicium in both the flower types. Hardin (1956) concluded that the term andromonecious (having perfect and staminate flowers on the same plant) best characterized the flowering biology of the genus since both functionally staminate flowers and at least a few complete flowers could be found within individual plants. The functionally staminate flowers have a vestigial gynoecium with an undeveloped ovary and stunted style. Complete flowers have fully developed androecium and gynoecium (Hardin, 1956). Production of two types of flowers (developed and underdeveloped) in *A. indica* is likely to be the effect of genetic and physiological factors. The environmental factors affecting floral sex expression in woody species which includes moisture and nutrient availability, light levels, temperature extremes and predation (Benseler, 1968; Bertin, 1980; Charnov and Bull, 1977; Shifriss, 1956; Wolfe and Drapalik, 1999). Benseler (1968) found that temperature and soil moisture altered the number of complete flowers in California buckeye with plants grown in more moderate and moist climates producing more complete flowers per panicle than those grown in hot and dry climate. Bertin (1980) found no effect of crown position (height from the ground) or cardinal direction on the frequency of complete flowers, but plants in open habitats had more complete flowers than those in wooded habitats. He also reported that the distribution of complete flowers was uneven.

The flower with stunted or under developed style do not produce seed even though the stigma is receptive, this may be because the style are too short for the pollen to undergo changes that is for producing the pollen tube and not enough nutrients are present in it for supporting the growth of the pollen. In nature water, sugar, amino acids are supplied by the style to nourish the growing pollen tube. Boron is also provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986). Throughout the study, it was found to be all complete flowers within trees are not converted into mature fruit and seed. Benseler (1968) and Hardin (1955) both reported that most of the panicles that contain female flowers, fail to produce any mature fruits. Immature fruit fall has been encountered in all the sample trees. It can be assumed that the fruits turned into the mature stage are the winner of selection pressure from mother tree and nature. High temperature, water stress, or both appear to accentuate the fruit drop in crops (Coit *et al.*, 1919; Jennings, 1934). Blumenfeld *et al.* (1990) and Cutting and Bower (1990) reported that fruit fall, fruit retention/fruit drop in both crops is characterized by (i) competition between the young fruit and vegetative growth; (ii) sensitivity to temperature extremes; and (iii) water-deficit stress. Fruit borne on leafy inflorescences are faster-growing and have a high potential to set and survive to harvest. Fruit borne on leafless inflorescences are slower-growing and tend to abscise early in their development (Erner, 1989, Hofman, 1988; Lovatt *et al.*, 1984). Erner (1989) has proposed that fruit borne on leafy inflorescences have a greater ability to withdraw water from the transpiration stream than those borne on leafless inflorescences. It would be expected that fruit borne on leafless inflorescences would be more susceptible to water-deficit stress.

### 5.2 *Anthesis*

The anthesis is an active process based on the physiological and ecological behavior of a plant. Flowers open at the definite time of the day. The exact time however, depend upon temperature and other weather conditions. In *A. indica* diurnal pattern of anthesis has been recorded; the peak period of anthesis was found to be 06:00 to 08:00 hours of the day during this time the activity of floral visitors was also observed maximum. The flowering pattern of *A. indica* was asynchronous *i.e.* buds and flowers are at different stages of development even on the same tree. In shisham and *Bombax ceiba*, the flowering pattern was also observed asynchronous (Bangarwa 1993;

Khosla *et al.*, 1982). Intra-specific variation in the frequency, duration, amplitude and synchrony of flowering by individuals also has been proposed as an important factor that affects the reproduction and the genetic structure of tropical plant populations in disturbed habitats (Murawski *et al.*, 1990; Newstrom *et al.*, 1994). Plants with asynchronous flowering may experience a decrease in reproductive output, the amount of pollen, the number of pollen donors and the levels of outcrossing compared to individuals blooming during the same period (Schwartz, 2013). For plants with temporally separate sexual phases to outcross, population level flowering asynchrony is necessary, but this can decrease the resource base available for pollinators (Bronstein *et al.*, 1990).

The present study shows that tree to tree variations did not influence the opening of the flower while the time of the day significantly influenced the flower opening which implies to change in the microclimatic conditions like temperature, humidity, rainfall, and photoperiod varied from time to time in a day. The effect of climatic conditions on anthesis pointed out by many authors as the climatic conditions partly influence the time of anthesis, anther dehiscence and density of flower that can ultimately affect the quantity of pollen produced by an individual (Stanley and Linskens, 1974). The above findings are strongly supported by the statements of Khanduri and Sharma (2003) which states that variation in the time of anther dehiscence and stigma receptivity could be due to the rapid fluctuation in temperature, humidity and light exposure. Even though the anthesis period is genetically fixed it is influenced by the external factors. The knowledge of anthesis and pollen production is essential to study of pollination, developing a functional model for forecasting pollen concentrations and to understand more about the ecological background of pollen dispersal.

### 5.3 *Pollen production*

The number of pollen grains per anther and per flower in *A. indica* has not been considered of much difference which reveals that the total pollen production per flower within a tree is genetically fixed but slight variation arises due to the morphology and microclimatic conditions. Subba Reddi and Reddi (1984) stated that the level of pollen production in a particular species is a function of its genotype. However, in the present study, it is very clear that the total pollen production among trees has significantly

varied which is because in the differences in the number of total inflorescences, flower production, and micro-environmental conditions. Agnihotri and Singh (1975) stated that pollen production varying greatly from one species to another even within the same genus, was directly related to the size of anther and inversely related to the size of the pollen grains and that, therefore, those species with large anthers and small pollen grains were the most productive ones.

Tangmitcharoen and Owens (1997) noted that pollen intensity influences the quality of seeds produced, meant high pollen loads intensify competition among male gametophytes to fertilize the available ovules on that, of this only the most vigorous gametophytes effecting fertilization and producing zygotes vigorous enough to form high-quality seeds. Faegri and Iversen (1989); Campbell and Halama (1993) also mentioned that the estimation of total pollen production per plant is useful not only from an aerobiological but also from an agronomical stand point, as the production of seeds often depends on the production of pollen.

### 5.4 *Pollen viability and Pollen germination*

The percentage of viable pollen in *Aesculus* indica ranged between 87.54 ± 4.02% to 90.04 ± 0.82% in flowers with developed style and 85.34 ± 3.23% to 89.57 ± 3.65% in flowers with underdeveloped style. A higher percentage of viable pollen per anther augments the output and enhances the autogamy as well as xenogamy. This trend of pollen viability with prolonged flowering time was caused by its physiological change as well as the real-time temperature and relative humidity (Chen *et al.*, 2013). Pollen viability is an important aspect that can help in determining the successful adaptation of a plant species. It is necessary to identify the factors influencing pollen germination and elongation of the tube (Char *et al*., 1973; Qureshi *et al*., 2002). Temperature is important in determining the effective pollination period, stigma receptivity (Burgos *et al.*, 1991), ovule longevity (Eaton, 1959; Postweiler *et al.*, 1985), pollen germination (Escobar *et al.*, 1983), and pollen-tube growth both *in* v*itro* (Escobar *et al*., 1983) and *in* vivo (Cuevas *et al.*, 1994). All of these may affect fertilization and fruit set.

In *Aesculus indica* the maximum pollen germination was obtained from 20% sucrose concentration 87.57% in flowers with developed style and 87.18 % in flowers with under sucrose media 82.67% in flowers with developed style and 81.86% in flowers with under

developed style. Maximum pollen germination was observed after 5 hrs of immersing the pollen in the sucrose concentrations. Artificial germination of the pollen grains is the surest test of pollen fertility (Nautiyal *et al.*, 2009). Sucrose is the carbohydrate source for pollen germination and tube growth (Tupy, 1960). Sugar in the culture medium serves functions like (a) maintaining osmotic pressure of the medium and (b) as a substrate for metabolism of pollen. In 20% sucrose solution maximum germination was obtained. In *A. chinensis*, the highest percentage of pollen germination and greatest pollen tube length was observed on medium containing 20% sucrose over both fresh and stored pollen samples (Ostrolucka and Bencat, 1987).

*In vitro* pollen germination is an efficient technique for realizing the basic (Mascarenhas, 1993) and applied aspects of pollen biology (Herrero, 1991; Kristen and Kappler, 1990). Satisfactory pollen germination requires solution of carbohydrate, especially sucrose (Patel *et al.*, 1997), with or without other substances.

### 5.5 *Pollination and Breeding system*

Mode of pollination in a species is vital to the choice of breeding procedures to be adopted for its genetic improvement. Genius research of Camerarius in 1694, about sex in plants provided much-needed insight (Stanley and Linskens, 1974). Controlled pollination which is basic to plant improvement depends upon pollination mechanisms. The results on controlled pollination suggest predominantly self-incompatibility in *A. indica* as there is no fruit setting observed from autogamous (self-pollination). Although, the percentage of fruit set in open cross pollination was found to be slightly more than open pollination. Nevertheless, the pollen ovule ratio indicates that the breeding system of the species supports xenogamy.

The pollinator observations reveal that insect would also have a major role in performing pollinations as the species is mostly visited by *Bombus spp.* during the morning hours and *Tagiades menaka* (butterfly) during the evening hours. However, Sharma and Khanduri (2007) reported that pollination in *A. indica* is anemophilous. Flowers of *A. indica* own up high pollen production, the high pollen ovule ratio, vertically projected inflorescence and most importantly the pollen grains were dry, which may transfer though wind also. These characteristics indicate that the species is also wind pollinated. The incidence of high populations of insect activity in flowers would be assisting wind-pollination in *A. indica*. Huang *et al.* (2002) reported that

honeybee-assisted wind pollination in bamboo *Phyllostachys nidularia.* Huang *et al*. observed that the pollen grains were scraped from the anther and transferred to the hind legs and abdomen, but some of them were dispersed into the air like a cloud. When groups of honeybees visited at the same time, large numbers of pollen grains were observed to disperse from the anthers in clouds.

It is also evident that few of the insect visitors are acting as foragers and pollen robbers. The most pollen theft occurs by vectors that pollinate other plants and the most frequent pollen thieves are bees (Hargreaves *et al*., 2009). Flowers of *Primula secundiflora* were commonly robbed by bumblebees (Zhu *et al.,* 2010). Pollen robbing has been reported frequently in *Rhododendron spp.* also (Stevens, 1985; Ng and Corlett, 2000). The frequent incident of Bees, Thrips, and Butterflies in *A. indica* flowers were assumed to increase the chances of pollen theft, as there was low fruit and seed set observed. Similar results were also reported by past workers that the robbing negatively affect the seed production (Arizmendi *et al.*, 1996; Irwin *et al.*, 2001; Lara and Ornelas, 2001).

**5.6 *Seed abortion***

It is evident that fruit production in *A. indica* is very low when compared to the abundance of flowers commonly produced in it. Hardin (1955) suggested that fruit production within a species was limited by effective pollination, fertilization, and fruit development on the tree. The seed set percentage of *A. indica* ranges from 0.53% to 0.58% in lower canopy, 0.68% to 0.97% in middle canopy and 0.82% to 1.01% in upper canopy layer. The seed set percent is similar to that of fruit set percent as number of seed formed per fruit is one. Surprisingly there are six ovules in a flower out of which only one ovule developed into a seed, indicating five of ovules likely to be aborted. Boyer (1982) stated that the most growing conditions enable many plant species to initiate sufficient numbers of ovules to maximize reproduction. But most plants do not experience optimal growth conditions and hence they inevitably abort some of these ovules. Schussler and Westgate (1995) stated that dramatic drop in photosynthesis results in reduced sugar transport into the embryo sac and in effect, it limits resources for seed development.

Seed abortion, which occurs at different developmental stages within the fruit, has been shown to be non-random and based on the genotype of the embryo

(Korbecka *et al.*, 2002); however, lower seed: ovule ratios can also result from the abortion of unfertilized ovules within the fruit (hereafter, ovule abortion). Once fertilization has occurred, fruit and seeds start to mature. However, not all will mature. There are several factors responsible for the selective abortion of ovules and seeds at different stages of development (Charleswhorth, 1989). The seed: ovule and the fruit: flower ratios are the main parameters for evaluating species fecundity and can be used to measure the degree of reproductive efficiency of a population (Cruden, 1972).

Many workers suggest that pollen competition may be an important component of natural selection through gametophytic selection (Mulcahy, 1979; Snow, 1986; Mulcahy and Mulcahy, 1987; Walsh and Charlesworth, 1992). Shaanker and Ganeshaiah (1990) found that pollen deposition patterns regulate the seed number per fruit in multi-ovulated species. In *Dalbergia sissoo*, a tropical tree with wind-dispersed pods, exhibits a highly positively skewed distribution of seeds per pod with predominantly only one of the four or five ovules maturing into seed. The abortion cannot be attributed to lack of pollen or resources. The abortion may be due to an intense rivalry among the developing sibs to gain dispersal advantage (Raju *et al.*, 1996). In some of the leguminous species studied, seed abortion has been found to depend on pollen quality and on the position of the ovule or fruit. The direct consequence for the mother plant is that fruit costs increase as the seed: ovule ratio decreases. However, because ovule abortion occurs earlier than does seed abortion, the former can reduce the biomass invested per seed (i.e., fruit costs) more efficiently than does the latter (Ana Calvino, 2014). Several authors, Levin (1972), Bawa and Webb (1984), Wiens *et al.* (1987) and Dalling and Hubbell (2002), argue that the pollination mechanism can be a limiting factor for fruit and seed abortion and consequently, in the reproductive success of a species.

### 5.7 *Flower Visitors*

Five insect species from three orders Hymenoptera, Thysanoptera and Lepidoptera were observed, collected, and identified from the flowers of *Aesculus indica.* The inflorescence received most *Bombus spp.* in the morning hours from 06:00- 08:00 which is also the peak anthesis time and *Tagiades menaka* during the evening hours. It was noticed that *Bombus spp.* and Butterfly were not observed at the same timing of the day; both of them had different visiting hours. Bumble bees tended to visit the

outward facing flowers and the flowers at the base of the panicle first. Most pollinators to visit the flowers were observed between 08:00-10:00 hours of the day. Even though insects visit the flowers but still the fruit formation is very low when compared to the number of flowers and pollen produced. This may be because after visiting one flower and collecting the pollen it will visit the flower having underdeveloped style as Bertin (1980) has stated that there is no difference in the visitation rates of insects between the staminate or complete flowers which will not result in the fruit formation or it may be because the pollinators visit the flowers with the intention of robbing the pollen from the flower. Most pollen theft occurs by vectors that pollinate other plants and the most frequent pollen thieves are bees (Hargreaves *et al*., 2009). Pollen theft occurs because of mismatched pollination vectors and flowers in terms of flower structure (particularly with regard to sexual dichotomy) and timing of differential sex maturation. Several strategies have been documented which allow plants to either tolerate pollen theft or resist pollen theft. One tolerance strategy is increasing pollen production (through an increase in flower numbers or pollen quantity per flower). This is suggested as a form of masting and an adaptive advantage of a mass-flowering strategy (Hargreaves *et al*., 2009).

### 5.8 *Reproductive Success in different crown layer*

In *Aesculus indica*, there is significant variation between total number of flowers produced and a total number of fruit set. *Aesculus indica* exhibits relatively lower percentage of fruit set. Reproductive success of trees depends not only on the total fruit yield but also quantity of seeds produced, successful seed dispersal and germination. Reproductive success in plants results from a number of processes. Fruit production (the number of seeds that the tree produces initially) is the starting point for measuring reproductive potential. Fruit production in individuals can be affected by factors such as the amount of available resources (e.g., light, water, and nutrients) and competition by the surrounding neighborhood of plants for those resources (Lee and Bazzaz, 1982; Graham *et al*., 2003).

Reproductive success in *Aesculus indica* was found to be more in the upper canopy layer of the tree likely to be the effect of optimum conditions such as temperature, light availability, high photosynthetic rate and free of biotic pressure. Fruit development depends strongly on photosynthesis and light availability (Brady, 1987; Raven *et al*., 1992; Taiz and Zeiger, 1998). Variation in light availability can affect photosynthetic

rate, photosynthate production, dry matter allocation, and various leaf phenotypic traits and physiology including leaf conductance, chloroplast size, and mean stomatal densities (Lynch and Gonzalez, 1993; Mehrotra *et al*., 1998; Proietti *et al.*, 2000; Raven *et al.*, 1992; Sellin and Kupper, 2005; Taiz and Zeiger, 1998). Because leaves in the upper part of trees shade those below, one would expect a vertical stratification of light within the tree. Consequently, upper leaves are expected to produce more photosynthates than lower, shaded leaves. Zones of the canopy that receive more sunlight are expected to distribute more photosynthates locally, from the leaf to the adjacent fruit: local translocation of resources hypothesis (Lynch and Gonzalez, 1993; Proietti *et al.*, 2000; Raven *et al*., 1992; Taiz and Zeiger, 1998).

It is possible that the putative intra tree vertical stratification of fruit varies as a function of light availability or other factors (Houle *et al*., 2007). It is particularly true that the upper crown represents a sunnier, warmer, and drier microhabitat than the lower crown layers. Upper crowns produced fruits were bigger, more abundant, and in higher densities than those in lower crown layers of *A. indica*.

# CHAPTER - 6

# CONCLUSION

# 6. CONCLUSION

The information on floral biology, pollination, and breeding system is prerequisite for knowing the life history of *Aesculus indica* for initiation of future breeding works and its effective management. The inflorescence type of *A. indica* is compound raceme (Tyrsus) bearing terminal panicles, broad at the base forming a pyramidal shape. The flowers are pedicellate and are zygomorphic in nature. Two types of flowers have been observed in these species one with the developed style (perfect) and other with underdeveloped style (male), the sex expression of *A. indica* is andromonoecious in nature. The term andromonoecious nature (having perfect and staminate flowers on the same plant) best characterized the flowering biology of this genus since both functionally staminate flowers and at least a few complete flowers could be found within individual plants. The functionally staminate flowers have a vestigial gynoecium with an undeveloped ovary and stunted style. Complete flowers have fully developed androecium and gynoecium. The production of flowers with developed and under developed style within an inflorescence is significant. The distribution of flowers with developed style was uneven. The flowers with underdeveloped style does not produce fruits and all the fruits produced by flowers having developed style does not mature as pre mature fruit fall was observed during June and July. The fruit drop during this period is necessary to balance crop load and improve fruit size. Reproductive phenological studies in *A. indica* shows that the flower bud initiation takes place in the month of March and the first fruit developed in the last week of April fully ripened in October to November and took an average duration of 7.5 months for the completion of one reproductive cycle. Floral characteristics play an important role in facilitating both pollen transfers from anthers and deposition on stigmas. A detailed understanding of the floral biology needed for performing effective breeding programme.

In *Aesculus indica* diurnal pattern of anthesis was recorded. The flowering pattern of *A. indica* was asynchronous *i.e*. buds and flowers are at different stages of development even on the same tree. Within a cincinnus, the flower bud closest to the rachis was the first to mature and subsequent development proceeded outward. The average time taken for complete blooming of one inflorescence was 56.24 ± 1.85 days. The peak period of blooming was recorded between morning hours 06:00 to 08:00 of the day at all the selected trees for which the anthesis percent ranged from 49.7% to 52.87%. Anthesis has no significant effect of tree to tree difference but it has

significant effect for variation in time. As the anthesis is an active process based on the physiological and ecological behavior of a plant. Flowers open at the definite time of the day. This is due to change in the microclimatic conditions like temperature, humidity, rainfall, and photoperiod varied from time to time in a day. It was conformed that anthesis in a species is an interactive results of genetic, physiological and microclimatic factors. The knowledge of anthesis in *A. indica* is the prerequisite to understand the ecological background of pollen dispersal.

The copious pollen production in *A. indica* was found to be characteristic feature of anemophily. An average pollen production per flower ranged between 53183.69 ± 643 to 54177 ± 1189 and 18363790 ± 476673 to 23914032 ± 524987 per inflorescence and 1010008499 ± 26217045 to 8078958841 ± 97679046 per tree. The number of pollen grains per anther and per flower in *A. indica* has not been considered of much difference which reveals that the total pollen production per flower within a tree is genetically fixed but slight variation arises due to the morphology and microclimatic conditions. However, the total pollen production among trees has significantly varied. The total pollen output among trees to be varied with differences in the number of total inflorescences, flower production, and effect of climate on trees. The variation of pollen production and pollen viability in between flowers with developed style and flowers with underdeveloped style was not significant. The effect on pollen germination due to time interval and sucrose concentration is significant. In nature water, sugar, amino acids are supplied by the style to nourish the growing pollen tube but it is clear that the flowers with the underdeveloped style is too short to provide the desired condition for the growth of pollen tube results failure of pollen germination.

The results on controlled pollination suggest predominantly self-incompatibility in *A. indica* as there is no fruit setting observed from autogamous self-pollination and the percentage of fruit set in open cross pollination was found to be slightly more than open pollination. The average seed set per fruit is one in all the pollination treatments. Nevertheless, the pollen ovule ratio indicates that the breeding system of the species supporting xenogamy. Pollination observations have shown that wind pollination in *A. indica* is likely to be assisted by insects. The species is mostly visited by *Bombus spp.* during the morning hours and *Tagiades menaka* during the evening hours. The high pollen production, the high pollen ovule ratio, and vertically projected inflorescence are also the characteristic features of wind pollinations. The magnitude of fertilization

success, which was larger in the case of artificial pollination than obligatory selfing, indicated that pollinating agents are responsible for a better success.

The result of fruit production is very less when compared to the total flower production in *A. indica*. The number of seed formed per fruit is one indicating that the percentage of seed set and fruit set are equal. The seed set varied for different canopy layers, in lower canopy it ranged from 0.53% to 0.63%, in middle canopy 0.63% to 0.84% and in upper canopy 0.82% to 1.01%. The number of ovules per ovary was 6. The number of ovules that converted into seed was one, likely to be results of high rates of ovule abortion. It may be due to pollen competition for natural selection through gametophytic selection or the abortion may be due to an intense rivalry among the developing sibs to gain dispersal advantage. The reproductive success (Number of fruits/ number of flowers) in canopy layer showed marked variations. The variation in reproductive success among the canopy layers may be the effect of optimum conditions such as temperature, light availability, high photosynthetic rate and free of biotic pressure. So the present investigations about the floral biology and reproductive effort of *A. indica* will assist to study the breeding system, pollination ecology, pollen – pistil interactions, distribution and composition of the *A. indica* populations in future.

# SUMMARY

## SUMMARY

The present investigation entitled "Floral biology and Reproductive Effort of *Aesculus indica*(Wall. exCamb.) Hook: An Andromonoecious species in Temperate region of Garhwal Himalaya, India", has conducted from April 2016 to November 2016. The genus *Aesculus* has an unusual reproductive strategy including a large portion of functionally staminate flowers, and a high microspore to mature seed ratio. Reproductive biology is important to combine phenology, pollinator's activity and incompatibility systems as a whole approach in studying reproductive ecology. The knowledge of reproductive biology is essential for the effective protection of plants, especially for species with small populations, restricted distributions and to improve desired traits or to develop new varieties. The study has been framed to find out the objectives of (1) Examination of floral developmental stages and floral architecture along with anthesis and stigma receptivity in relation to the time of the day and associated weather conditions, (2) Estimation of variability in pollen production, pollen viability, and pollen germinability, (3) Determination of reproductive success and reproductive potential in terms of fruit and seed set percentage in different crown layers and (4)Documentation of major pollen vectors of *Aesculus indica*. For each objective standard methodologies have been given in the chapters. It includes 6 Chapters. The brief summary of the study has been described below.

1) **Floral morphology, structure, and development-** The studies pertaining to floral, structure and development were recorded from the very beginning of floral bud initiation by tagging randomly selected 20 inflorescences from three sample trees. The morphological parameters were measured and recorded from twenty randomly selected flowers.
2) The *Aesculus indica* tree sex expression is andromonoecious in nature as it bears two types of flowers one with the developed style can be called functional flower and underdeveloped style can be taken as the male flower. Floral morphology studies showed that tree produces compound raceme (Tyrsus) bearing terminal panicles inflorescence type. Total no. of flowers/ inflorescence is 341.9±65.67 to 441.4±74.62 out of which 13.3 ± 3.19 to 20.15 ± 6.68 is the flowers with developed style. The average time taken for all the buds in one inflorescence to open was 56.24 ± 1.85 days. The mean number of days for fruit development from bud initiation was 142.34 ± 5.2 days.

3) **Anthesis-** Ten inflorescences were randomly tagged in three sample trees. Inflorescences were monitored daily at every two hour (0600h, 0800h, 1000h, 1200h, 1400h and 1600h) interval to determine the anthesis for successive days. Opened flowers were counted and removed to avoid error due to duplication and over counting.
4) In *A. indica* acropetal blooming pattern and asynchronous flowering was observed. The average time taken for the complete blooming of one inflorescence was 56.24 ± 1.85 days. Anthesis occurred between 06:00 to 16:00 hr of the day. The peak period of blooming was recorded between morning hours 06:00 to 08:00 of the day at all the selected trees for which the anthesis percent ranged from 49.7% to 52.87%. Anthesis has no significant effect of the tree to tree difference but it has the significant effect for variation in time.
5) **Pollen production-** The total pollen production in *A.indica* was conducted on buds near to anthesis. Pollen production within anther was estimated by Noon loop method using glycerol solutions. Pollen production within an anther, flower, inflorescence and tree was assessed subsequently (it was assessed separately for flowers with developed and under developed style).
6) An average pollen production per flower ranged between 53183.69 ± 643 to 54177 ± 1189 and 18363790± 476673 to 23914032 ± 524987 per inflorescence, and 1010008499 ± 26217045 to 8078958841 ± 97679046 per tree. The variation of pollen production in between flowers with developed style and flowers with underdeveloped style was not significant.
7) **Pollen ovule ratio-** Pollen-ovule ratio was determined by dividing the mean pollen quantity per flower by the mean ovule quantity per flower. For estimating the number of ovules per flower, cross section of ovary was taken and ovule number was counted.
8) There are six ovules per flower; hence the pollen ovule ratio varied from 8855 ± 65 to 9078 ± 198 pollen grains per ovule in flowers with developed style and 8863 ± 107to 9029 ± 198 pollen grains per ovule in the flowers with under developed style for *Aesculus indica.* There is not much variation in the pollen ovule ratio between two types of flowers. Thus, the pollen ovule ratio of *Aesculus indica* falls within the reported range of Xenogamy.

9) **Stigma receptivity-** Stigma receptivity was determined by immersing stigma of flowers in three percentage aqua's hydrogen peroxide; bubbling indicated that peroxide activity and stigma receptivity.

10) The stigmas of both flowers with developed style and flower with under developed style were receptive. The stigmas were receptive before the opening of bud. This implies that it possesses protogyny (dichogamy). The receptivity retained for a period of five to six days. The peak period of stigma receptivity was on the first day of flower opening. The receptivity constantly reduces from the first day to the sixth day.

11) **Pollen viability-** Pollen viability was assessed by using acetocarmine staining methods on pollen grains in *in vitro* condition. Five anthers were selected from five inflorescence of each sample tree. Pollens were dusted on a clean microscopic slide with hair brush in which a drop of 0.2% acetocarmine solution added on it and was kept for 10-15 minutes to allow the pollen to absorb the stain. The slides were then examined under the microscope.

12) The percentage of viable pollen per slide ranged between 87.54 ± 4.02% to 90.04 ± 0.82% in flowers with developed style and 85.34 ± 3.23% to 89.57 ± 3.65% in flowers with under developed style. Not much variation was observed in pollen viability percent between the two flower types.

13) **Pollen germination-** Pollen germination test *in vitro* on *A. indica* was done by taking pollen grains separately from flowers with developed and under developed style and was germinated in sucrose (10%, 20% and 30%). Pollen was collected from undehised anthers of flowers of different inflorescences. The germinated pollen grains were observed under microscope from each solution at an interval of (1h, 2h, 3h, 5h, 8h, and 24h) in order to check the percentage increment in germination with varying time under different solutions.

14) Among the sucrose concentration, maximum germination of 87.57% in flowers with developed style and 87.18 % in flowers with under developed style was observed in 20% sucrose concentration. The maximum percent of germination was observed in 5 hours after immersing in the media in both the flowers. Significant effect of both time interval and sucrose concentration was observed.

15) **Breeding system-** Assessment of breeding system was done by applying different treatments on randomly selected twenty inflorescences each on the selected trees. The breeding system examined by different pollination treatments

were obligate selfing, open cross pollination, natural pollination and apomixes under field condition. The fruit set and seed set was assessed in each treatment.

16) There is not much difference on fruit set between the cross pollination and the open cross pollination. The reproductive success was 0.367 for open cross pollination 0.367 and 0.267 in case of open pollination. Reproductive potential was same for both open cross pollination and open pollination (0.167). Only one ovule gets converted into seed. No fruits formation from the bagged flowers which demonstrates that *A. indica* does possess self-incompatibility.

17) **Flower visitors-** Determination of pollinators and their visitation rates was done by counting the visiting insect species on all the chosen trees manually. Each tree was observed over the course of the whole day from 0600 to 1600h in every 2 hr interval.

18) Five insect species from three orders Hymenoptera, Thysanoptera and Lepidoptera were observed, collected, and identified from the flowers of *Aesculus indica.* Among the pollinators, the most frequent visitors in flowers were *Bombus spp.* and Thrips. No differences between the visitation rates by insects to staminate (under developed) or complete (developed) flowers. The inflorescence received most *Bombus spp.* in the morning hours from 06:00-08:00 and almost absent during the noon. The insect *Tagiadesmenaka* was found more frequently during the evening hours from 16:00 - 18:00.

19) **Reproductive success in different crown layer-** The crown length of the selected sample trees was measured and accordingly the total crown length was divided into three equal portions namely upper canopy layer, middle canopy layer and lower canopy layer. The average number of flowers per inflorescence was estimated by counting 20 randomly selected inflorescences in each sample trees. The total number of inflorescence in different canopy layer was estimated by manual counting. The average number of flowers per inflorescence was then multiplied by the number of inflorescence to get total flower production in different canopy layers. The fruits were then counted in different canopy layers evaluated the reproductive success. Reproductive potential estimated by the ratio of average seed set from a flower with average number of ovules within the flower.

20) Total number of fruit set per inflorescence in lower canopy layer ranges from 2 ± 0.63 to 2.45 ± 1.39, 2.33 ± 1.07 to 3.25 ± 1.95 in middle canopy layer and 3.47

± 2.09 to 3.75 ± 2.64 in upper canopy layer. The seed set percentage of *Aesculus indica* ranges from 0.53% to 0.63% in the lower canopy, 0.68% to 0.84% in middle canopy and 0.82% to 1.01% in upper canopy layer. The estimated reproductive potential was 0.167.

**21)** Based on the present study it could be concluded that the sex expression of the tree can be characterized as andromonecious since both functionally staminate flowers and complete flowers were found within individual plants. Reproductive phenological studies in *A. indica* shows that the flower bud initiation takes place in the month of March and the first fruit developed in the last week of April fully ripened in October to November and took an average duration of 7.5 months for the completion of one reproductive cycle. In *A. indica* diurnal pattern of anthesis was recorded. Asynchronous flowering pattern was observed in *A. indica.* This is due to change in the microclimatic conditions like temperature, humidity, rainfall, and photoperiod varied from time to time in a day. From the study it was observed that *A. indica* was pollinated by both insects and wind. The results on controlled pollination suggested self-incompatibility in *A. indica.* The fruit set and seed set shows remarkable variations among crown layers.

**22)** Overall, present findings about the floral biology and reproductive effort of *A. indica* providing some important information regarding the reproductive biology of the species; it will assist to study the breeding system, pollination ecology, pollen – pistil interactions, distribution and composition of the *A. indica* populations in future.

# PHOTO PLATES

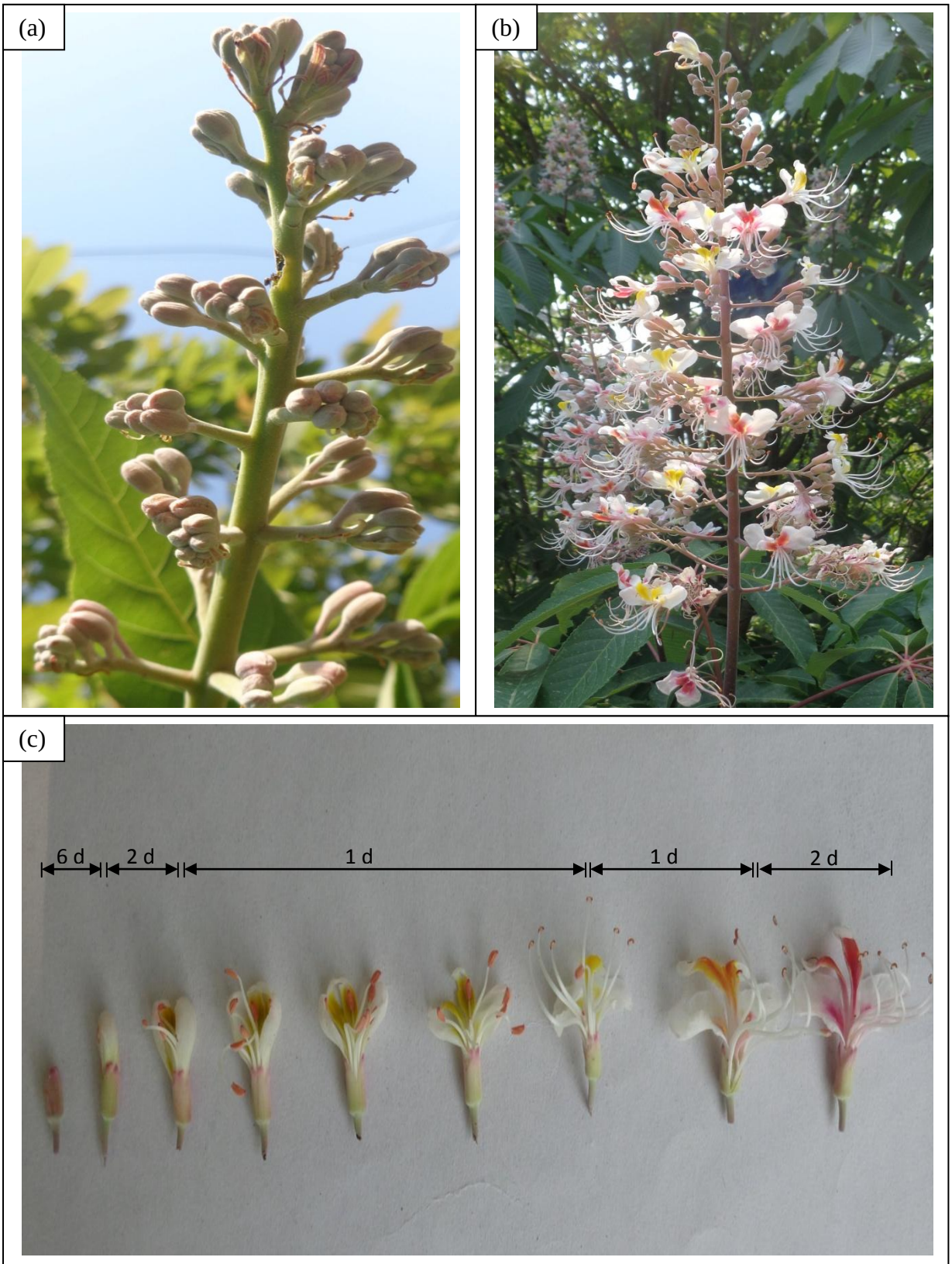



**Plate 1:** Flower developmental stages; (a) Bud stages; (b) Flowering stage; (c) Developmental stages from flower opening to senescence (d= Time intervals in days)

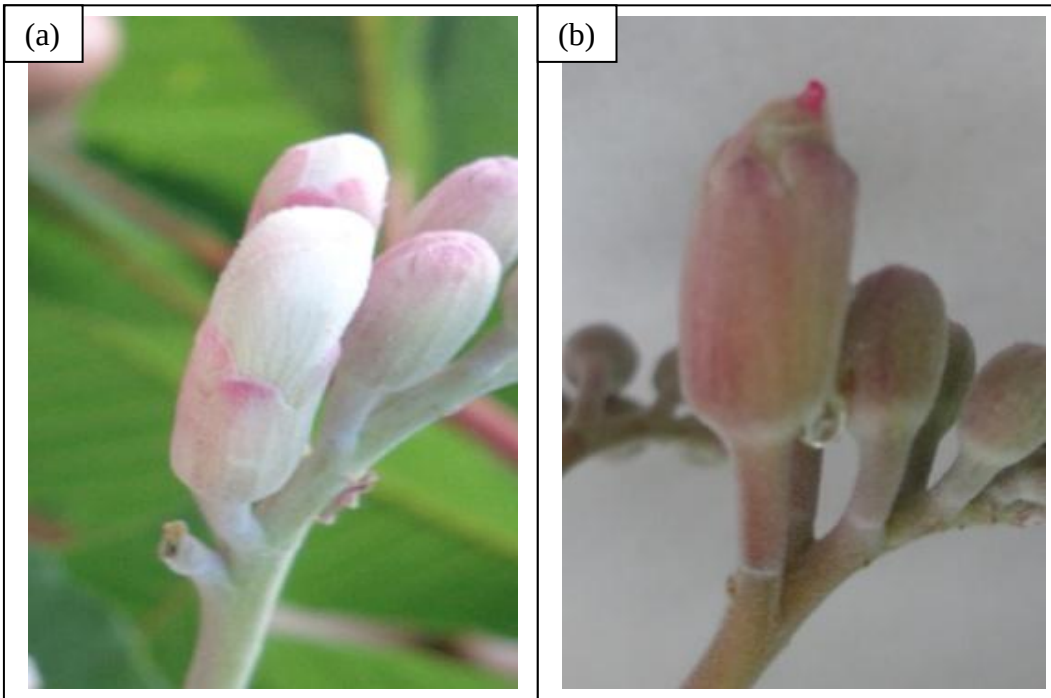



**Plate 2:** Bud stage of flowers with (a) underdeveloped (b) developed pistil

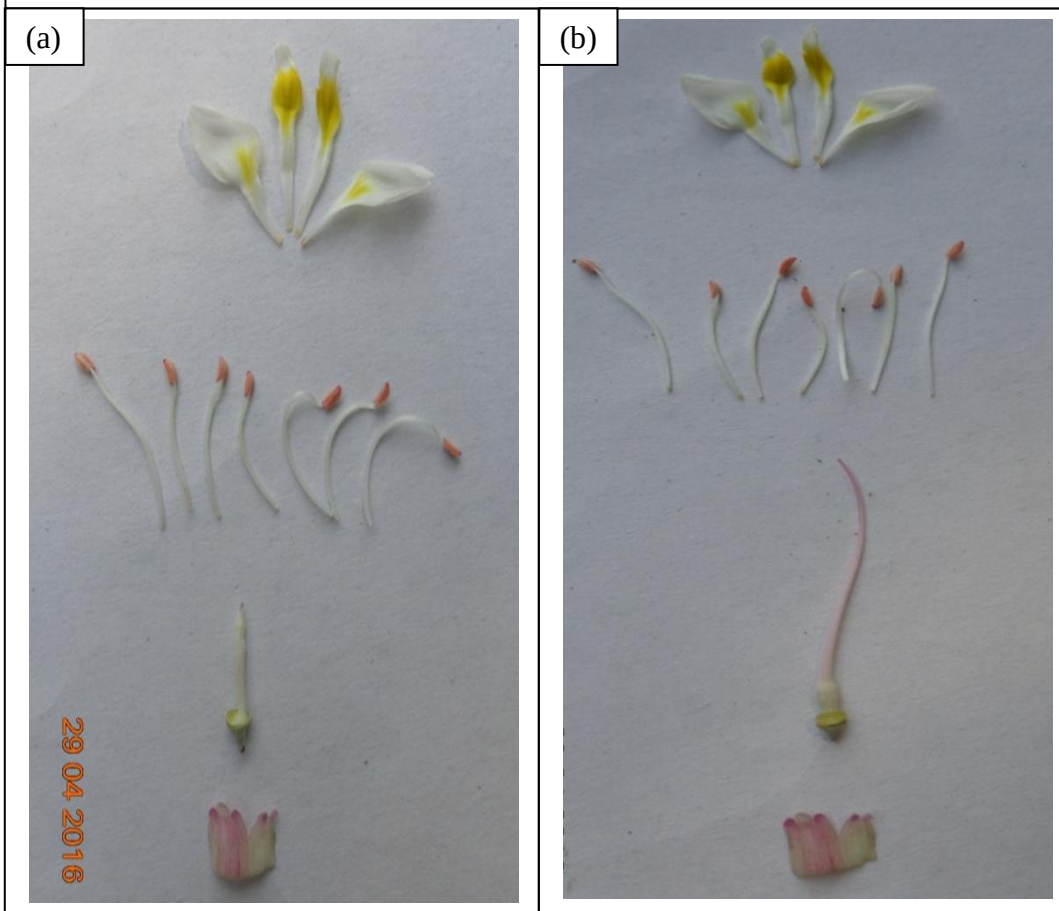



**Plate 3:** Floral parts of flower having (a) underdeveloped (b) developed pistil

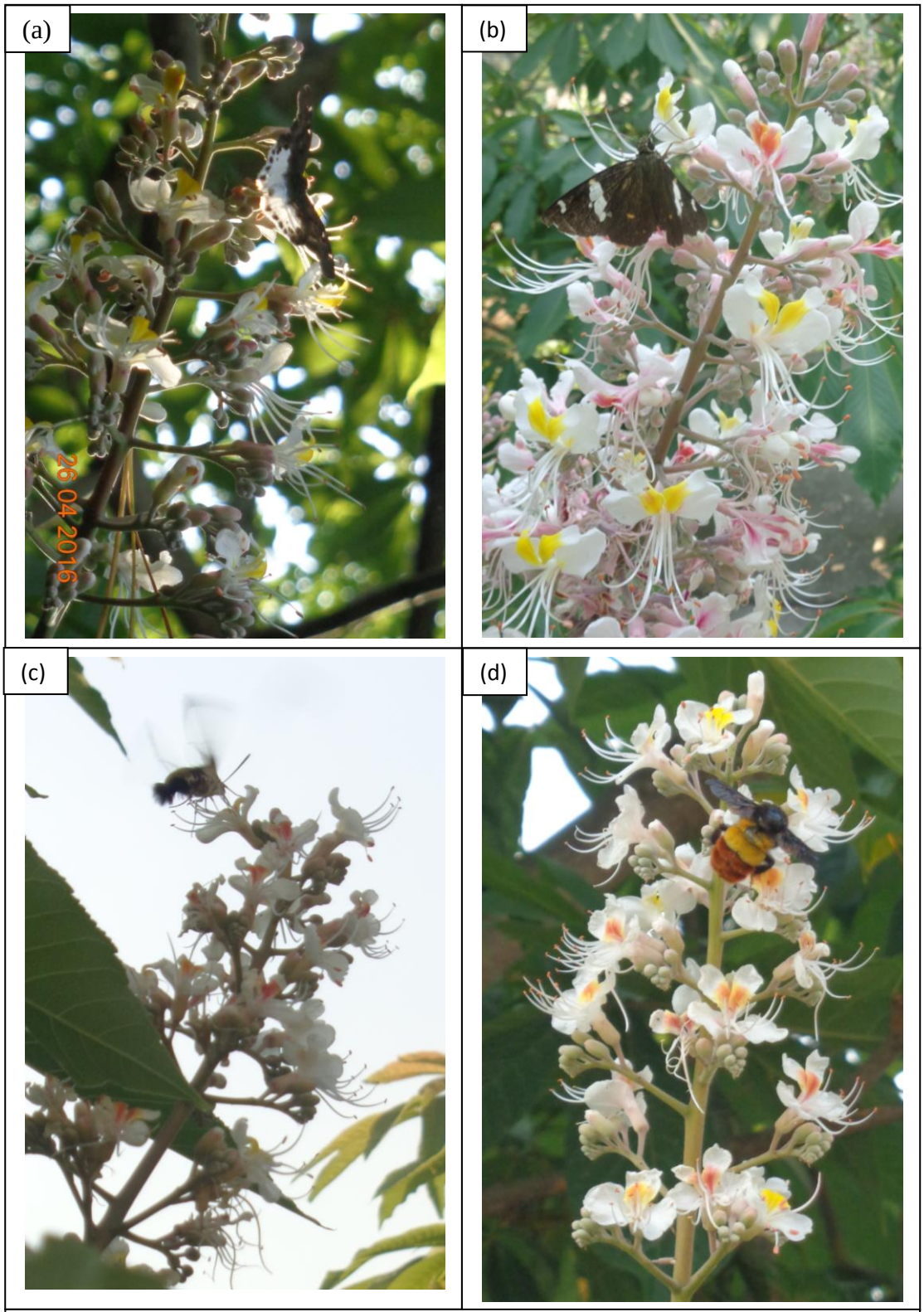



**Plate 4:** Pollinators of *Aesculus indica* (a) *Tagiades menaka*, (b) *Celaenorrhinus munda* , (c) *Hemaris spp.* and (d) *Bombus spp.*

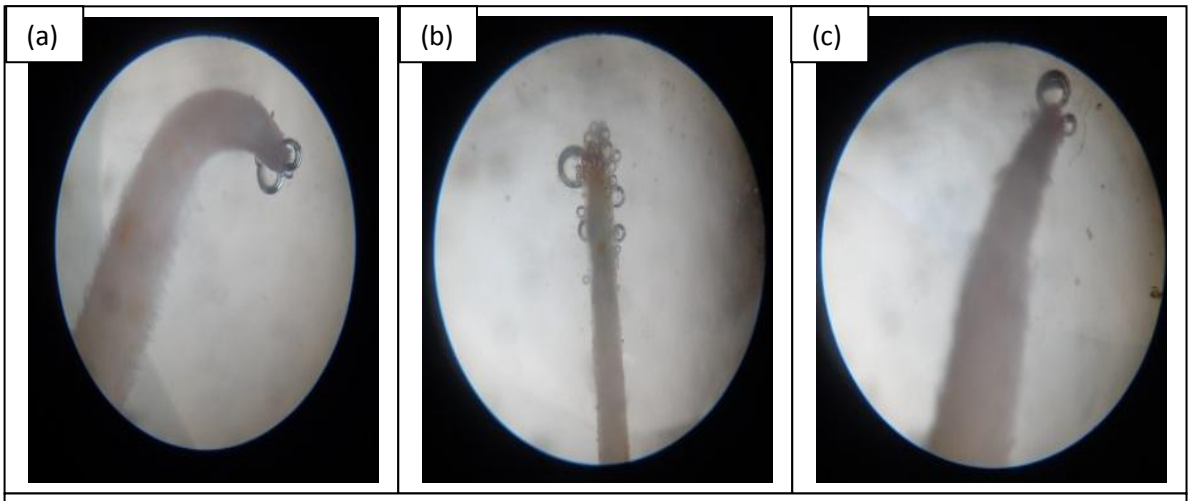



**Plate 5:** $H_2O_2$ testing for stigma receptivity in (a) flower prior to opening, (b) first day of flower opening and (c) fifth day of after flower opening

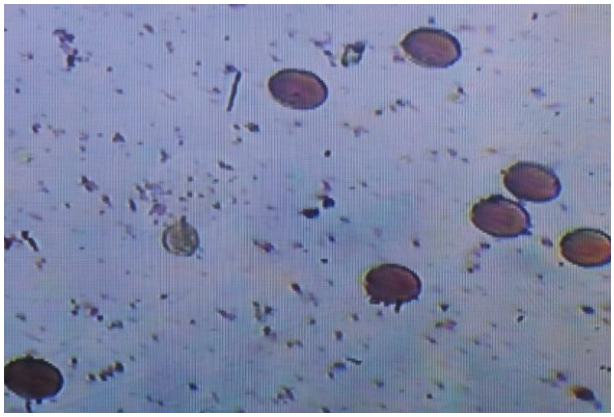


**Plate 6:** Pollen viability test (round and coloured are viable pollen and shrunk and colourless is non viable pollen)

**Plate 7:** Ovules of one flower

**Plate 8:** Riped fruit of *Aesculus indica*

**Plate 9:** Seed and the aborted ovule of a flower

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# APPENDICES

# APPENDICES

**Appendix-1:** Meteorological data showing maximum and minimum temperature from 20 April to 19 June 2016

| | max | min | mean | | max | min | mean |
|---|---|---|---|---|---|---|---|
| 20-30 Apr | 25.6 | 15 | 20.3 | 21-30 May | 27.6 | 18.4 | 23 |
| | 27.6 | 14.8 | 21.2 | | 25.4 | 16.4 | 20.9 |
| | 25.8 | 15 | 20.4 | | 24.8 | 16.4 | 20.6 |
| | 24.8 | 10.2 | 17.5 | | 25.9 | 18.4 | 22.15 |
| | 23.3 | 11.4 | 17.35 | | 27.6 | 12.8 | 20.2 |
| | 23 | 11.6 | 17.3 | | 24.4 | 13.1 | 18.75 |
| | 24.5 | 13.4 | 18.95 | | 24.9 | 13.5 | 19.2 |
| | 26.8 | 14 | 20.4 | | 24.8 | 16.2 | 20.5 |
| | 26.7 | 13.8 | 20.25 | | 27.7 | 15.9 | 21.8 |
| | 26.6 | 14.5 | 20.55 | | 27.2 | 11.9 | 19.55 |
| 01-10 May | 25.7 | 13.4 | 19.55 | 31 May-9 Jun | 22 | 14.1 | 18.05 |
| | 26 | 14.8 | 20.4 | | 22.7 | 12.5 | 17.6 |
| | 28.6 | 17.4 | 23 | | 23 | 15.2 | 19.1 |
| | 28.8 | 17.4 | 23.1 | | 25 | 16.9 | 20.95 |
| | 25.5 | 14.2 | 19.85 | | 25.5 | 17.1 | 21.3 |
| | 26.5 | 12.7 | 19.6 | | 24.8 | 19 | 21.9 |
| | 23.5 | 10.4 | 16.95 | | 25.9 | 19.4 | 22.65 |
| | 23.4 | 12 | 17.7 | | 25.2 | 14.6 | 19.9 |
| | 22.4 | 16.1 | 19.25 | | 25.8 | 14.9 | 20.35 |
| | 23.2 | 16 | 19.6 | | 18.9 | 14.5 | 16.7 |
| 11-20 May | 29.9 | 16.5 | 23.2 | 10- 19 Jun | 25.6 | 16 | 20.8 |
| | 22.4 | 14.5 | 18.45 | | 25.6 | 16.4 | 21 |
| | 24.8 | 13 | 18.9 | | 27.2 | 15.8 | 21.5 |
| | 24.2 | 14.1 | 19.15 | | 27.4 | 16.2 | 21.8 |
| | 25.6 | 15.9 | 20.75 | | 27.8 | 16.5 | 22.15 |
| | 26.2 | 15.4 | 20.8 | | 27.3 | 15.1 | 21.2 |
| | 28.3 | 15.9 | 22.1 | | 23 | 16.8 | 19.9 |
| | 28.8 | 17.1 | 22.95 | | 23.5 | 16.5 | 20 |
| | 29.2 | 17.9 | 23.55 | | 21.5 | 16.4 | 18.95 |
| | 29.2 | 17.8 | 23.5 | | 24.2 | 17.8 | 21 |

**Appendix-1.1:** Meteorological data showing maximum and minimum relative humidity from 20 April to 19 June 2016

| | max | min | mean | | max | min | mean |
|---|---|---|---|---|---|---|---|
| 20-30 Apr | 50 | 28 | 39 | 21-30 May | 82 | 62 | 72 |
| | 51 | 37 | 44 | | 81 | 66 | 73.5 |
| | 42 | 37 | 39.5 | | 51 | 61 | 56 |
| | 47 | 25 | 36 | | 90 | 56 | 73 |
| | 43 | 19 | 31 | | 76 | 43 | 59.5 |
| | 30 | 19 | 24.5 | | 52 | 44 | 48 |
| | 26 | 14 | 20 | | 55 | 52 | 53.5 |
| | 27 | 14 | 20.5 | | 63 | 50 | 56.5 |
| | 33 | 17 | 25 | | 62 | 85 | 73.5 |
| | 31 | 23 | 27 | | 87 | 68 | 77.5 |
| 01-10 May | 32 | 18 | 25 | 31 May-9 Jun | 92 | 65 | 78.5 |
| | 34 | 22 | 28 | | 72 | 63 | 67.5 |
| | 37 | 28 | 32.5 | | 83 | 66 | 74.5 |
| | 42 | 52 | 47 | | 75 | 74 | 74.5 |
| | 54 | 35 | 44.5 | | 91 | 75 | 83 |
| | 64 | 50 | 57 | | 88 | 68 | 78 |
| | 75 | 44 | 59.5 | | 86 | 64 | 75 |
| | 69 | 55 | 62 | | 80 | 58 | 69 |
| | 76 | 63 | 69.5 | | 81 | 89 | 85 |
| | 77 | 51 | 64 | | 71 | 52 | 61.5 |
| 11-20 May | 73 | 96 | 84.5 | 10- 19 Jun | 57 | 54 | 55.5 |
| | 83 | 62 | 72.5 | | 59 | 54 | 56.5 |
| | 77 | 61 | 69 | | 77 | 57 | 67 |
| | 75 | 61 | 68 | | 63 | 77 | 70 |
| | 63 | 48 | 55.5 | | 67 | 64 | 65.5 |
| | 59 | 65 | 62 | | 84 | 78 | 81 |
| | 52 | 36 | 44 | | 96 | 84 | 90 |
| | 55 | 35 | 45 | | 100 | 90 | 95 |
| | 50 | 40 | 45 | | 91 | 71 | 81 |
| | 66 | 44 | 55 | | 84 | 85 | 84.5 |

# ABSTRACT

## ABSTRACT

**Name: Priya Pradhan** **I.d. No.: UUHF/15341**
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**Topic: "Floral biology and Reproductive Effort of *Aesculus indica* (Wall. exCamb.) Hook: An Andromonoecious species in Temperate region of Garhwal Himalaya, India"**

*Aesculus indica* a large deciduous tree is widely distributed along the Himalayan lowlands. The studies in relation to floral biology and reproductive effort of *A. indica* conducted at Ranichauri exhibited that the sex expression of *A. indica* is andromonoecious. Total number of flowers per inflorescence was 341.9 ± 65.67 to 441.4 ± 74.62 of which13.3 ± 3.19 to 20.15± 6.68 flowers were with developed style. An average duration of 7.5 months is required for the completion of one reproductive cycle. The flowering pattern of *A. indica* was asynchronous. The peak period of anthesis was recorded between 06:00 to 08:00 hrs in a day. Pollen production (2016) per tree varied from 1010008499 ± 26217045 to 8078958841 ± 97679046. Viable pollen of 87.54 ± 4.02% to 90.04 ± 0.82% and 85.34 ± 3.23% to 89.57 ± 3.65% were observed in flowers with developed and underdeveloped style respectively. Maximum germination was observed in 20% sucrose concentrations. The pollen ovule ratio varied from 8855 ± 65.38 to 9078.61 ± 198.43 (developed) and 8863.95 ± 107.17to 9029.61 ± 198.23(underdeveloped) flowers supporting xenogamy. Pollination observations have shown that *A. indica* being pollinated by wind while insects also play a major role in performing pollination. Major visitors were *Bombus spp*. and *Tagiades menaka*. The study demonstrates that *A. indica* donot have much difference on fruit set between the cross pollination and the open cross pollination but it possesses a type of barriers to selfing. The average seed set per fruit is one in all the pollination treatments. The seed set and fruit set varied for different canopy layers, *i.e.*, 0.53% to 0.63% (lower canopy), 0.68% to 0.84% (middle canopy) and 0.82% to 1.01% (upper canopy). The reproductive success in canopy layer showed marked variations. Therefore, the current findings may be useful in studying breeding system, pollination ecology, pollen – pistil interactions, distribution and composition of the *A. indica* populations in future.

(Priya Pradhan)

## सारांश

**नाम: प्रिया प्रधान** **आई. डी. न.: यू. यू.एच.एफ./15341**
**डिग्री: एम. एस. सी. वानिकी (वृक्ष सुधार)** **प्रमुख: वृक्ष सुधार**
**प्रवेशकावर्ष: अगस्त 2015** **विभाग: वानिकी**
**वी. सी. एस. जी. यू. यू. एच. एफ., वानिकी महाविद्यालय, रानीचौरी**
**सलाहकार: डॉ. वी. पी. खंडूरी (सह-प्राध्यापक)**

**विषय: *एस्प्यूलस इंडिका* (Wall.exCamb.) भारत में गढ़वाल हिमालय के शीतोष्ण क्षेत्र की एक एंड्रोमोएनेएएस प्रजाति का पुष्प विज्ञान एवं प्रजनन प्रयास।**

*एस्क्यूकलस इंडिका* एक बड़ा, पर्णपाती वृक्ष है तथा हिमालय के निचले इलाकों के साथ व्यापक रूप से वितरित है। रानीचौरी में *ए. इंडिका* के पुष्प विज्ञान और प्रजनन प्रयासों के संबंध में किये गये अध्ययन ने यह दर्शाया कि *ए. इंडिका* के लिंग वर्णन एंड्रोमोएनेएएस है। प्रत्येक पुष्प क्रम में पुष्पों की संख्या 341.9 ± 65.67 से 441.4 ± 74.62 थी, जिसमें कि 13.3 ± 3.1 9 से 20.15 ± 6.68 पुष्प विकसित वर्तिका वाले थे। एक प्रजनन चक्र के पूरा होने के लिए 7.5 महीने की औसत अवधि आवश्यक है। *ए. इंडिका* का पुष्पन अतुल्यकालिक था। एक दिन में एनाथीसिस की अनुकूल अवधि सुबह 06:00 से 08:00 बजे के बीच दर्ज की गई। प्रति वृक्ष परागकणों के उत्पादन में 1010008499 ± 26217045 से 8078958841 ± 97679046 भिन्नता पायी गयी। विकसित व अविकसित वर्तिका में क्रमश: 87.54 ± 4.02% से 90.04 ± 0.82% और 85.34 ± 3.23% से 89.57 ± 3.65% के जीवक्ष्य पराग कण पाये गये। अधिकतम अंकुरण सूक्रोजकी 20% सांद्रता में पाया गया। पराग बीजाणु अनुपात 8855 ± 65.38 से 9078.61 ± 198.43 (विकसित) और 8863.95 ± 107.17 से 9029.61 ± 198.23 (अविकसित) पुष्पों को एक्सनोगैमी के समर्थन से अलग करता है। परागण के अवलोकनों से पता चला है कि *ए. इंडिका* का परागण हवा द्वारा होs रहा है, जबकि कीटों की भी परागण करने में प्रमुख भूमिका देखी गई। *बोम्बस* और *टैगियडे मेनैका* प्रजातियां प्रमुख आगंतुक थे। अध्ययन दर्शाता है कि *ए. इंडिका* में पर-परागण और खुले पर-परागण के बीच फल स्थापन में ज्यादा अंतर नहीं होता है लेकिन स्वपरागण के लिए इसमें गतिरोध/ बाधा होती हैं। सभी परागण उपचारों में औसत बीज स्थापन एक बीज प्रति फलपाया गया। बीज स्थापन और फल स्थापन विभिन्न आच्छादन परतों के लिए मिन्न पाया गया जो कि क्रमश: 0.53% से 0.63% (निचला आच्छादन), 0.68% से 0.84% (मध्यम आच्छादन) और 0.82% से 1.01% (ऊपरी आच्छादन) था। आच्छादन परत में प्रजनन की सफलता में विविधताएं देखी गयी। अतःवर्तमान निष्कर्ष भविष्य में *ए. इंडिका* की आबादी की संरचना प्रजनन प्रणाली, परागण पारिस्थिति की, पराग–पुष्प योनि, पारस्परिक प्रभाव तथा वितरण का अध्ययन करने में उपयोगी होगा।

(प्रिया प्रधान)
शोधार्थी

# VITA

The author Priya Pradhan was born on January 12, 1994 at Gangtok, Sikkim. She graduated her High school education and intermediate from Paljor Namgyal Girls' Senior Secondary; Gangtok in 2009 and 2011. She received B.Sc. in Forestry from Central Agricultural University, Imphal in 2015. She joined V.C.S.G. University of Horticulture and Forestry, Bharsar in 2015 for perusing the degree of M.Sc. (Forestry) Tree Improvement through J.R.F. (ICAR-AIEEA-PG-2015).

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