Reproductive Phenology, Pollen Production and Pollen Mediated Gene Flow in *Alnus nepalensis* D. Don.

Thesis
By

ANCHAL BISHT
Id. No. - 18336

Submitted in partial fulfilment of the requirements for the degree of

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Department of Tree Improvement
College of Forestry, Ranichauri
Veer Chandra Singh Garhwali Uttarakhand
University of Horticulture and Forestry, Bharsar
Pauri Garhwal - 246123, Uttarakhand, India
2021

Veer Chandra Singh Garhwali Uttarakhand Universityof
Horticulture & Forestry, Bharsar, Pauri Garhwal
246123, Uttarakhand, India

Department of Forestry,
College of Forestry, Ranichauri

Prof. (Dr.) V.P. Khanduri

CERTIFICATE - I

This is to certify that the thesis entitled **"Reproductive Phenology, Pollen Production and Pollen Mediated Gene Flow in *Alnus nepalensis* D. DON"** submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Forestry in the discipline of Tree Improvement of VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, Uttarakhand is a bonafide research work carried out by **Ms. Anchal Bisht, Id. No. UUHF/18336** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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

Place: Ranichauri
Date: 28.10.2021

(V.P. Khanduri)
Chairman
Advisory Committee

CERTIFICATE-II

This is to certify that the thesis titled, **"Reproductive phenology, pollen production and pollen mediated gene flow in *Alnus nepalensis* D.DON"** submitted by **Ms Anchal Bisht, I.D. No. UUHF/18336** to VCSG Uttarakhand University of Horticulture & Forestry, Bharsar, Pauri Garhwal, Uttarakhand, India in partial fulfilment of the requirements for the degree of Master of Science **Forestry** in the discipline of **Tree Improvement** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner on **07th September, 2021 (11.00AM).**

07/09/2021

Chairman Advisory Committee
Dr. V.P.Khanduri
Professor

External Examiner
Dr. V.K. Sah
Professor(Retd), Forestry
G.B.P.U.A.&T., Pantnagar
Udham Singh Nagar, UK

Members, Advisory Committee

07/09/2021

(B.S. Butola)
Member

(R.S. Bali)
Member

(Sneha Dobhal)
Member

Head of the Department

Countersigned

Dean
College of Forestry, Ranichauri

CERTIFICATE - III

This is to certify that all the mistakes and errors pointed out by the external examiner have been incorporated in the thesis entitled "Reproductive Phenology, Pollen Production and Pollen Mediated Gene Flow in *Alnus nepalensis* D. DON" submitted to VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Pauri Garhwal, Uttarakhand, India, by Anchal Bisht, Id.. No. UUHF/18336 in partial fulfillment of the requirement for the award of Master of Scinence in Forestry in the discipline of Tree Improvement.

Prof. V.P. Khanduri
Chairman, Advisory committee

Dr. Amol Vasishth
Head, Department of Forestry
College of Forestry, Ranichauri

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Anachal Bisht
(Author)

ABSTRACT

Name	**: Anchal Bisht**	**Id. No. : UUHF/18336**
Year of Admission	**: August 2018**	**Degree: M.Sc. Forestry (Tree Improvement)**
Major	**: Forestry (Tree Improvement)**	**College: College of Forestry, Ranichauri**
Department	**: Forestry**	**V.C.S.G Uttarakhand University of**
Advisor	**: Dr. V. P. Khanduri**	**Horticulture and Forestry**
	(Professor)	

Alnus nepalensis is a medium sized, deciduous important tree not only occurs in the Indian sub-continent, but also in South America, Hawaii and China. It is cultivated in the hills of Himalayas around 2000 m (amsl). It is a multipurpose tree of Himalayan region growing in temperate and subtropical areas. Being non leguminous it has nitrogen fixing capacity due to the symbiotic nitrogen fixing bacteria, i.e., Frankia residing in its roots. It is a prolific pioneer species in freshly exposed soil in landslide affected areas. It is used as a commercial timber production. The study of reproductive phenology, pollen production, pollen germination and pollen flow was conducted at Ranichuari, Tehri Garhwal, Uttarakhand. The observation revealed that the *A. nepalensis* is a monoecious tree. The male flowers are composed of staminate flower called "cymules". Importantly, it was observed that, the presence of previous year female cone, bifid stigma and protoandry conditions was the unique feature of the species. Flowering in male phase was started in the last week of September and continued till November. Time between onset and peak flowering was 2 weeks 4 days and the total average duration of flowering period was about 24.8 days. Temperature played a major role in pollen release and maximum pollen was released in 30^0 C at 1300 hrs. Average pollen grains per tree were 2.20×10^{10}. However, pollen production varied significantly among trees.

The mean pollen viability was found 100%. The maximum pollen germination of 3.69% was observed in Boric acid (25 ppm), followed by Boric acid + Sucrose 10% (2.03% in 50 ppm). The pollen ovule ratio suggests that breeding system of *A. nepalensis* coming under the class xenogamy. Pollen mediated gene flow study was carried out to assess the travel distance of pollen of study species. It was observed that pollen of *A. nepalensis* can travel upto 80 m. Detailed research on reproductive phenology were conducted to determine the temporality of key events in a species' life cycle, pollen release in relation to temperature to determine the maximum pollen concentration in the atmosphere, pollen production to determine the reproductive success and health of a natural population, and long distance dispersal to eliminate population isolation. The current findings in *A. nepalensis* will be useful for formulating effective forest management plan and initiating various tree improvement programmes in future.

(V. P. Khanduri)
Advisor

(Anchal Bisht)
Author

सारांश

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संस्थान का नाम :वानिकी महाविद्यालय वीर चन्द्र सिंह गढ़वाली औद्यानिकी एवं वानिकी विश्वविद्यालय, रानीचौरी परिसर टिहरी गढ़वाल।

अलनस नेपलेंसिस एक मध्यम आकार का, पर्णपाती महत्वपूर्ण वृक्ष है जो न केवल भारतीय उप–महाद्वीप में, बल्कि दक्षिण अमेरिका, हवाई और चीन में भी होता है। इसकी खेती हिमालय की पहाड़ियों में लगभग 2000 मीटर (msl) पर की जाती है। यह हिमालय क्षेत्र में एक बहुउद्देशीय वृक्ष है जो समशीतोष्ण और उपोष्णकटिबंधीय क्षेत्रों में होता है।नॉन लेग्युमिनस होने के कारण इसमें नाइट्रोजन फिक्सिंग क्षमता होती है, जो सहजीवी नाइट्रोजन फिक्सिंग बैक्टीरिया अर्थात, इसकी जड़ों में रहने वाले फ्रेंकिया के कारण होता है। यह भूस्खलन प्रभावित क्षेत्रों में मिट्टी में अग्रणी उपजाऊ है। इसका उपयोग व्यावसायिक लकड़ी के उत्पादन के रूप में किया जाता है। वर्तमान प्रजनन संबंधी फेनोलॉजी, पराग उत्पादन, पराग के अंकुरण और पराग के प्रवाह का अध्ययन रानीचौरी, टिहरी गढ़वाल, उत्तराखंड में, किया गया। अवलोकन में पता चला है कि अ. नेप्लेन्सिस एक द्विलिंगी पेड़ है। महत्वपूर्ण रूप से, यह देखा गया कि, पिछले वर्ष मादा शंकु, बाइफिड कलंक और प्रोटॉन्ड्री स्थिति की उपस्थिति प्रजातियों की अनूठी विशेषता थी। नर चरण में फूल सितंबर के अंतिम सप्ताह में शुरू हुआ था और नवंबर तक जारी रहा। महत्वपूर्ण रूप से, यह देखा गया कि, पिछले वर्ष मादा शंकु, बाइफिड कलंक और प्रोटॉन्ड्री स्थिति की उपस्थिति प्रजातियों की अनूठी विशेषता थी। नर चरण में फूल सितंबर के अंतिम सप्ताह में शुरू हुआ था और नवंबर तक जारी रहा। आमतौर पर सबसे जयादा पराग मात्रा 1–2 सप्ताह तक चरम ग्रहणशीलता को बढाती है। शुरुआत और चरम फूलों के बीच का समय 2 सप्ताह 4 दिन था और फूलों की अवधि की कुल औसत अवधि लगभग 24.8 दिन थी। पराग मुक्ति में तापमान की प्रमुख भूमिका थी और अधिकतम पराग 30^{0} सेंटीग्रेड में 13.00 बजे जारी किया गया था। प्रति पेड़ औसत पराग कन 2.20×10^{10} थे। हालांकि, पेड़ों के बीच पराग का उत्पादन काफी भिन्न होता है। औसत पराग जीवन क्षमता 100 : पाई गई। बोरिक एसिड (25 पीपीएम में 3.69% का अधिकतम पराग अंकुरण देखा गया इसके बाद बोरिक एसिड सुक्रोज 10% (50 पीपीएम) में 2.03% अंकुरण देखा गया। पराग के अण्डाणु अनुपात से पता चलता है कि अ. नेप्लेनसिस की प्रजनन प्रणाली जेनो गामी के अंतर्गत आती है। पराग द्वारा जीन प्रवाह अध्ययन, प्रजातियों के पराग की यात्रा दूरी का आकलन करने के लिए किया गया था। यह देखा गया कि अ. नेप्लेन्सिस के पराग 80 मीटर तक यात्रा कर सकते हैं। एक प्रजाति के जीवन चक्र में महत्वपूर्ण घटनाओं की अस्थायीता निर्धारित करने के लिए वातावरण के अधिकतम पराग संकेंद्रण का निर्धारण करने के लिए, तापमान के संबंध में पराग जारी, और लंबी दूरी की फैलाव आबादी अलगाव को खत्म करने, एक प्राकृतिक आबादी की प्रजनन सफलता और स्वास्थ्य का निर्धारण करने के लिए पराग उत्पादन संभंधित विस्तृत शोध, प्रजनन फलोदगदिकी पर आयोजित किया गया था। अ. नेप्लेन्सिस में वर्तमान निष्कर्ष प्रभावी वन प्रबंधन योजना तैयार करने और भविष्य में विभिन्न वृक्ष सुधार कार्यक्रमों को शुरू करने के लिए के लिए उपयोगी होंगे।

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आँचल बिष्ट

आंचल बिष्ट

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ABBREVIATIONS USED

S.No.	Symbol/Notation	Meaning
1.	%	Percentage
2.	asl	above sea level
3.	cm	Centimetre
4.	mm	Milimeter
5.	DBH	Diameter at breast height
6.	et al.	et alia
7.	%	Percent
8.	&	And
9.	/	Per
10.	^{0}C	Degree Calsius
11.	ETC	and so on
12.	Fig.	Figure
13.	hrs.	Hours
14.	i.e.	that is
15.	m^2	Meter
16.	Max.	Maximum
17.	Min.	Minimum
18.	Min.	Minimum
19.	S. No.	Serial Number
20.	RH	Relative humidity
21.	Spp.	Species
22.	S.Em ±	Standard error mean
23.	SD	Standard deviation
24.	*viz.*,	Namely

CHAPTER-1 INTRODUCTION

Himalayan mountain ranges are admired for being remarkably diverse and globally important playing a significant role in contributing towards the best ecosystem services **(Sharma et al. 1992)**. Though these ranges are recognized among the most frangible mountain systems in the world and are now threatened due to continuous human intervention and climatic factors **(Bargali 2011)**. Recurring landslide during rainy season have become a disastrous phenomenon in most of the hilly region **(Sharma et al. 2004)**. Due to landslides and anthropogenic activities, i.e. deforestation, overgrazing, forest fire action **(Skilodimou et al. 2018)** leads to the low concentration of nutrient like nitrogen, symbolic change in the soil carbon structure **(Neff et al. 2005)** and higher concentration of noncombustible element such as calcium, potassium, magnesium and phosphorus **(Diagnee et al. 2013; Sharma et al. 1998)** and contamination of soil with zinc and lead **(McGrath and Zaho 2003)**. Thus, soil fertility is the major problem in hill forestry and agriculture systems in such area. Therefore, Alder based hill forestry and agroforestry systems (example, Alder and cardmom, Paddy with Alder, horticulture crops) provide a significant tangible (improved production of the food, fuel wood and timber) and intangible (carbon sequestration, hydrological balance, soil fertility recovery and slope stability) benefits **(Maikhuri et al. 1997)**, in spite of being non-leguminous (**Rathore et al. 2009)**. This system enhance and restore through its symbiotic relationship with nitrogen fixing bacteria, i e., Frankia, Actinohorizal plants **(Sharma and Ambasht 1984; Luna 2005)** forming 80 nodule per plant (**Pokhriyal et al. 1993)** which increased performance in degraded land and also provides multiple alternative and opportunities to farmers and rural people to improve their livelihood by improving farm production, income for these regions due to the high concentrations of nitrogen and phosphorus in their tissues as compared to those of forest mixed species **(Sharma et al. 1994; Sharma et al. 2006)**. In mountains, traditional agroforestry systems are very close to the natural ecosystem providing services identical to the forest **(Sharma et al. 2006)**. Thus reviewing the above problem and ecological importance, *Alnus nepalensis* D.Don is considered to be the best for bio amelioration of the soils, as the tree cover had more root - shoot biomass and leaf litter fall, extensive root system increased soil organic carbon, better aggregation, improved water transmissivity and infiltration in turn reduced soil erosion **(Saha et al. 2007)**. It is a prolific pioneer species in freshly exposed soils in landslide affected area, rocky and degraded slopes **(Sharma et al. 1998)**. *A. nepalensis* is a fast-growing tree **(Barakoti 2001)** and has the potential to improve Zn, Pb, and Cd contaminated soils **(Jing et al. 2014)** showing species potential to reclaim mined degraded soil **(Rathore et al. 2010)**. *A.*

nepalensis is a native of Himalayas, commonly known as Himalayan Alder, Nepalese alder (**Sharma et al. 1998**), Indian alder in English, Utis in Hindi (**Troup, 1921; Luna, 2005**). It is a state tree of "Nagaland state of India". *A nepalensis* is a monoecious tree belonging to the family Betulaceae, also known as "Birch family" (**Jhonsan 1917**) and order "Fagales". The family Betulaceae is subdivided into two families Betulaceae and Corylaceae (**Prantl 1893; Winkler 1904; Li and Skvortsov 1999**). It includes 150 species within six important genera, i.e. *Alnus*, *Betula*, *Carpinus*, *Corylus*, *Ostrya*, and *Ostryopsis*. About 35 species of Alnus are reported **(Sharma et al. 1998)** which are an important group of trees not only in the Indian sub-continent, but also into South America, Hawaii and China. It occurs in the hills of Himalayas around 2000 m (amsl) (**Troup 1921; Chen 1994a; 1994b; Luna 2005**). *.Alnus* refers to the ancient Latin name for the "Alder" and *nepalensis* designate to its type locality Nepal **(Hanum and Maesen 1997; Pagaget al. 2010)**. *Alnus nepalensis* is a deciduous tree with smooth and slivery grey bark, resembling that of birch **(Chaudhari and Pandey 2016)**. *Alnus nepalensis* prefers moist climate with mean annual temperature of 13-26^{0}C **(Chaturvedi et al. 2018)**, shady ravine near water, though it can withstand imperfectly drained areas and flooding but cannot withstand water logging. It shows best performance on well drained deep loamy alluvial soils but can grow on wide range of soils from gravel, sand and clay **(Troup 1921; Luna 2005; Rathore et al. 2009)**. It is a multipurpose tree of Himalayan region growing at high altitudes both in temperate and subtropical region **(Chaturvedi et al. 2018)**. It is a wind throw species when grown alone, a good coppicer and has the ability to compete with the weeds (**Luna 2005; Jing 2014)**. Due to moderately soft wood and charcoal feedstock, it is used as a commercial timber production. Moreover, it is a chief associate in various commercial timber plantations, where it helps to raise the nitrogen levels of fields (**Luna 2005)**. *Alnus nepalensis* has the potential of yielding a red dye and tannin from its bark **(Gaur 2007)** and also considered as a bee forage plant of the Garhwal Himalaya **(Gaur 2007)**. Being a pioneer species, *A. nepalensis* regulates the important ecosystem functions. It regulates many ecological services such as soil development, regulation of nutrient and water cycling, forest structure development, and production of flowers and fruits for pollinators and dispersers interactions. It is a pioneer species in the landslides areas of the Himalayan region (Champion and Seth 1968) apart from these importance's, basic life-history data on the autecology of temperate pioneers, especially phenological reports are scarce **(Bentos et al. 2008)**. It has been assumed that pioneers begin reproduction at a young age, and then reproduce annually, often continuously throughout the year **(Swaine 1982)**. India with a considerable amount of variation in climate, altitude and physiography displays variation in the life cycle of different plant species **(Koul and Bhatnagar 2005)**. These variations are advantageous to find their effect on phenology. The timing of life history of events taking place over a period of time can be characterized and quantified in terms of number of parameters **(Newstorm et al. 1994)**, i.e., timing, frequency, duration, degree of synchrony. These are probably the consequences of variety of selective pressure such as seasonal climatic change, resource availability, predators and seed dispersal. For many species, phenological events occur at specific times of year or under specific environmental conditions, and time-based shifts in these seasonal phenomena are widespread and well-documented responses to climate change **(Chmura et al. 2018)**. So, study of reproductive phenology becomes important to study the significant variation among them in the timing and quantity of reproduction.

Phenology is the study of seasonal temporal events of a plant consists of seedling, vegetative and reproductive stages and plays a key role in the matching between organisms and their environment. Vegetative phenology consists of growth of leaves and stems, and flowering, fruiting and seed dispersal are covered under reproductive phenology (**Upadhaya et al. 2017; Segrestin et al. 2019**). Plant reproductive phenology plays a key role in the dynamics of plant and animal communities in many ways **(Paritsis et al. 2006)**. Thus, studies on phenology of population / communities are important to understand the long-term adaptation of species to prevailing environmental conditions for reproductive success **(Shivanna and Tandon 2014)**. The flowering and fruiting phenology of plants is very sensitive to environmental cues such as temperature, moisture, and photoperiod **(Bernier et al. 1981)**. In other hand reproductive phenology is of prime importance for assessing the mating system and reproductive success of individuals in a population **(Khanduri and Sharma 2010**). The number and feature of flowers may have profound consequences for reproductive success. Large floral displays often attract more pollinator visits, increasing outcross pollen receipt **(Karron and Mitchell 2012)**. The most prominent features of flower of *A. nepalensis* is the presence of compact aggregate clusters of small yellow flowers or staminate. Catkins hanging in cluster at the end of the twigs, grouped in a terminal panicle separate or on the same twigs, which flowers from September to November, pollinated through wind (anemophillous pollination) **(Schopmeyer 1974)**. Female catkin or cone appearance strobili grouped in short axillary raceme of 3-8 catkins, rarely solitary occurring on the branching side of the twigs **(Troup 1921; Sharma et al. 1998; Rathor et al, 2010)**. The staminate flower is unisexual, compound spikes, the partial inflorescence is reported as “dichasium” or a “cymule” (three flowered) with sessile flower makes Betulaceae family, morphologically interesting and discrete composition making it distinguishable from the catkins of other members **(Abbe 1935)**. The pedicle of individual floret being extremely short brings these florets together so closely that they appear to form a "diminutive cyme", or "cymule", these lateral members of the inflorescence may be racemose since they are racemosely arranged on the ament axis **(Abbe 1935)**. Pistillate inflorescence is composed of large number of cymules arranged helically on the primary ament axis **(Abbe 1935)** in each pistillate cyme, there are primary, secondary and tertiary bracts forming a "bract complex" **(Abbe 1974)**. Fruits which resemble the cone of the pine family are dark brown upright on short stalk, elliptical, composed of many hardwood scales **(Rathor et al. 2010)** and small winged fruits or nuts **(Grimm and Renner 2013)**. The empty catkins remain on the tree for long time which is the most distinguishable features of the alders to the birches **(Troup 1921)**. Seeds are light brown, circular and flat with two board membranous wings nutlets **(Orwa et al. 2009)** or short winged called samara **(Munro 2014)**. Reproductive phenology is attributed to affect the prolific nature of individuals in a population **(Khanduri and Sharma 2010)**. As timing have a great impact on phenological patterns, among individuals mating pattern is highly influenced by large modification in flowering date and short flowering period **(Khanduri and Sharma 2010)**. Similarly, different floral parts have different maturation phase and they have profound effect on reproductive mechanism such as; a lengthened pollination period increase selfing **(Fashler and El-Kassaby 1987)** exclusively during the early and late flowering period due to the increased density of flowering trees during these periods **(Khanduri and Sharma 2010)**. Conversely compaction in pollination period improves panmixis and increase the frequency of outcrossing **(Fashler and Devitt 1980)**. The anther dehiscence follows pollen release, which gives an accurate estimate of the time of pollination as pollen release and pollination occurs at the same time of maximum pollen release **(Khanduri and Sharma 2003; Sharma and Khanduri 2007)**. The flowering time (when to flower) of each species is genetically fixed, which shows high variability with environmental factors, i.e. precipitation and temperature. Climate change affecting many features of tree biology and its effect on first flowering dates of plants would be of great significance **(Khanduri 2012)**. The mating system of seed orchard are effected by biological factor and management practices these include variation in reproductive phenology timing and cone production **(Kasalby et al. 1984)**. Within a population synchronization in flowering is important for seed set, maximum outcrossing level, determine segregation of genetic variation in the population and asynchronisation leads to detrimental effect on panmitic equilibrium and minimising selfing **(Khanduri and Sharma 2010)**.

The reproductive phenology of individuals in a population is important to researchers, field biologists and plant breeders, as it determines the distribution of genotypes within populations and influences the degree of differentiation among populations **(Khanduri 2012)**, reproductive success or mortality is associated with phenological traits **(Sakai et al. 1999)**. Flowering phenology of tree species at population level has not been given the required heed thus far and therefore a detailed knowledge of flowering phenology and fruiting pattern is critical for successful management resources **(Khanduri et al. 2013)**. On the other hand pollen production is the key attribute of reproductive success and fitness of a natural population **(Ladeau and Clark 2006)**. The first and complete study of pollen grain was carried out by **Pohl (1937)**. The pollen grain is the carrier of the male gamete or their progenitor cell **(Knox 1984)**. Each pollen grain is a multicellular, mobile, organism that is produced by meosis and carries half of the chromosomal complete set of its parent **(Di-Giovanni and Kevan, 1991)**. As a result of anther dehiscence the pollen grain are released into the atmosphere for pollination and subsequent fertilization **(Shivanna and Tandon 2014)**. It is essential to study the behaviour of male gamete in order to know about the inheritance pattern for subsequent improvement and breeding programmes **(Khanduri 2013)**. The knowledge of pollen production per individual tree along with flowering phenology would be useful for estimating the pollen season to aerobiologists and tree breeders for making crosses for enhancement of wood quality through provenance testing and mating design, foresters, silviculturists and ecologists for natural selection and progression of plant life history **(Khanduri 2014)**. Successful pollination depends on pollen production **(Allison 1990)** such as; anemophillous plants have developed extensive pollen production due to their haphazard dispersal mechanism in order to reimburse for their restricted efficiency **(Teresa Gómez-Casero et al. 2004).** The amount of pollen produced by anemophilous plants is higher than insect pollinated plants whether per stamen, per flower, or per inflorescence **(Proctor 1996)**. Pollen production per plant is determined by number of pollen grain per anther, number of anthers per flower, number of flowers per plant, and plant size (**Khanduri and Sukumaran 2019)**. It is important to estimate the total production of pollen per plant because of the dependency of the seed production on pollen **(Molina et al. 1996)**. The total production of pollen grains is influenced by various factors **(Stanely and Linskens 1974)**, showing variability for year to year **(Rogers 1993)** and also from lower altitude and higher altitude **(Khanduri, 2011)**. Climatic conditions plays important role, the foremost conditions generally promoting pollen grain production are warmth, dryness and sunshine during formation of flower primodia on the previous year, favourable precipitation during the vegetative growing season, lack of winter killing and sunshine prior to pollination **(Khanduri 2011)**. According to **Faegri and Iversen** (**1964)** pollen production is also dependent upon the frequency of flowering years. Some forest trees flower, only intermittently. This may be partly due to morphological reason, the flower or inflorescence being situated in such a position that no flower can be formed in that place in the following year and partly to physiological factors a period of rest being necessary before next profuse flowering **(Kramer and Kozolowski 1960)**.

> ***"If any entomophilous species ceased altogether to be visited by insects, it would probably perish unless it were rendered anemophilous, or acquired a full capacity for self pollination*" (Darwin 1826)**.

Thus, reflecting that wind pollination might evolve as an alternate to selfing when pollinators are unavailable.

Gene flow studies in plant species emphasized the role of pollen movement and mode of pollination. The degree of mode of pollination depends on the spatial distance between the individuals of species **(Bohn et al. 2016)**. Gene flow is a key factor in the evolution of species, influencing effective population size, hybridization and local adaptations **(Gerber et al. 2014)**. Gene flow (dispersal or migration) is the transfer of genetic material from one population to another **(Fundamental of molecular evolution 2014)**. Gene flow is important in shaping genetic structure of population and is a key factor for determining the composition of ecosystem (**Sharma and Khanduri 2007)**. Spatial restriction of gene flow can lead to non-random mating and to subdivision of a population into genetic neighbourhood **(Schaal 1980)**. Numerous studies on gene flow in plants have shown that pollen flow is the major contribution to the gene flow **(Ellstrand et al. 1989; Ellstrand 1992; Kaufman et al. 1998; Bohn et al. 2016; Khanduri 2019; Khanduri and Sukumaran 2019; Zhang et al. 2019)**. Gene flow predominantly occurs through dispersal of seed and pollen, and the contemporary distribution of neutral genetic diversity across the landscape is largely, though not entirely, due to the extent and relative importance of these two dispersal processes i.e. seed and pollen **(Gerber et al. 2014)**. For wind-pollinated trees, pollen-mediated gene flow can be considered as the most important mechanism to maintain genetic connectivity over long distances **(Beghe et al. 2017)**. Wind borne pollen grains of widely distributed tree species have the potential to travel dozens or hundreds of kilometre **(Khanduri and Sharma 2012)**. Genetic differences between individuals are crucial for the adaption and evolution of population as a new gene combination are generated by recombination process, mating between unrelated individuals increase genetic variability **(Bona et al. 2019)**. Any differences between populations that cannot be explained by locally selected adaption must have evolved by genetic drift **(Ellstrand 2003)**. Gene flow within and between plant populations has been of continuous interest to the plant breeders and seed producers for many decades. Economic consideration have stimulated studies of gene flow as a function of distance, breeding system, pollinating agent and planting design in numerous domestic plants **(Levin and Kerster 1974)**. Long distance dispersal events are very important in colonization and in breaking down population isolation **(Gillesple 2012)**. Early studies of seed and pollen dispersal appear to have systematically underestimated actual level of gene flow **(Levin 1981)**. Many studies revealed that pollen flow might be quite restricted in forest trees **(Levin and Kerster 1974; Khanduri and Sharma 2002; Burczyk et al. 2004; Sharma and Khanduri 2007; Cuartas-Hernández et al. 2010)**. Research on gene flow in trees should receive high priority as it is poorly understood **(Khanduri and Sharma 2007)**. Keeping view the above facts and problems, the objectives of the study were:

1) Flowering phenology, extension of the catkins and anther dehiscence in relation to time and temperature of the day.
2) Estimation of variation in pollen output per anther, catkin and per tree.
3) Estimation of pollen viability and *In-vitro* pollen germination following hormones media composition.
4) Pollen mediated gene flow by considering a patch of trees as a pollen source.

This study will be helpful to achieve the breeding goal, because it requires complete review of species in terms of Silviculture, Biology and Genetics.

CHAPTER-2. REVIEW LITERATURE

As *A. nepalensis* is a newly described tree species in Garhwal Himalaya in terms of reproductive phenology. Hence literature regarding its biology is almost in cross references are explained under this chapter.

2.1 Floral morphology structure and development

The floral morphology and inflorescence of wind pollinated plants largely reflect the aerodynamics requirement for successful pollen liberation from anthers and capture by stigma **(Niklas 1992).** Compact panicles act as a bluff – bodied obstruction to air flow, so that enternained pollen is deflcted from windward stigma and is instead captured by sedimentation from eddies on the leeward side of the inflorescence. In contrast, diffuse panicles disrupt airflow less, so that most pollen collides with windward facing stigma **(Frideman and Harder 2004)** In angiosperm the flower is the unit of sexual reproduction, therefore studies on reproductive ecology should start with an understanting of structural and functional aspects of flower **(Shivanna and Tondon 2014).**

Case study

In **1935 Abbe** studied the phylogeny of the Betulaceae. He studied by using herbarium material and fresh specimen. Floral and inflorescence morphology by using herbarium material and fresh specimen, the results proposed the concept of compound spike for staminate inflorescence, the partial inflorescence was reported as dichasium or a cymule “ three flowered” . Cymule refers to cymose but was elucidated through presence of vestigial vascular bundle. The inflorescence was found to be uniform but at the base less crowding of partial inflorescence was found, the florets were sessile, stamens more or less deeply divided. The pistillate inflorescence, pistil in the axil of “ bract complex” *Alnus* have primary, both secondary and abaxial pair of tertiary bract present, and is composed of numerous cymules arranged sprially on primary axis, ovary inferior, bicarpellate with two anatropous and unitegmic ovules.

In **Hjelmqvist 1948** studied on the floral morphology and phylogeny of Amentiferreae. His conclusion were same as Abbe only the partial inflorescence in the family have the possibility that the floret in *Alnus* may be pseudanthium because of certain tetralogical forms which he observed.

In **1990 Furlow** examined genera of betulaceae family in the South eastern united states and established a summary of morphological nature of cymules of flower in betulaceae family even though his conclusion were same as Abbe, some character reported were, at maturity the pistillate catkin becomes woody and cone like and the five bracts of each cymules fusing into a single persistent scale bearing two fruits, the infrutisence vary in shape, size and thickening and those distinctive feature of *Alnus* makes it distinguishable from the *Betula*. *Alnus* fruit are small, smooth surfaced, light dry, indehiscent and laterally winged, the summit of the fruit is crowned by two persistent styles and are termed as nutlets or samara. The fruit outer body is elliptical in shape. In these species the dispersal is through water.

Kubitzki (1993) reported in Betulaceae family that staminate catkin is pendulous conspicuously bracteates (1-5) bracts, fused flower, small lacking perianth, anthers terta sporangiate, two locular drosifixed extrose and opening by longitudinal slit. The pistillate inflorescences erect to pendulous, the scales consisting of 1-5 fused bracts, flower small, perianth usllay lacking adenate to ovary, linear style, ovule axile 1-2 per locules.

Harrington et al (1994) worked on the biology and ecology of red alder and reported that the tree were monoecious which posseses separate male and female catkins growing on the previous year twig. Staminate catkin occurred on pendulous group usually on terminal position elongating from 2-3 cm to 7-8 cm long. Several pistillate catkins are borne per floral bud, located on a bud proximal to staminate catkin 5-8 mm long and reddish and green when receptive, pistillate catkin are usually upright and become pendulous when they mature.

Lin et al. (2010) studied the inflorescence, cymules and the flowers of *Betula alnoides* using scanning electron microscopy and found that Betula *alnoides* is monoecious with pendulous inflorescence that have compound spike comprising of several cymule arranged helically along an inflorescence axis. Each cymule usually consists of three flower, neither a cymule with less than three flowers has been discovered in this study. Both male and females are laterally borne on the branchlets. The primary bract of the cymule are pelate woody and conspicuous, covering the inner floral organs tightly in the male inflorescence they are much more relaxed in female. The anthers poses two theca connected by a short bifurcated filament, each theca bears two anther sacs. Pistillate flower consist of two carples each with linear styles. The ovary is inferior. Male inflorescence of *Betula alnoides* develop earlier than those of female and experience longer development.

As only few studies have been conducted on the micromorphlogy of flower and inflorescence in this family **Zhu et al (2017)** studied floral ontogenic patterns of Betulaceae based on SEM observation on inflorescence and flower to evaluate their phylogenetic implication in the context of recent development in molecular phylogentics of Betulaceae. He reported that, in each pistillate cyme there are one primary bract, two secondary bract and only in *Alnus* there are two tertiary bracts on the abaxial side.

2.2 Flowering phenology

It provides important data in forecasting concentration to air borne pollen allergens **(Dabrowska et al. 2012)** in plant organism, reproductive phenology is critical detriment of reproductive success and offspring genetic diversity **(Grogan and Loveless 2013)**. such kind of study help in the understanding of biological processes in trees, as well as their relationship with other species and the dynamics and functioning of the ecosystem **(Kebede and Isotalo 2016)**.

Case study

Farmer et al. (1985) studied reproductive characteristics *Alnus virdis* in Northerwestern Ontario and concluded that, there was synchronistion among clumps. Blossoming and fertilization occurred from mid-May to early June, lateral bud containing female catkins were about one third and a average of 60% catkins was fertilized. Pollen season was observed 18 – 20 days after maturation of catkin.

Brown (1995) reported that peak shedding of pollen generally precedes peak receptivity by a few days but synchrony in pollen shed and receptivity have been observed in some trees of *Alnus rubra*

Raju and Ezradanam (2002) studied a monoecious and protoandrous species *Jatropha curcas* and reported that, both flower sexes open synchronously the male flower first and produce flower daily till the completion of male buds and the female flower bloom between the second – sixth days, the flowering lasts for 11 days. The average male to female flower ratio was 29:1, Thus it reflects both Geitnogamy and Xenogamy, geitonogamy which is considered to be adaptive for colonization of *J. curcas.*

Sogo and Tobe (2005) reported that *Alnus firma* and *A. sieboldiana* are dichogamous i.e. the anther (male flower) mature before the pistil (female flower) on the same tree so that the pollen grain dispersal is earlier before the pistil become receptive in these species therefore the pollen grains are received from other trees thus avoiding self-pollination.

Bai et al. (2006) studied the flowering phenology of *Juglans mandshurica* and concluded that there was no synchrony between sexual function within individual tree. Blooming period of two mating time were synchronous and reciprocal, maximum pollen longevity was four hours for protoandrous individuals, and three hours for protogyny. The stigma of the flower turned dark red and wilted after 6-7 days, thus dichogamy or the temporal separation in male and female flowering within an individual is sufficient to reduce selfing.

Aboulaich et al. (2008) studied the male phenolgy and pollen production of *Cupressus semipervirens* in Tetouan. Five phenological phases were described according to size, colour, stage of microsprangia and blossoming event, starting date – the bud differentiation correspond to bud burst, Immature cone increasing in size, Pre flowering - the changes in the cone colour was seen with the increment in the size of microspore lead to male cone swelling, Blossoming male -the sporangia open and release pollen the microsporangia dehisce asynchronsly, Senescence- the microsporangia are empty turning brown colour this is the termination of pollen period some cones drop down and some remain on the tree. These phenological events take place over a month and anthesis occur two months after bud burst. Thus, the description of phenological stage is helpful way to determine the starting date of pollen emission.

Siljamo et al. (2008) studied the phenological *Betula* data collected in different parts of Europe and result concluded that station specific data varied 3-8 days.

Khanduri and Sharma (2010) studied reproductive phenology of *Cedrus deodara* and concluded that reproductive phenophase was of four months while flowering and pollination period for one month, flowering showed high level of asynchrony among individuals within a population leading to possibility for geitonogamy thus it becomes important to study the reproductive phase because it reflects opportunities for genes to move among conspecifics in the population through pollen and seed dispersal.

Dabrowska et al. (2012) studied the floral phenology of *Alnus* taxa. The length of male inflorescence in *Alnus* taxa measured range from 12-70 mm, during flowering the inflorescence was seen elongation by 100 % Inflorescence development was mostly related to temperature and air humidity thus weekly associated with wind speed and precipitation. In majority of *Alnus* taxa flowering lasted for 17 days and overlapped with various phases of pollen season except *Alnus subcordata*

Chavan et al. (2012) studied phenology of *Casuarina* species order Flagles in agricultural research station at Ganga research station at Gangavati University of Agricultural Sciences, Raichur phenological observation revelead that the flowering occur twice among the five species studied. Synchronization of flowering of male and female inflorescences was recorderd in both species of *C equsetifolia* and *C cunninghamiana* with lesser duration of fruit maturity, hence these two species are recommended for hybridization and tree improvement programme.

Khanduri et al. (2013) worked on reproductive phenology of *Schima wallichii* concluded that anthesis started 15 days after the initation of floral bud and the overall duration of flowering period was one month. Flowering synchrony within a population was calculated by Primack method and result concluded that there was a partial synchronization within a population which varied from 33-42 days, mean synchrony of 0.54 -0.68 within a population and silmultaneous within an individual during flowering period. Synchrony within population is important for seed set, outcrossing level and genetic variation in the population, synchrony among individual increases cross pollination.

2.3 Pollen release in relation to diurnal temperature

Pollen availability is a major constraint of plant reproductive success and reproductive performance of plant largely depends on amount of pollen release. Climatic factors, such as temperature has a strong correlation with pollen release (**Ejsmond 2015**).

Dabrowska-Zapa et al. (2018) established the correlation between pollen release and temperature in *Alnus miler* which reveals that there is a statistically significant positive correlation with the air temperature.

Jato et al. (2002) studied the pollen concentration of *Pinus*, *Platanus*, *Quercus*, *Betula*, Poaceae and *Castanea*, for eight years they analyse the influence of the temperature on pollen concentration, and obtained a positive correlation with temperature.

Further studies of pollen release with relation to climate change by increased temperature were also done. **Vliet (2002)** studied the influence of temperature and climate change on the timing of pollen release in the Netherlands and concluded that increased temperature leads to early pollen release in study area. The pollen grains of family Betulaceae considered one of the major pollen allergens considering this fact **Meng et al. (2016)** studied dynamics of airborne pollens with relation to meteorological factors i.e., temperature and found that airborne pollen amount of Beijing urban area was significantly affected by increased temperature.

2.4 Pollen production and Pollen ovule ratio

The level of pollen production of a particular species is a function of its genotype **(Reddi and Reddi 1986)** and the number of pollen grain per anther might be genetically fixed **(Khanduri and Sharma 2002)**. The total pollen production is useful in estimating the number of pollen grains that could be in air during a certain season once the density of plant per surface is known **(Mollina et al. 1996).** Pollen ovule ratio in essential part of plant breeding system and also be correlated with habitat or successional stage **(Cruden 1977)**.

Case study

Pohl in 1937 reported that pollen output per inflorescence in *Alnus glutinosa* was 4.4×10^6 and pollen output of ten-year-old branch of Alnus *sibirica* is 302 million.

Etardman (1954) in his book "An introduction to pollen analysis" reported that the pollen output per inflorescence in *Betula pubescens* was 6,000,000 and *Betula verrucosa* 5,450,000. **Ogden et al. (1974)** reported that 10,000 the pollen grain per anther were found in Betulaceae.

Cruden (1977) studied the pollen ovule ratio of some flowering plants and divided breeding system into five classes ie Xenogamy (P/O s 5859.2 ± 936.5), Facultative xenogamy (P/O s 796.6 ± 87.7), Facultative autogamy (P/Os 168.8 ± 22.1), Obligate autogamy (P/Os 27.7 ±3.1), Cliestogamy (P/Os 4.7 ±0.7). Thus, pollen ovule ratio show the sufficient pollen grain reaching each stigma to result in maximum seed set.

Reddi and Reddi (1986) studied pollen production for 82 angiosperm plants and reported that pollen production varied within the same genus thus such variation could be related to mode of reproduction. The non poaceae and non cyperaceae members were high pollen producers hence high pollen producers are cross pollinated whereas low pollen producers are either self-pollinated or apomatic.

Moe (1998) studied the pollen production in *Alnus incana* and reported that 1×10^{6} pollen was produced per catkin.

Pitroswaka (2008) studied the pollen yield in *Corylus avellana* and *Alnus glutinosa* and reported that the male of Black Alder contain 580 flowers and stamen on an average produce 8420 pollen grain, whereas inflorescence produce 19534000 pollen grains. Thus, under adverse condition bees should be fed with amount of pollen from Alder inflorescences.

Khanduri and Sharma (2002) studied pollen production and pollen ovule ratio in *Pinus roxburghii*. The pollen ovlule ratio is reflected as Xenogamus type of breeding system thus the high male to female ratio and large pollen production capacity shows intraspecific competition among trees for successful outcrossing within a population. Pollen production value ranged between 552.36 $\times 10^3$ and 895.76 $\times 10^3$, per strobilus between 54,352.51 $\times 10^3$ and108, 834.50 $\times 10^3$ per strobilli group and 1247.5 $\times 10^9$ -3503.96 $\times 10^9$ pollen grain per tree which varied individually.

Khanduri (2014) reported that annual pollen production per tree in *Lagerstroemia speciosa* during mass production year was 6.4 $\times 10^8$ to 1.3 $\times 10^8$ thus higher pollen production reflects higher fruit set percentage.

2.5 Total ovule production

For a broad and taxonomically diverse plant species, there one should know the ovule number and for successful pollination and fertilization which leads to floral mating success. In forest tree species very few studies has been done such as, **Burd et al. (2009)** looked at the number of ovules per flower in 187 different angiosperm species and came to the conclusion that spontaneous variation in mating performance at the floral level has influenced the evolution of ovule packaging in angiosperms. One of such study has been done in actinomorphic and zygomorphic flower species to know the correlation between numbers of ovule with flower size. It was observed that in the actinomorphic flower species is correlated with size of flower while it was not observed in the zygomorphic flower species **(Mochizuki et al. 2019).**

Wetzstein et al. (2013) studied ovule number per flower in Pomegranate flowers and found that it can contain large numbers of ovules, reaching to over 3000 ovules per flower.

2.6 Pollen viability

The capacity of pollen grain to germinate, but some time it does not germinate due to unsuitable conditions. It may even germinate, but not have the ability to fertilize an ovule because of some form of incompatibility can be stated as pollen viability **(Dafni and Firmage 2000**). In most plants effective pollination is an essential for fruit- and seed-set. Information on pollen viability is required for any rational approach to increase plant productivity. To ensure the success of hand pollination, it is important to determine pollen viability prior to pollen application **(Tangmitcharoen and Owens 1997)**. Pollen viability is important for species distribution, fitness, and survival of the next plant generation. It is also vital for directed plant breeding and, consequently, crop improvement. Pollen viability includes diverse aspects of pollen performance such as fertilization ability, germinability, and stainability **(Dafni and Firmage 2000)**.

Case study

Pollen viability in *Betula pendula* was examined by using Trypan blue by **Cuinica et al. (2013)** and reported that pollen showed viability upto 93 ± 1%.

Bazhina (2019) analyzed pollen viability starch content, *in vitro* percentage and pollen tube length and reported that the viability of pollen grain varied between plots at control plots from 53.6 - 95.1 % of pollen grain gave a positive reaction on starch content.

2.7 Pollen germination.

In vitro pollen germination is of utmost importance because it solve physiological and biochemical conditions required for successful pollen germination and pollen tube development **(Kumar et al. 2016).** Pollen germination varies with respect to species, composition of medium, temperature humidity and time **(Stanley and Liskens 1974)** pollen longevity and sucrose level **(Pacini 1996).** The pollen having lower germination in vitro may have the potential for fertilization because germination in vivo is enhanced by natural secretions that cannot is duplicated in artificial media **(Falleri 1993)**.

Case study

Bensimon (1985) studied the pollen germination of *Alnus glutinosa* and reported that 60-80% germination of control pollen. He also reported the importance of germinating media, several media was tested without success for *Alnus glutinosa thus* pointing to high sensitivity of germination behavior to environment condition. Only germination occurs where pollen were previously hydrated.

Falleri (1992) performed germination test on *Alnus cordata* by using vapour method modified by **Giordano and Bonechi (1956)** thus showing germination capacity of 32% on the fresh pollen samples.

Steiner and Gregorius (1998) studied invitro pollen germination in *Alnus* by using four different methods media A and B were taken from **Eschrich (1976)** and **Linares (1985)** and ensured that pretreatment was given before inserting it to media, C only pretreatment was given and no chemicals were used, D was modification of B with 50% of the original conc. the germinated pollen were counted after 24 hours, result reflected that pollen hydration might be a necessary pre condition for germination not only for A but possibily for other media also The pollen germination rarely exccceds 50%, the diffferention of germination caused by different media comprise a broad range from 0- 33 % from a single pollen source, for different media it was reported that they are not consistent and predictable neiher quantity nor the direction of superiority. Thus experiment revealing the interaction of pollen donors (genotype), physiological state of pollen when entering the germination experiment, germinating media is necessary.

Kumar et al. (2016) studied pollen *germination* of *Spathodea campanulata*, *Bauhinia purperia* and *B. racemosa* to assessthe efficacy of growth hormone IAA, IBA, GA_3, Kenetin and sucrose in three different concentration (100ppm, 200ppm, and 300ppm) and sucrose (5 and 10%). Observations reveal that pollen germination under control is very low and fairly good germination were found with all regulators. Observation concludes that germination of pollen can be enhanced by the use of proper regulators in appropriate concentrations.

2.7 Pollen mediated gene flow

Gene flow is important in conservation biology and because of its influence on effective population size of threatened species **(Burczyk et al. 2014)** Pollen mediated gene flow is likely to be important for sustaining population connectivity and may help to mitigate the risk of genetic degradation caused by colonization, bottle neck, inbreeding and genetic drift **(Ellastarand 2014)**

Case study

In **1901 Holst** with analysis by Lagerhiem reported that Hazel pollen frequency has reduced strongly from 61.75 percent in the lower layers to 25.78 percent in the upper layer (pollen frequency were expressed as percentage of total forest tree pollen). The decreasing rate in *Alnus* and Betula in upper part was 40.17 and 11.11 percent and in lower part was 44.37 and 17.50, thus referring that elm slightly decrease in number upwards.

Wodehouse (1935) in his book "Pollen grain" reported that in Birch pollen season lasted from May to 10 June and the maximum frequency was 70 i.e. (the number of pollen grains caught per sq.cm in 24 hours.

Silen (1962) studied pollen dispersal for Dogulas Fir and concluded that only a small fraction of pollen dispersal by a tree falls at a distance beyond 5-10 times tree height, this isolation distance beyond 5-10 times the height of mature tree provide safety in a seed orchard. The maximum pollen count were found at 50 feet from the trees averaged 5,116. But high amount was pollen i.e. 2000 grain per square inch was found at 2,000 feet from the trees which was far away from the pollen source thus reflecting that this pollen was mostly from surrounding stands.

In recreation of past vegetation and assessing the vegetation pattern around the habitat of species, the importance of long-range dispersal of pollen grains has been recognized by many palynologists. The scattering of pollen grains is influenced by weather conditions (wind direction, wind velocity, temperature, relative humidity, precipitation, pressure and solar radiation) and on the micro and macrotopography within the area where the transport takes place. **Hjelmroo (1991)** gave an Evidence of long-distance transport of Betula pollen from three places in Sweden. The investigation concentrates on data from usually prior to the local birch flowering season at the localities. Pollen count was done by using a Burkard Seven Day Recording Volumetric Spore Trap. Results shows that the travelling times for pollen grains transported were mostly in the range of 9 to 20 hours with distance of over 2000 km, depending on the topography.

Khanduri and Sharma (2002) studied pollen migration from isolated tree and reported that pollen migration was not uniform in all direction and long-distance transport was observed in the downhill than uphill thus knowledge of average distance that pollen dispersed is essential for estimating the extent to which isolation is important in race formation and for successful seed set.

Khanduri (2007) studied pollen mediated gene flow in *Pinus roxburghii* and reported that the pollen travelled upto 2.5 km in regard to downhill direction thus revealing that scattering of pollen grain was not uniform in all direction while in regard to uphill direction the pollen grain was constricted to 320 m from the pollen source. As the distance from the pollen source increased the pollen frequency declined rapidly with highest density within 50-100 m, thus reflecting that most intermating within local population occurs among neighbouring individuals.

Picornell et al (2020) studied medium and long-range transport event of *Alnus* pollen, pollen sampling was done by means of three Hirst type of volumetric pollen traps and sample were mounted and counted following the recommendation of Spanish Aeriobiology network and the European Aeroallergen Society. The result concluded that, a high inter annual variability in the annual pollen integral was found in the station favoring meteorological condition, a long range of transport and therefore high concentration detected in specific days, thus *Alnus* pollen seem to have a heterogenous origin with prevalence of long-distance transport which would suggest a possible reproductive connection among distant population.

CHAPTER-3 MATERIAL AND METHODS

The study entitled "Reproductive phenology, pollen production and pollen mediated gene flow in *Alnus nepalensis* D. Don" was carried out in the premises of College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand on way to Dandachali road from August 2019 to December 2019 with the purpose to evaluate the objectives mentioned in introduction section. The study site lies in between $30^0$18" North latitude and 78^0 24" East longitude with an altitude of 1600-2192 m above sea level (asl) (Fig. 1). The soil of the study area is silty clay loam, brownish black in colour, coming under the series of orthents (Entisol). The pH of soil ranges between 5.3-5.6. **(Sukumaran 2016).** The vegetation of the area is consisted of *Pinus roxburghii*, *Pinus wallichiana*, *Cedrus deodara*, *Quercus leucotrichophora*, *Lyonia ovalifolia*, *Myrica esculenta*, *Prunus cerasoides, Pyrus pashia, Rhododendron arboreum* etc. The study area typically experiences moist temperate climate with chilled winters. Average rainfall received during the study period was 85.56 mm experiencing highest rainfall in the month of August, i.e., 172.2 mm and lowest in the month of November, i.e., 27.5 mm. The recorded mean monthly maximum (19.26^0C) and minimum temperature (9.0^0C) along with RH, wind speed, sunshine and evaporation are presented in Table 1. Snow fall occurs in the months between mid-December and mid-January (Table.1).

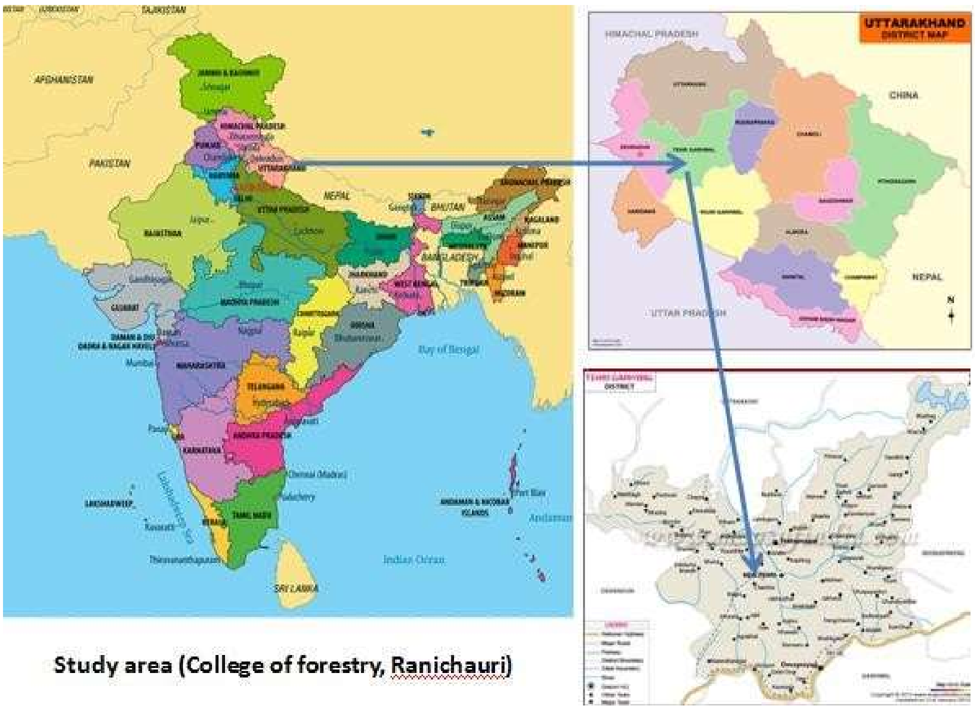

Fig. 1. Study area college of forestry Ranichauri (Tehri Garhwal)

Table 1 **Monthly Meteorological Data during 2019**

Month	Tmax (^{0}C)	Tmin (^{0}C)	Rainfall (mm)	RH1 (Morning)	RH2 (Afternoon)	Wind speed (kmph)	Sunshine (Hours)	Evaporation (mm)
Jan	10.5	0.1	98.6	79	63	4.6	5.1	1.0
Feb	11.3	1.5	136.8	84	70	4.9	4.5	0.9
March	15.9	4.4	40.9	73	52	4.8	7.2	2.0
April	22.4	10.0	22.5	66	50	4.1	8.7	3.5
May	26.0	12.1	21.3	53	35	4.3	9.3	4.4
June	27.7	14.3	39.5	64	50	3.9	9.0	4.6
July	23.4	15.3	175.6	93	82	2.0	4.2	1.9
August	23.4	15.4	172.2	95	81	2.6	4.2	1.8
Sept.	22.6	14.1	139.1	95	79	3.8	5.1	1.7
Oct.	20.0	8.8	27.9	85	68	2.8	7.1	1.5
Nov.	17.5	6.1	27.5	77	63	3.9	6.1	1.4
Dec.	12.8	0.6	61.1	78	62	4.1	6.2	0.8

Five sample trees, which were situated under varying microclimatic condition and exposed to abundant sunlight were selected randomly, girth and the height of the trees were measured and marked by red paint prior to the bud initiation for studying different reproductive phenology parameters. It was ensured that the selected sample tree were accessible, convenient enough to take the data under study. Pollen germination, pollen grain estimation, pollen viability were conducted in the laboratory conditions. For pollen mediated gene flow, a patch of forest consisting of 13 *A. nepalensis* trees whose height and girth was measured and considered them as a pollen source was taken into account. The following information's on the selected topic was done.

3.1) Floral morphology, structure and development

3.2) Flowering phenology

3.3) Pollen release in relation to diurnal temperature

3.4) Pollen production

3.5) Total ovule production

3.6) Pollen ovule ratio

3.7) Pollen viability

3.8) Pollen germination

3.9) Pollen mediated gene flow

3.1) Floral morphology structure and development

In order to monitor the developmental stages, five representative branches were selected at random in each tree (i.e., total 25 branches) containing clusters of male catkins and female cones of *A. nepalensis* and were tagged by using colour tags, prior to bud initiation. Observations were recorded weekly from August 2019 to November 2019 on clusters of male catkins and female cones. The different stages of development were recorded with the help of hand lens.

The morphological parameters such as type of inflorescence, initial and final length of the male catkins and female cones were taken with the help of scale, the date of elongation of male catkins and date of expansion for female cone buds were recorded. Shape of the male catkins and female cones, the colour changes on the male catkin and female cone were closely observed daily with the help of hand lens to check the stigma development and recorded its time duration for anthesis. Number of cymules per catkin for male and scales per ovule for female, were counted for 20 randomly selected male catkins and female cones for each tree (i.e., 100 male catkins and 100 female cones). Number of catkins per cluster and number of cones per cluster were counted for 20 clusters, diameter of female cones were taken in every two weeks with the help of Vernier caliper.

To elucidate the anther structure and morphological changes of the stigma, more catkins and cone clusters were collected within the weeks and observed under stereoscope with the help of 20x lens.

3.2) Flowering phenology

The phenological parameters were examined on the five different trees that have been marked with the help of red paint. The flowering phenological data were observed in terms of; 1) Duration of the first and last flowering, 2) Peak flowering time and Synchrony (flowering overlap among individuals).

3.3) Pollen release in relation to diurnal temperature

Five clusters of male catkins were tagged prior to anthesis for three different trees to keep the track of pollen release. The trees and cluster of catkins were selected on the basis of accessibility to take frequent observations at short interval. At the time of peak blooming, microscopic slides coated with petroleum jelly (Vaseline) were kept to track the pollen release. The microscopic slides acted as pollen trap for every two hours intervals (i.e. 0700, 0900, 1100, 1300, 1500 and 1700 h) and the temperature was recorded in each interval with the help of digital thermometer. Three microscopic slides (T_1,T_2,T_3) in each interval were mounted vertically on the wooden sticks or either placed perpendicular to the prevailing wind direction or positioned all around the source of the tree (under the tree in between the branches). It was ensured that the slides were kept at height corresponding to the height of the catkins and also 1.5 m height above the ground. Slides were replaced at every two hours interval and the number of pollen grains per slide was counted in a 1cm^2 area under a binocular microscope. Pearson correlation was calculated between temperature and number of pollen release at different time dehisced anthers.

3.4) Pollen production

Five trees were selected for the estimation of pollen production. At first, the numbers of primary branches were counted and then secondary branches with male catkins and female cone were counted in each tree. Further, a random sample of 20 mature male catkins was collected from each tree (i. e. 20×5=100) and the number of cymules was counted manually. Similarly, anthers per cymule were counted manually. The undehisced anthers were viewed under sterio microscope and obtained from the cymules (anthers were removed with the help of needles) and counted under binocular micrscope. Due to the small size and large amount of pollen grains, the anther were placed at the centre of the slide, a small drop of water was added and anther was squeezed and crushed with the help of needles and forceps to release all grains on microscopic slide, the debris of anther on microscopic slides were removed gently. The pollen grains were counted using a binocular microscope. The number of pollen grains was counted for top, middle and bottom microscopic views (1st , 2nd, 3rd) by moving the slide from one end to the other and then adding all the microscopic view. The method used for pollen productivity analysis was modified after Molina et al. (1996). To estimate the total production of pollen grains per tree, the following equation was used: Pollen production per tree = Number of anthers per tree × average number of pollen grains per anther; Number of anthers per tree was calculated as: Number of primary branches with catkin clusters × average number of secondary branches per primary branch × Average number of catkin clusters per secondary branch × Average number of catkins per catkin cluster × Average number of cymules per catkin × Average number of anthers per cymule. Pearson Correlation between the number of catkins per tree, number of cymules per tree, number of anthers per tree, total ovules per tree with tree height and diameter was established. ANOVA with two factor was performed by using Microsoft excel.

3.5) Total ovule production

Five trees that were selected for the estimation pollen grains were also selected for estimation of ovule production. At first, the numbers of primary branches were counted and then secondary branches with female cones were counted in each tree. Further, a random sample of 20 mature cones was collected from each tree (i. e. 20×5=100). Than number of ovules were determined by counting them on scales for twenty cones on each tree (i. e. 20×5 = 100). Total ovules per tree was obtained as; number of primary branches × average number of secondary branches per primary branch × average number of cones on a secondary branch × average number of scales per cone × average number of ovules per cone. Pearson correlation was established between number of cone per tree, number of scales per tree, total ovule per tree with tree height and diameter.

3.6) Pollen ovule ratio

Pollen ovule ratio was determined by dividing the estimate of the number of pollen grains per flower (catkin) by the number of ovules per flower (cone) as per the method of Cruden (1977). The pollen estimation method was followed as mentioned earlier. Number of ovules was determined by counting them on the bract scale and the number of scales in a cone. The ratio was derived from the mean pollen quantity with the mean ovule's quantity.

3.7) Pollen viability

In vitro pollen viability was assessed by using acetocarmine staining method **(Alexender 1965)** Three anthers of three inflorescences from each sample trees were selected. Due to small size of anther and large amount of pollen grains per anther a single anther was examined for pollen viability, the anthers were crushed and squeezed to release all pollen grain, anther debris was removed and a drop of acetocrmine solution was added and kept for 24 hours to allow the pollen to absorb the stain. The slides were than examined under the microscope. Deeply stained normal looking pollen grains within microscopic slide were counted as viable pollen, shriveled 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 inflorescence collected from the rest of the sample trees.

3.8) Pollen germination

In vitro pollen germination test for the pollen of *Alnus nepalensis* was done by Hanging drop method (Van Tieghem 1869), using the matured undehisced anthers of different inflorescences of each selected sample trees. Fresh pollen grain were dusted with the help of brush on to a drop of germinating media placed on a cover slip with its edges, smeared with petroleum jelly and the slide was than inverted so that the drop is in the centre of the cavity slide hanging over a shallow depression to prevent evaporation of the media. The pollen grains were germinated to optimise media composition. The media used to check pollen germination were:

1) Sucrose (10%, 20%. 30%)

2) IBA (100ppm, 200ppm, 300ppm)

3) IAA (100ppm, 200ppm, 300ppm)

4) GA_3 (100ppm, 200ppm, 300ppm)

5) Kinetin (100ppm, 200ppm, 300ppm)

6) Boric acid (5 ppm, 15ppm, 25ppm)

7) Boric acid + sucrose 10% (25ppm, 50 ppm, 75ppm, 100ppm)

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 2 hours interval under different solutions were recorded and tabulated in order to assess the effect of different concentrations of growth regulators on pollen germinations.

3.8) Pollen mediated gene flow

Pollen mediated gene flow was recorded for two directions from the source trees (uphill direction and downhill direction. 1) For five isolated trees as pollen source, pollen mediated gene flow was carried out for uphill direction upto 80m and downhill direction upto 10 m because of the unfavorable geographical conditions. 2) The same experiment was conducted upto 80m considering thirteen group of trees as pollen source (focal trees) located on an isolated patch. Measurement of height and diameter of focal trees was also taken. For both the directions, the pollen source trees were in the one patch and isolated from other possible pollen contamination from foreign pollen, and no other flowering trees of other pollen sources. Petroleum jelly coated microscopic slides were used as pollen traps. The slides were mounted vertically on wooden rods and placed perpendicular to the direction of the prevailing wind around the source tree at geometrically increasing intervals (i.e., 0 m, 5 m, 10m, 20m, 40m and 80 m) as per the method used by **Sharma and Khanduri (2007).** Three pollen slides (T_1, T_2, T_3) were placed between 9 am, which were collected after 24 hr during the peak flowering period. The pollen count was made directly from the exposed glass slides (area 1.0 cm^2) under binocular microscope. The effect of distance and direction on pollen dispersal was tested with regression analysis. Pollen dispersal was modeled as a function of different directions, and the distance from the source tree using regression analysis with pollen grains per slide was taken as dependent variable, distance from the source tree as independent variable.

CHAPTER-4 RESULTS

4.1 Floral morphology structure and development – The inflorescence structure and floral morphology is highly compact and inconspicuous in *A. nepalensis*. The observations revealed that *A. nepalensis* is a monoecious tree, i.e., male and female flowers are present separately on the same plant. The female part is known as cone or strobilli which is composed of bract enclosing two ovules within. The male part is known as catkin which is composed of cymules to which anthers are attached. It was observed that the catkin developed from the axil of the leaves which gave clusters of catkins, some catkins occur solitary as well. It was observed that despite whole tree, flowering some of the axillary buds remained dormant in the month of August to November but lately burst in the month of November, however, could not grow into complete catkin due to decreased temperature in the month of November. The presence of previous year female cone, bifid stigma and protoandry condition were the unique features of the species.

The advent of bud bursting of male catkin occurred in first week of August. At initial stage the male catkin were tender, short and erect (Fig. 2a), with orange like tinge at the top (Fig. 2b) which disappeared with the increase in length. At initial stage, the catkins were called spike. At about 0.5 cm length, the male catkins developed pink feather like structure giving the whole catkin a dusting brush like look (Fig. 2c) and average 15.8 catkins per cluster produced which eventually turned yellow in colour (Fig. 2d). The catkins were erect and highly conspicuous and spike like till the third week of September (Fig. 2e,f) and drooping of spike was seen in the last week of September (Fig. 2g) with cymules becoming clear and distinct and started loosing white powdery and silvery colour (Fig. 2f) and average number of cymules per catkin was 113.92. Prior to anthesis, the male catkins partially seggregate and the anthers turned red in colour (Fig. 2h). The cymules start unfolding and arrange themselves in the thread like structure and turned in to yellow colour and bursting occurred (Fig. 2i). Thus, the final anther dehiscence occurred (Fig. 2j). The length of catkin prior to elongation was 3.98±0.17 cm while after complete elongation of catkins, length was 10.02±3.02 cm (Table 2).

During the study period it was observed that the female cones were erect, spherical, highly compact and occurred in the raceme (Fig. 3a) pattern with whitish stigma and scales curved at the top forming a condense spherical cone or strobilli (Fig. 3b). The female cones were dark green in colour, comprising of scales (Fig. 3c). A single scale bearing two ovules, (Fig. 3d), which became more spherical during the growing period and the final diameter was 2.13±0.12

cm and length was found to be 1.83±0.35 cm (Table 3). The cones varied in shape, size and thickness and an average of 5-6 cone were found in a cluster. The change in colour of the female cone was seen 1-2 weeks after blooming of the male part (i.e., catkins). However, synchrony in pollen shed and female receptivity was also observed. Thus, average date of first flowering was observed in the first week of October. The purplish colour was seen in the tip of the scales which indicate the extension and maturity of the bifid stigma, opening of the cones, ripening of the cones and stigma receptivity of the female part (Fig. 3f). The change in colour of the stigma was also seen from white to red and the declined receptivity was reflected through the stigma forming an arc in the scales (Fig. 3g). In the first week of November, the cones changed into hard woody structure thus giving the advent of the seed set (Fig. 3h). *Alnus* fruits were small, smooth surfaced, light, dry, indehiscent and laterally winged the summit of the fruit is crowned by two persistent style and are termed as samara, the dispersal of the seed occurred through wind. The empty cone remains attached to the tree even in second year also (Fig. 3i).

Table 2: Elongation of the catkins

Tree	Number of catkin per cluster	Number of cymules per catkin	Length Prior to elongation (cm)	Final length (cm)	% increase
Tree 1	13.2	117.6	4.21±0.88	10.85±3.34	157.71
Tree 2	20.2	123.55	4.10±0.75	10.97±3.18	167.56
Tree 3	15.74	124.35	3.80±0.93	8.92±2.50	134.74
Tree 4	19.06	114.75	3.94±0.96	10.91±3.06	176.90
Tree 5	10.8	89.35	3.86±0.70	8.46±2.99	119.17
Average	15.8	113.92	3.98±0.17	10.02±3.02	151.76

Table 3: Length and diameter of cone or female part or strobili of *Alnus nepalensis*

Tree	Length (cm)	Diameter (cm)	Scale per cone	Ovules per cone
1	1.77	2.25	127.2	227.65
2	2.26	2.17	131.4	245.20
3	1.30	2.18	115.7	212.85
4	1.83	2.12	123.1	221.32
5	1.98	1.94	97.95	187.05
Avg	1.83	2.13	119.07	218.81
SD	0.35	0.12	13.15	21.36

4.2 Flowering phenology

During the study period, it was observed that flowering in male phase was started in the last week of September and continued till November. Asynchronisation in flowering was seen within and among the individual trees. Time between onset and peak flowering was 2 weeks 4 days and the total average duration of flowering period was 24.8 days (Table 4) representing asynchronistion pattern of flowering among individual trees. Early flowering was seen in Tree No. 1 and partial synchronization was observed in Tree 2 and Tree 4 followed by Tree 3 and Tree 5 on which flowering occurred lately.

Table 4: Variation in the phenophases of the male catkins of *Alnus nepalensis*

Observed variable	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Average Date
First flowering	21^{th} September	1^{st} October	18^{th} October	27^{th} September	21^{st} October	5^{th} October
Peak flowering	10^{th} October	15^{th} October	1^{st} November	15^{th} October	31^{st} October	20^{th} October
Last flowering	14^{th} October	23^{th} October	11^{th} November	21^{th} October	15^{th} November	29^{th} October
Flowering duration (days)	24	24	25	25	26	24.8

Protoandry was the unique features of the species. The opening of the female cones occurred 1-2 weeks late of the male flowering. It was also observed that female cone opening occurred after the peak flowering (Fig 4).

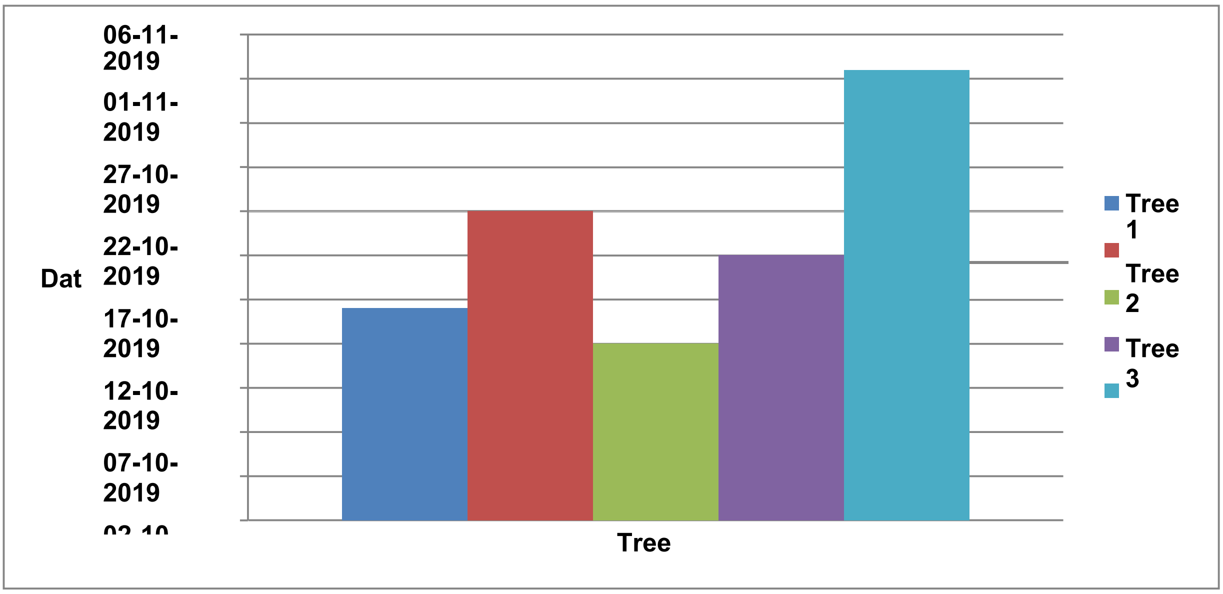

Fig 4: Dates of opening of the female cone

4.3 Pollen release in relation to diurnal temperature

To assess the pollen release in relation to diurnal temperature microscopic slide were placed at different distance (Fig. 5 a, b, c, d). Table 5.0 represents the maximum and minimum time and temperature of pollen release. The observations revealed that the male catkins followed asynchronisation pattern in opening of the anthers. The anthers were bilobed and minimum 6 anther per cymule were observed. No pollen release occurred early in the morning at 0700 hrs with temperature of 17.5^{0}C. The proportions of pollen release increased with the increasing temperature and peaked (average total pollen per slide 73±4.58) at around 1300 hrs with temperature 30^{0}C in day 2. Average pollen release was 66.33±5.51 in day 1 at 1300 hrs with temperature 29.2^{0}C and 62.67±7.09 pollen at temperature 29.2C in day 3. Thus, reflecting a strong positive correlation of 0.8976 with temperature. This gives important information about how abiotic factors and microclimatic conditions are directly proportional to pollen release (Fig.6).

Table 5: Pollen release in relation to diurnal temperature

Day	Time	Temperature (^{0}C)	Date – 13.10.2019 to 15.10.2019				Cumulative anther dehiscence %	Average Anther dehiscence
			1	2	3	Total		
1	7.00AM	17.5	0	0	0	0	0.00	0±0
	9.00AM	21.1	2	2	6	10	0.88	3.33±2.31
	11.00AM	24.1	15	19	11	45	3.96	15±4.00
	1.00PM	29.2	**60**	**70**	**69**	199	17.52	66.33±5.51
	3.00PM	22.1	29	33	37	99	8.71	33±4.00
	5.00PM	20.2	1	0	2	3	0.26	1±1.00
2	7.00AM	17.3	0	0	0	0	0.00	0±0
	9.00AM	21.1	3	2	2	7	0.62	2.33±0.58
	11.00AM	27	25	20	27	72	6.34	24±3.61
	1.00PM	30	**72**	**69**	**78**	219	19.28	73±4.58
	3.00PM	25.2	40	38	47	125	11.00	41.67±4.73
	5.00PM	20.1	2	1	1	4	0.35	1.33±0.58
3	7.00AM	17.5	0	0	0	0	0.00	0±0
	9.00AM	21.1	4	10	11	25	2.20	8.33±3.79
	11.00AM	24.1	14	10	17	41	3.61	13.67±3.51
	1.00PM	29.2	**55**	**69**	64	188	16.55	62.67±7.09
	3.00PM	22.1	23	38	33	94	8.27	31.33±7.64
	5.00PM	20.2	0	3	2	5	0.44	1.67±1.53
Total number of observed pollen /trees			**346**	**386**	**410**	**1136**		

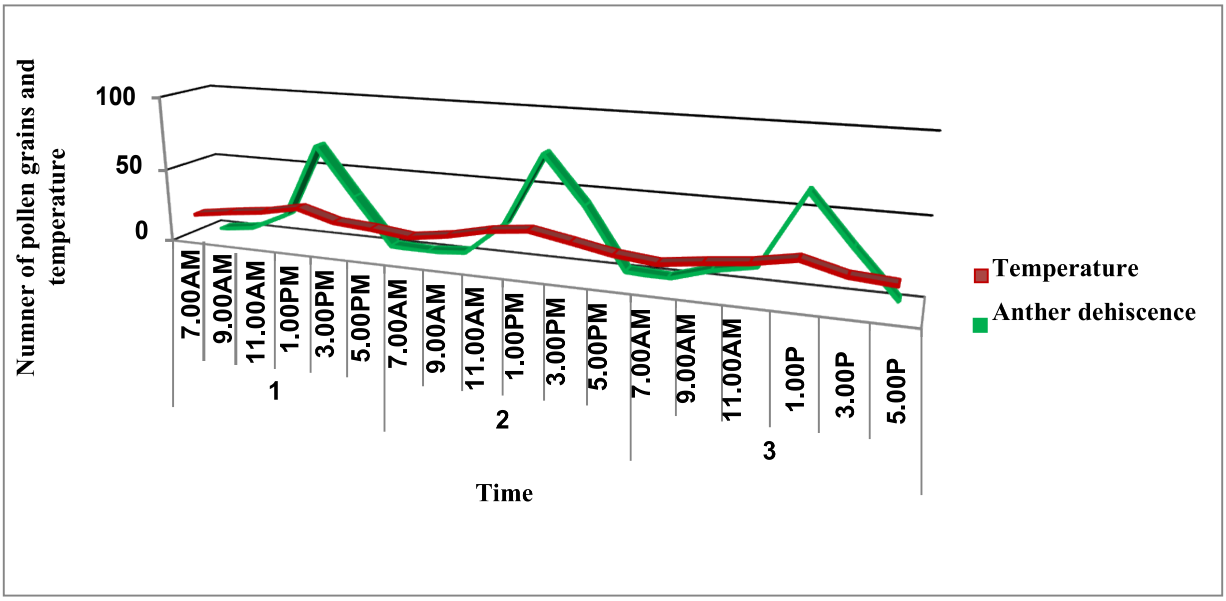

Fig.6 Day-wise relationship between temperature and average pollen release

4.4 Pollen production

In *A. nepalensis*, number of anthers per tree, number of pollen grains per anther, pollen grains per catkin varied from tree to tree. Similarly, total pollen grains per tree (Fig.7 a - 7 b) were differing from tree to tree. Average number of anthers per tree was observed as 4.84×10^6 anthers. Total anthers per tree were ranged between 1.11×10^6 and 8.08×10^6. Pollen grain per anther ranged between 4430.33 and 5146.33 with an average of 4774.13. Moreover, average pollen grain per catkin was found between 2.52×10^6 and 3.84×10^6. Average pollen grain per tree was 2.20×10^{10}, which was oscillating between 5.20×10^9 in tree no 5 and 3.66×10^{10} in tree number 2 (Table.6).

Table 6: Variation in pollen production per anther, catkin and per tree

Tree Number	1	2	3	4	5	6	7	8	9	10	11	12
	Diameter (m)	Height (m)	Number of Primary Branches	Number of secondary branches per Primary Branch	Number of catkin clusters per secondary branch	Number of catkins per catkin cluster	Number of cymules per catkin	Number of anthers per cymule	Number of anther per tree	Number of pollen grains per anther	Pollen grains per catkin	Total pollen grains per tree (9x10)
				(averg.)	(averg.)	(averg.)	(averg.)		(8x7x6 x5x4x3)	(averg.)	(7x8x 10)	
											(averg.)	
1	0.61	12	21	5.43	4.17	13.2	117.6	6	4.43×10^6	4430.33	3.13×10^6	1.96×10^{10}
2	0.96	14.5	17	7.44	4.27	20.2	123.55	6	8.09×10^6	4523.33	3.35×10^6	3.66×10^{10}
3	1.16	15	9	6.56	5.78	15.74	124.35	6	4.01×10^6	5146.33	3.84×10^6	2.06×10^{10}
4	0.50	10	13	7.31	4.43	19.06	114.75	6	5.53×10^6	5069.33	3.49×10^6	2.80×10^{10}
5	0.34	8	5	7.6	5.03	10.8	89.35	6	1.11×10^6	4701.33	2.52×10^6	5.20×10^9
Average	0.71	11.9	13	7.29	4.73	15.8	113.92	6	4.84×10^6	4774.13	3.26×10^6	2.20×10^{10}

Result for correlation

The total production of catkins, cymules, anthers, pollen grains, cone scales and ovules per tree are correlated as one is multiplied by the next to obtain the total production per tree. The height and diameter of the tree has positive correlation with number of catkins, cymules, anthers, pollens, cone scales and ovules per tree due to comparatively moderately height which leads to higher production. It is a known fact that the anemophillous plants are characterized by high pollen production, as the vector of pollination they employ, the wind, sometime not specific and to compensate the reduction of pollen efficiency. For the tree height moderately positively correlated variables was apparent, such as number of catkin per tree (r=0.58), number of cymules (0.64), anthers (0.64), pollen grain per tree (r=0.64), number of cone per tree (r=0.63) On the other hand, moderately positive correlation was observed for tree diameter with number of catkins, cymules, anthers, number of pollen grains (r= 0.44, 0.50, 0.50, 0.52, respectively).

Table 7: Correlation between number of catkin per tree, number of cymules per tree, number of anther per tree, number of pollen grain per tree, with height and diameter.

Variable	Tree height	Tree diameter
Number of catkins per tree (averg.)	0.58	0.44
Number of cymules per tree (averg.)	0.64	0.50
Number of anther per tree (averg.)	0.64	0.50
Number of pollen grains per tree (averg.)	0.64	0.52

Result for ANOVA

The results of Analysis of variance indicate that there is significant ($p < 0.01$) variation among individual for pollen production per tree. Pollen production per branch is non-significant.

Table 8: ANOVA of pollen production per tree

Source of variation	Df	F	P-value
Treatment (trees)	4	6.51	0.0026
Replication (branches)	4	1.81	0.18
Error	16		

4.5 Ovule production

The female cones are greenish brown in colour. Table 9 summarise that the average number of cones developed on secondary branches was 3.97±0.50, The average number of cones produced per tree was 376.24 ± 137.51. The total ovule production per tree was estimated as

82324.40±37030.30. Relation between tree height and diameter for parameters, such as cone per tree, scales per tree and ovule production per tree was moderately correlated. As cone per tree (r=0.63), number of scales per tree (r=0.61). The total ovules per tree reflected weak positive correlation (r=0.21) with tree height. However, moderate correlation was observed for diameter with scales, and ovules per tree (0.44, 0.42, and 0.44, respectively).

Table 9: Variation in number of scales, ovule and total ovule per tree.

Tree Number	1	2	3	4	5	6	7	8
	Diameter (cm)	Height (m)	Number of Primary Branches	Number of secondary branches per Primary Branch (averg.)	Number of cones per secondary branch (averg.)	Number of scales per cone (averg.)	Number of ovules per cone (averg.)	Total ovule per tree (7 x5x4 x3)
1	0.61	12	21	5.43	3.48	127.2	227.65	90337.07
2	0.96	14.5	17	7.44	4.15	131.4	245.20	128703.52
3	1.16	15	9	6.56	4.64	115.7	212.85	58309.32
4	0.50	10	13	7.31	3.45	123.1	221.32	72560.54
5	0.34	8	5	7.6	4.11	97.95	187.05	29213.47
Average	0.71	11.9	13	7.29	3.97	119.07	218.81	82324.40

Table 10: Correlation between number of cones per tree, number of scales per tree, total ovule per tree with height and diameter

Variable	Tree height	Tree diameter
Number of cone per tree (averg.)	0.63	0.44
Number of scales per tree (averg.)	0.61	0.42
Total ovule per tree (averg.)	0.21	0.44

4.6 Pollen ovule ratio

The number of anthers per catkin was 683.52±85.88 and the number of pollen grains per anther was 4770.00 ± 335.52. Thus, it was calculated that total number of pollens in a catkin was 3263213 ± 490538. The total number of ovules per cone was observed as 218.81±21.36. Hence, pollen grains per ovule were 14913.46 ±2003.79 (Table 11). The difference in pollen production per anther and ovules per cone between trees differs significantly. Also, the pollen ovule ratio among trees was significantly different. On the basis of pollen ovule ratio indices of **(Cruden 1977)**, *A. nepalensis* falls under xenogamy i.e., out breeding system.

Table 11: Showing variation in pollen ovule ratio

Trees	Number of ovules per cone (averg.)	Number of pollen grains per anther (averg.)	Number of anthers per catkin	Number of pollen per catkin	Pollen ovule ratio
1	227.65	4534.67±112.45	705.6	3199661	14055.18
2	245.2	4373.67±429.20	741.3	3242199	13222.67
3	212.85	5146.33±129.55	746.1	3839679	18039.37
4	221.32	5076.00±99.08	688.5	3494826	15790.83
5	187.05	4719.33±139.00	536.1	2530035	13525.98
Average	218.81	4770.00	683.52	3263213	14913.46
SD	21.36	335.52	85.88	490538.8	2003.79

4.7 Pollen viability

Viability of pollen grain was measured by using acetocarmine test. The percentage of viable pollen per slide were 100% (Table.12), The pollen grains deeply stained after 12 hours. No shriveled, weakly stained and non-viable pollen was recorded. This gives important information about the reproductive potential of the species as no biotic agents are involved and pollen has to travel a long distance through wind for mating success.

Table 12: Pollen viability of *Alnus nepalensis*

Tree		Total pollen	Viable pollen	pollen viability %
1		4939.333	4939.333	***100***
	SD	150.6748	150.6748	0
2		5019.333	5019.333	100
	SD	12.65789	12.65789	0
3		4912.667	4912.667	100
	SD	358.0748	358.0748	0

4.8 Pollen germination

Table 13 summarizes that the seven different media Sucrose, IBA, IAA, GA_3, Kenetin, Boric acid Boric acid + sucrose 10%) were used to promote *In-vitro* pollen germination in *Alnus nepalensis.* The observation revealed that the pollen grains took about 7-12 hours to germinate from immersing them into the germinating media and the species shows quiet low germination in the germinating media. Even though the germination was low some broken pollen tubes were seen in microscopic view. The maximum pollen germination of 3.69% was observed in Boric acid (25ppm), followed by Boric acid + Sucrose 10% (2.03% in 50 ppm) than Sucrose 10% 1.67, Boric acid +Sucrose 10% (1.43 in 25ppm) . The germinating media IAA, IBA, GA_3, and Kinetin were failed to achieve pollen germination.

The germinating media used in the experiment was incompatible with the stigmatic conditions required for successful pollen germination and pollen tube development. Thus, proper germinating media and different experiment will be conducted in future for further validation.

Table 13: The germination percentage of pollen grains in different media

Media		Germinated pollen	total pollen	Germination percentage
SUCROSE	10%	3	180	1.67
	20%	0	200	0
	30%	0	217	0
IBA	No germination			
IAA	No germination			
GA3	No germination			
KENETIN	No germination			
BORIC ACID	5ppm	0	321	0
	15ppm	3	100	3
	25pmm	6	234	3.69
BORIC ACID +SUCROSE10%	25ppm	4	280	1.43
	50ppm	8	394	2.03
	75ppm	0	432	0
	100ppm	0	245	0
Control		0	403	0

4.9 Pollen mediated gene flow

Table 15 summarizes the results on pollen dispersal of *A. nepelensis* from isolated source trees for uphill and downhill direction but upto 10 m due to geographic barrier and another location for downhill for a chunk of 13 trees with average diameter 21.5 cm and height 18.5 m (Table 14). The pollen dispersal at two different directions was not uniform and varied with different distance from source trees. Results shows that the frequency of pollen flow were increased with increasing distance upto 10 m from source trees in uphill and downhill direction. Maximum average pollen grains was found in 10m distance from source trees i.e. 44.00±6.00 per slide in uphill direction and for downhill side maximum pollen grains per slide was found at 10 m distance from source tree, i.e. 139±17.2 per slide for isolated source of trees as a source in downhill direction per slide was 22. ±4.04. Pollen grains can travel upto 80 m distance from source tree towards downward directions, (1.3±0.6 pollen grains per slide). However, for uphill directions it can travel upto 40m only (Fig. 8). From recorded observations, a linear equation was developed for both directions (uphill and downhill) as follows: for uphill direction: Y= -

0.764x+60.91 R^2= 0.198 and downhill direction Y= -1.46x+213.9 (R^2= 0.065) where Y is the magnitude of pollen grains per slide and X is the distance from the source tree.

Table 14: Height and diameter of chunk of pollen trees.

S no	Height(m)	Diameter(cm)
1	19	28.66
2	18	27.07
3	21	30.25
4	20	28.66
5	18	27.07
6	16	22.29
7	19	36.62
8	18	28.66
9	14	27.07
10	20	20.70
11	18	28.66
12	22	34.59
13	18	25.48

Table 15: Pollen grains per slide for uphill and downhilll directions for selected source trees.

Distance from source (m)	Pollen frequencies (1cm^2) towards downhill distance from a chunk of 13 trees					Pollen frequency (1 cm^2) from isolated 5 trees									
						Downhill					Uphill				
	T1	T2	T3	Total pollen1 cm^2	Average	TI	T2	T3	Total pollen	Average	TI	T2	T3	Total pollen	Average
0m	0.0	0	0	0.0	0.0±0.0	0	0	0	0.0	0±0	0	0	0	0.00	0.0±0.0
5m	34.0	25	26	85.0	28.3±4.9	17	15	11	43.0	14±3.05	17	15	21	53.00	17.7±3.1
10m	153.0	120	145	418.0	139±17.2	23	18	26	67.0	22±4.04	50	38	44	132.00	44.0±6.0
20m	104.0	96	112	312.0	104±8.0						20	13	24	57.00	19.0±5.6
40m	80.0	71	86	237.0	79.0±7.5						2	1	2	5.00	1.7±0.6
80m	1.0	2	1	4.0	1.3±0.6						0	0	0	0.00	0.0±0.0

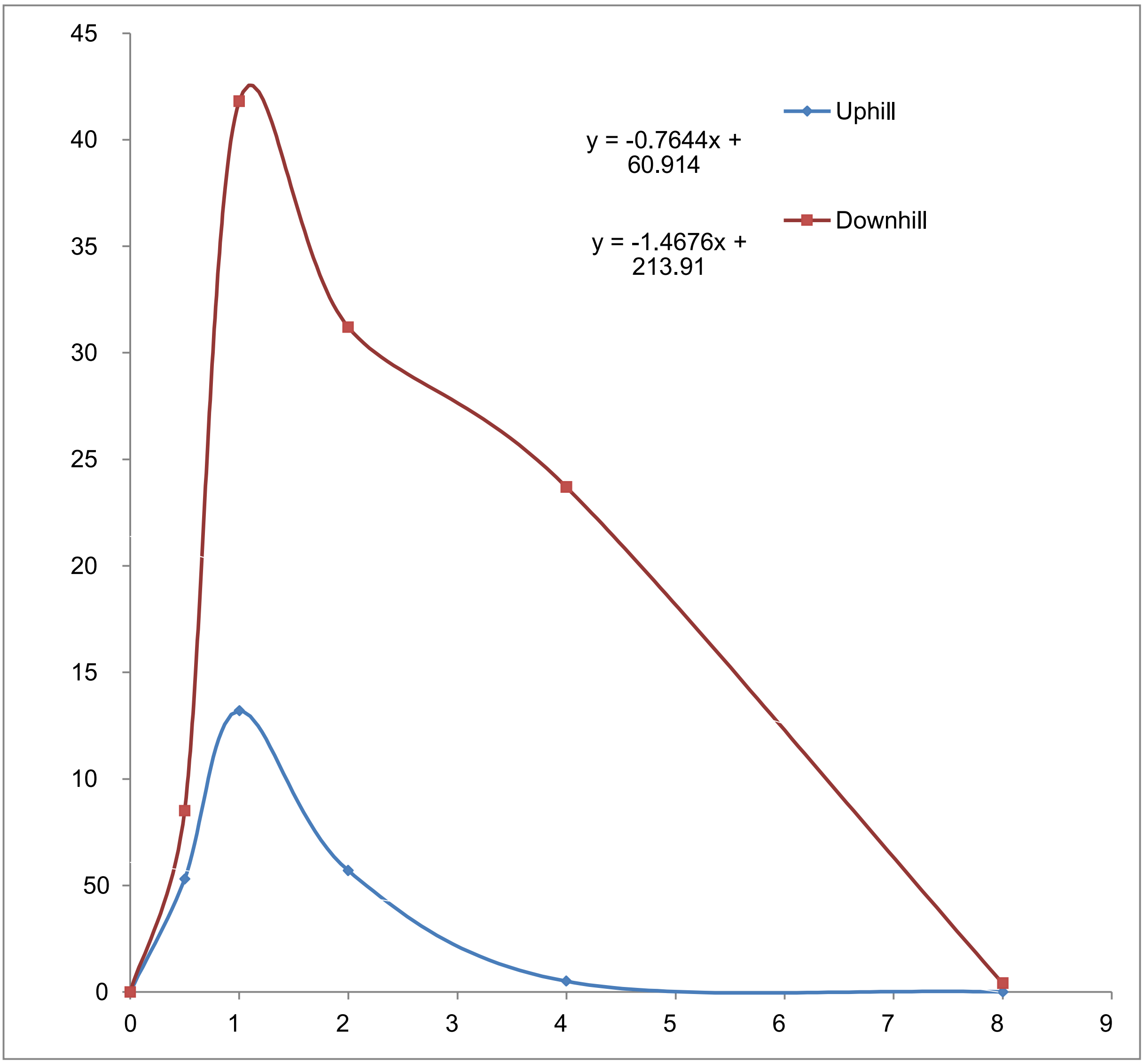

Fig. 8 Graph representing pollen mediated gene flow in uphill and downhill direction

CHAPTER 5 DISCUSSION

5.1 Floral Morphology, Structure and Development

The broad reflection of the aerodynamics requirement that leads to efficient pollen liberation from anthers and capture by stigma has been discovered in studies on structural and functional aspects of flowers of wind pollinated plants **(Niklas 1992; Shivanna and Tandon 2014)**. Herein, the inflorescence structure and floral morphology of *Alnus nepalensis* were studied, and it was observed that *A. nepalensis* is monoecious tree, the male catkins are composed of sessile flower or "cymule". The advent of bud bursting started in the first week of August and fully developed in last week of September, with strong differentiation of cymules. Importantly, after this phase, the cymules began to unfold and organize themselves in the thread-like structure, and the cymules began to burst and color changed from red to yellow. However, the female cone or strobili was tall, spherical, and clumped together. It had a raceme pattern. Cone scales bent at the top form a compact spherical cone called strobili. Female cones are usually made up of scales that are dark green in color. The color of the cone changed after the male portion (catkins) bloomed for 1-2 weeks. Similar researches on family Betuleaceae supports our study, which has led to the idea of compound spike for staminate inflorescences; while partial inflorescences have been classified as dichasium or a cymule (three flowered). In female inflorescence the ovary is inferior, bicarpellate with two anatropous and unitegmic ovule **(Abbe 1935; Hjelmqvist 1948)**. Furthermore, staminate catkin is pendulous with prominent bracteates (1-5) bracts, fused flower, small deficient perianth, tetrasporangiate anthers, two locular dorsifixed extrose, and opening by longitudinal slit consisting of 1-5 fused bracts. The ovary is normally absent from the flower, the style is linear, and the ovule axile is 1-2 per locule (**Kubitzki 1993)**. Similarly, the floral morphology and phylogeny of the Betulaceae family indicates that the pistillate cone grows into wood and cone-like structures, and the pistillate flower is made up of two carpels with linear designs. The anthers have two theca, each with two anther sacs and a short bifurcated filament connecting them. Staminate catkins appeared in a pendulous cluster, typically at terminal position ranging in length from 2-3 cm to 7-8 cm. Several pistillate catkins were borne per floral bud, located on a bud proximal to staminate catkin 5-8 mm long and reddish green when receptive **(Harrington et al. 1994; Lin et al. 2010)**. All of the previous research performed by other researchers in the same genus and family supports this analysis.

5.2 Flowering Phenology

Accurate phenology assessment allows us for better understanding of population dynamics and picking of provenance that is ideally suited to new climatic conditions **(Cleland et al. 2007)**. The flowering phenology of *Alnus nepalensis*, according to **Luna (2005)** is from September to October. In comparison, the current study found that male phase of flowering began in September and lasted until November. Male flower cycle started in August where bud bursting happened 20 days after the initiation and remain erect for 40 days and drooping of catkin started after 11 days. Similarly, cone initiation started in August and enlargement took 30 days. The average duration took to complete the reproductive cycle was 2.5 months. Peak shedding of pollen precedes peak receptivity but synchrony in receptivity and pollen shedding is also seen during the study period. Similar results have also been reported by **Brown (1995)** in *Alnus rubra.* Moreover, present study reveals that *A. nepalensis* flowered for approximately 3 week which is well supported by the other study in *Alnus* taxa which shows that in average, taxa flowered for 2 to 3 weeks (**Dabrowska et al. 2012)**. According to **Fashel and El-kassby (1987),** the prolonged pollination period increases the chance of selfing. **Nicholls and Dorken (2012)** pointed out that in monoecious species geitonogamous selfing occurs. However, the synchronous and asynchronous flowering has an impact on the reproductive success which determines the level of outcrossing and selfing **(Khanduri et al. 2013)**. Asynchronization in the flowering was seen within and among individuals. Asynchronization has shown to have adverse effect on random mating and minimizing selfing **(El- kassaby 1987)**. The unique feature studied about the species was the protandrous condition. The opening of the female cones occurred 1-2 weeks after male flowering. It was also observed that the female cone opening occurred after the peak flowering. Protandry is also reported in another species *Alnus firma* and *Alnus sieboldiana* which means that the pollen grains dispersal happens before the pistil become receptive. Therefore, the pollen grains are received from other trees, thus avoiding self-pollination (**Sogo and Tobe 2005**).

5.3 Pollen Release in Relation to Diurnal Temperature

It was clear from the observations that the pollen release depends on climatic factor, i.e., temperature and humidity. It was observed that at temperature 30^0C in day 2 at 1300 hrs, maximum pollen was released. The optimum temperature required for *A. nepalensis* was 30^0C to release pollen. In the case of most anemophilous trees, pollen release is mainly controlled by the temperature (**Pacini and Hesse 2004**). The present study reflected a strong positive correlation of 0.8976 between air temperature and pollen release. Similar observations was also observed by **Dabrowska-Zapa et al. (2018)** and showed a statistically significant positive correlation with the air temperature. Studies on airborne pollen was conducted with relation to temperature and found a good support for the result that temperature has positive correlation with pollen dispersal **(Jato et al. 2002; Meng et al. 2016; Ziska et al. 2019)**. Interestingly, pollen from *Alnus* trees is one of several sources of the annual atmospheric aerosol **(Sabela lvarez-López et al. 2010)** and identified that the *Alnus* as one of the most important allergic taxa, observing an increase in the incidence of pollen allergy diseases over the last half-century, which is the key biological contamination episodes caused by the aeroallergens of the major allergenic tree species in environments. *Alnus* pollen has been reported as one of the major causes of pollinosis in Central and Northern Europe **(D'Amato et al. 2007)**. Pollen grain occurrence in the atmosphere is closely related to meteorological influences. **Bartková-evková (2003)** discovered that the average daily temperature is a significant factor in pollen grain occurrence in the atmosphere. It was also observed that mean temperature during the previous month to pollination also increases the annual pollen amount **(Weryszko-Chmielewska et al. 2006)**.

5.4 Pollen Production

Anemophilous trees produce large amount of pollen to ensure successful pollination in long range distance and the amount of total pollen production per tree ensures the successful seed production which is important for reproduction and development of plant community **(Fageri et al. 1989; Alison 1990; Campbell and Halama 1993; Whitehead 1983; Khanduri 2012)**. The total pollen production is influenced by various factors and initial study on pollen production in anemophilous trees was done by **Pohl (1937)** that gives some values for the total pollen production per plant in several herbaceous species which lies between almost 3 million to 1300 million. **Erdtman (1945)** gives values for pollen production per flower for different trees, such as *Pinus sylvestris* (158×10^3 per strobilus) and *Quercus sessilifora* (41×10^3). Also, **Mollina et al. (1996)** reported that in *Pinus pinaster*, pollen production per strobilus ranged between 244×10^3 to 280×10^3 per strobilus, while 340×10^3 to 1100×10^3 per catkin for *Quercus rotundifoila*. In present study, total pollen production per tree ranged from 5.12×10^9 in tree no 5 to 3.66×10^{10} in tree number 2 with an average pollen grain production per tree was 2.20×10^{10}. In this analysis, average pollen grains per catkin ranged from 2.52×10^6 to 3.84×10^6, which is consistent with *Alnus incana* **(Pohl 1937; Moe 1998)**. In an analysis of pollen production in six oak species by **Fernández-González (2020)**, discovered that the average pollen amount per anther ranged from 3400 pollen grains in *Q. robur* to 6282 pollen grains in *Q. ilex*. The number of pollen grains produced per catkin varied between 355,237 and 716,385 for *Q. suber* and *Q. rubra*, respectively. Finally, pollen grains per tree varied between 436 billion and 1.6 billion for *Q. robur* and *Q. ilex*, respectively. **Gómez-Casero et al. (2010)** studied pollen biology in four Mediterranean *Quercus* species for two years. They discovered that production of pollen grains per tree in the years 2000 and 2001 for *Q. suber* was 24,046 × 10^6 and 55,045 × 10^6, *Q. ilex* ssp. *ballota* 11,919 ×10^6 and 24,500 ×10^6, and *Q. faginea* 16,715× 10^6 and 22,731 ×10^6 pollen grains per tree, respectively, In other cross pollinated species, such as *Acer negundo* produces 17,374-96,520 pollen grains per anther and per tree it ranged between 15.2 to 250.1 ×10^9 while, *Populus* produces 564.5 to 712.4 ×10^6 pollen grains per tree **(Molina et al. 1996)**.

Height and diameter of tree possesses the moderate correlation with number of catkins per tree, number of cymules, anthers, pollen grain per tree, and number of cone per tree indicates that with the increasing height and diameter of tree, number of catkin, cymules, anthers and pollen grains per tree increased. In contrary, height and diameter of *Pinus roxurghii* have negative significant correlation with pollen grain per tree (**Khanduri and Sharma 2002)** and height of tree in *Populus* and *Acer negundo* have negative significant correlation with pollen grains per tree tree **(Molina et al. 1996)**. In detail study of six oaks species, negative correlations were found between pollen grains per anther, number of anthers per flower, and number of flowers per catkin. A positive correlation was discovered between pollen grains per anther and pollen grains per catkin. The number of anthers formed per flower was positively correlated to the number of catkins per tree and pollen development per tree. Pollen grains per catkin were also positively co related to the number of flowers per catkin (**Fernández-González 2020**).

5.5 Total Ovule Production

The amount of ovule production by an individual tree in a population varies from tree to tree. It is a known fact that high ovule production means high pollen: ovule ratio, and high pollen: ovule ratio leads to the higher seed set in trees. The total ovule number is an important factor affecting the seed set **(Strelin and Aizen 2018)**. In present study, the total ovule production per tree was estimated to be 82324.40±37030.30. Relation between the total ovules per tree with tree height reflected weak positive correlation while moderate correlation was observed with diameter. Such correlation study for trees is not yet studied and needs further investigations.

5.6 Pollen: Ovule Ratio

Since the pioneering work of **Cruden (1977)**, pollen to ovule (P: O) ratios have been commonly measured by plant population biologists and often been related to the mating system, despite ongoing controversies concerning their indicative values. Within phylogenetic lineages, the ratio of pollen grains to ovules per flower may indicate the mating mechanism **(Cruden 1977; Michalski and Durka 2009)**. The pollen ovule ratio in *A. nepalensis* was ranged between 13222.67 to 18039.36 pollen grains per ovule with an average of 14913.46. According to **Pohl (1937)** an obligate out-crosser has a pollen: ovule ratio of approximately 57,310 pollen grains per ovule. Similarly, in an anemophilous plant, such as *Pinus roxburghii,* pollen: ovule ratio reported to be varied between 4978.52 and 5929.22 **(Khanduri and Sharma, 2002)**. The observations revealed that there was high pollen: ovule ratio in *A. nepalensis*. On the basis of the pollen: ovule ratio **(Cruden 1977),** *A. nepalensis* falls within the reported range of xenogamy. **Khanduri (2012)** and **Tangmitcharoen and Owens (1997)** conducted pollen:ovule ratio studies on *Tectona grandis*, a xenogamous plant, and found that the average pollen:ovule (P/O) ratio was 3597:1 and 3253:1 respectively.

5.7 Pollen Viability

High seed set, production of highly viable pollen, is critical for breeders and conservators **(Impe et al. 2020)**. In a genetic breeding program, information on pollen viability is a main factor for artificial hybridization (**Alam and Grant 1970; Soares et al. 2008)**. Pollen viability is influenced by a variety of factors, including humidity and temperature. The flowering of *A. nepalensis* occurred after the rainy season in the current study sites, resulting in high relative humidity and optimal temperature. Pollen viability is mainly determined by these two variables **(Stanley and Linskens 1974; Travers 1999)**. Furthermore, *in vitro* pollen germination tests are generally used to evaluate the percentage of pollen that germinates, but they can also be used to measure pollen vigour by monitoring the rate of germination over time or the length of pollen tubes (**Sulusogolu and Cavsolglu 2014)**. In this study, only the acetocarmine staining method **(Alexender 1965)** was used, and it successfully stained all pollen. When pollen viability was tested using Trypan blue in *Betula pendula*, a similar result was obtained **(Dafni and Firmage 2000)**. It was also found that pollen viability of *A. nepalensis* depends on genotype, pollen collection time and medium component, site conditions etc. This study is expected to be useful to plant breeders, geneticists, or gene bank curators who need pollen viability tests for *A. nepalensis.*

5.8 Pollen Germination

Pollen germination and tube growth are fundamental processes for reproduction of flowering plants. The development of reliable methods for determining the functional quality of pollen helps in monitoring pollen vigor during storage, genetics and pollen-stigma interaction studies, crop improvement and breeding, and incompatibility and fertility studies **(Sulusoglu and Cavusoglu 2014)**. The maximum (3%) *in vitro* germination in *A. nepalensis* was found in Boric acid 10% (25ppm), followed by Boric acid + sucrose 10% (50 ppm). 60-80% pollen germination was found in *Alnus glutinosa*, indicates the importance of germination media and favorable environment conditions **(Linares, 1985)**. Apart from this, germination capacity of *Alnus cordata* was 32% on the fresh pollen samples **(Falleri, 1992)**. The low germination of *A. nepalensis* pollen indicates the lack of functional pollen grains in individual trees of study sites that leads to the lower seed set and seed vigor. One of the studies on alders shows that after contact with rainwater, pollen grains from alder can release allergens into the atmosphere by a mechanism called abortive germination. Because fresh pollen grains of alder had pollen tube growth in rainwater in a way similar to pollen tube growth on the stigma of the female reproductive organ, which is required for fertilization. In contrast to normal fertilization, tube growth in rainwater stops after some hours and aborts **(Grote et al. 2003)**. Similar experiment *i.e. in-vitro* pollen germination of *Alnus* was conducted by **Steiner and Gregorius (1998)** and observed many factors which affect the pollen germination such as pollen donors, physiological state of pollen and germination media. However, similar media was used in this study *i.e.*, Boric acid + sucrose 10% leads to very poor germination. In contrary, maximum (98 ± 6.1%) pollen were germinated with germination medium contained 10% sucrose solution supplemented with 100 ppm H_3BO_3, 300 ppm $Ca(NO_3)_2$, 200 ppm $MgSO_4$ and 100 ppm KNO_3, after 6 h of incubation in *Betula utilis* **(Wani et al. 2020). Steiner and Gregorius (1998)** found relatively high percentage of pollen germination when pollens were subjected to high humidity in moist chamber. Incubation time is also an important factor for pollen germination **(Lin et al 2017)**. It can also be the possible reason of less germination percentage in the present study experiment.

5.9 Pollen Mediated Gene Flow

The results for pollen mediated gene flow for *A. nepalensis* indicated that the magnitude of pollen dispersed from source tree in both directions, i.e., uphill and downhill side was not uniform. Notably, pollen from trees on the uphill side capturing was isolated, while on the downhill side a patch of 13 trees were considered. Pollen flow frequency increased with distance up to 10m, then gradually decreased after that, for both conditions. However, the maximum pollen travel distance for isolated trees was 40 m, and the maximum pollen travel distance for trees in patches was 80 m. Similar results were found for Douglas fir where pollen dispersal range in pair trees was more than that of single tree source with maximum pollen count in 50 feet distance **(Silen 1962)**. This result proposed that three main factors influence pollen dispersal distance: gravity, wind speed, direction, and species distribution pattern **(Di-Giovanni and Kevan 1991)**, which is consistent with our findings. Similar results on pollen dispersal have also reported in several studies (**Wright 1952; Bramlett 1981; Sharma and Khanduri 2007; Sharma et al. 2012; Khanduri and Sukumaran 2019)**. Based on pollen trap technique, it was observed that the density of pollen decreases rapidly with the distance from male trees **(Noryśkiewicz, 2006)**. Similar pollen dispersal frequency was also observed for some anemophilous tree at various distances from the source trees e.g., *Myrica esculenta* 110 pollen grain per slide at 64 m from source tree (**Khanduri and Sukumaran 2019**) 3 pollen grains at 45 m, 8 pollen grains at 100 m, 2 pollen grains at 45 m, respectively for *Pseudotsuga taxifolia*, *Populus deltoides*, *Fraxinus americana* (**Wright 1952**). Similarly, frequency of pollen grains in *Cedrus deodara* at 190 m relative to the source frequency was 2.5-5% (**Khanduri and Sharma 2002**) and in *Pinus roxburghii* 4-6% relative to the source frequency at 600 m (**Khanduri 2012**). In *Malus sylvestris*, the average (50 percent) pollen flow distance was approximately 23 m, with a maximum distance of 60 m **(Larsen and Kjær 2008)**. In present study, the effect of direction was also observed for pollen dispersal. Total pollen count at different distances and direction was minimum for uphill and maximum in downhill. It indicated free flow of pollen in downhill side. This finding was also supported by **Sharma and Khanduri (2007)**. Pollen dispersion studies are important in the conservation management of forest tree species because they promote good natural selection efficiency by allowing the population to remove undesirable pollen from the outside, which would be useful in the establishment of seed orchards **(Larsen and Kjær 2008)**.

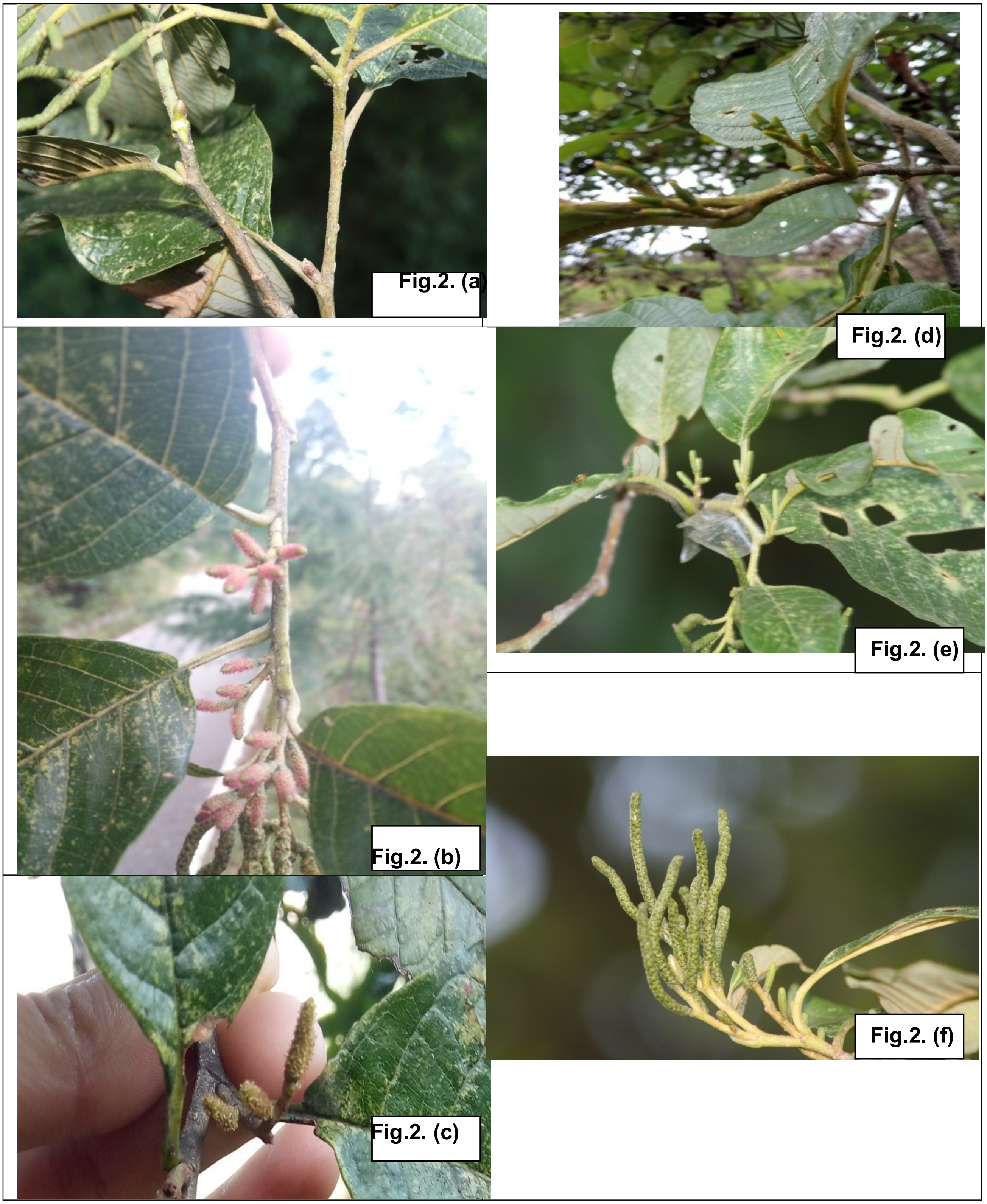

Fig.2. (a)

Fig.2. (b)

Fig.2. (c)

Fig.2. (d)

Fig.2. (e)

Fig.2. (f)

Fig.2. Different stages of catkin elongation

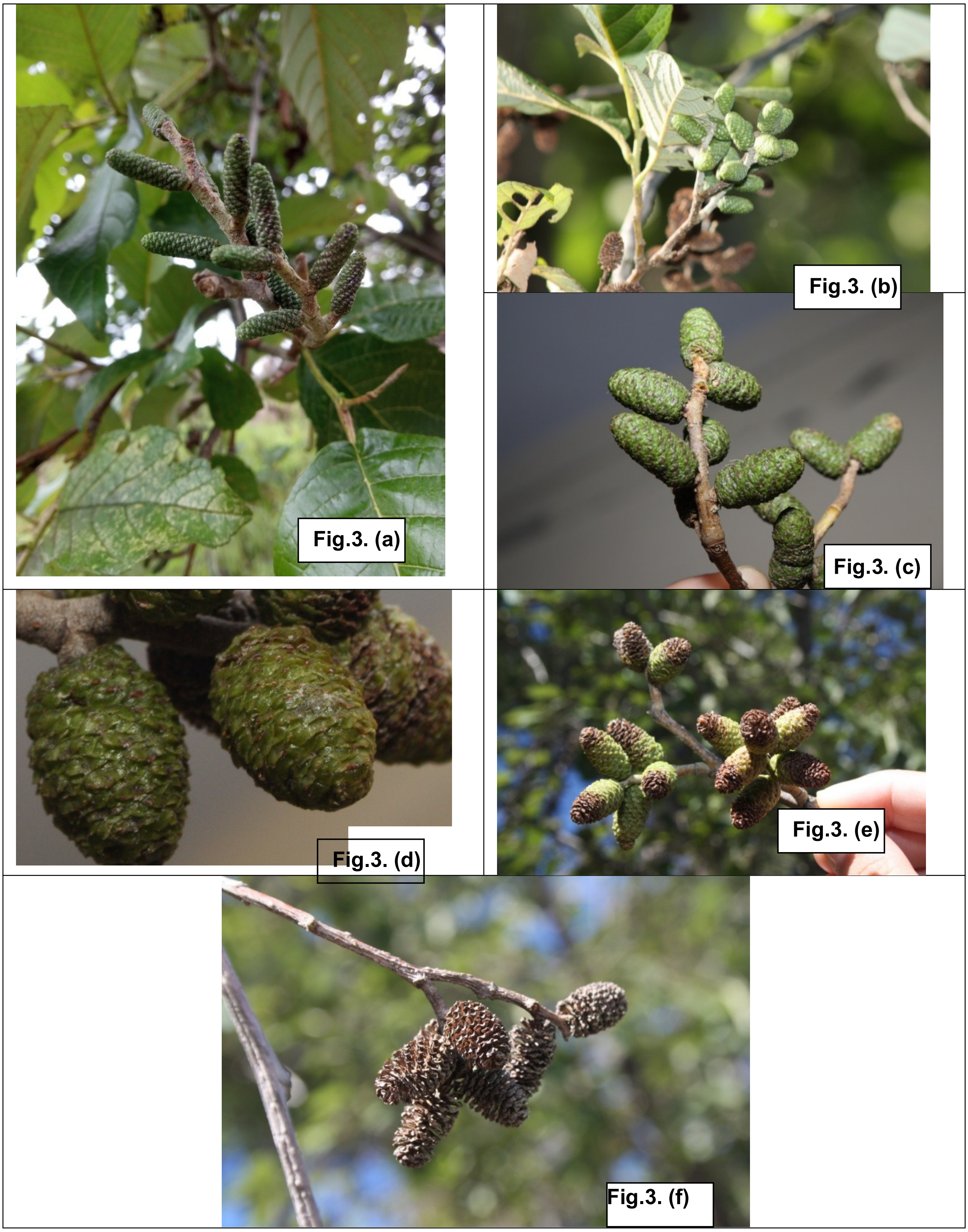

Fig.3. (a)

Fig.3. (b)

Fig.3. (c)

Fig.3. (d)

Fig.3. (e)

Fig.3. (f)

Fig.3. Different stages of cone developement

Fig.5. Petroleum jelly coated slides in experimental field

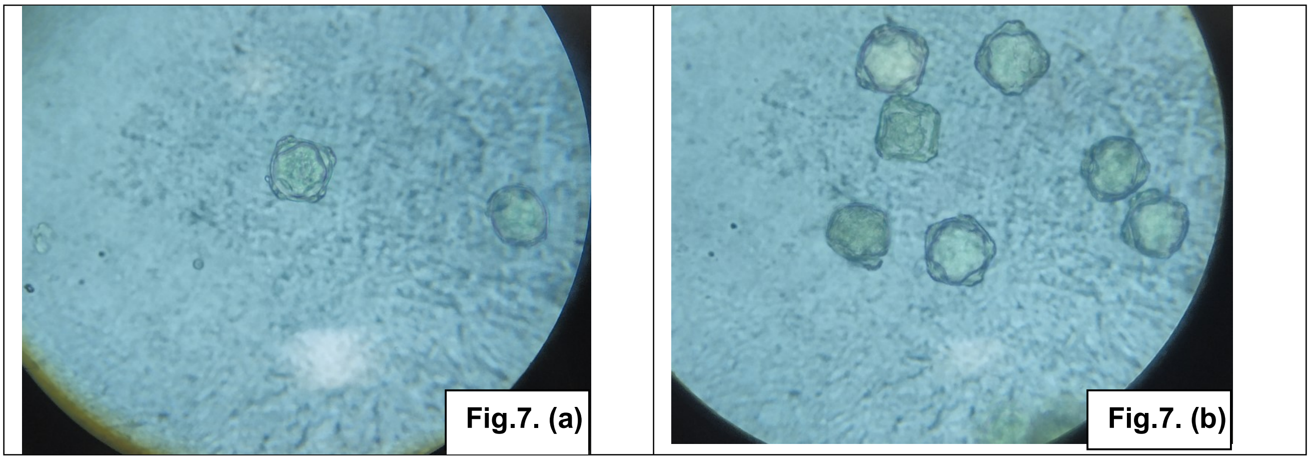

Fig.7. Pollen structure under microscope

CHAPTER- 6 SUMMARY CONCLUSION AND RECOMMANDATION

Reproductive phenological study of several temperate pioneer species is still lacking. Being a good pioneer species and ability of symbiotic relationship with nitrogen-fixing actinomycetes of the genus Frankia makes *Alnus nepalenisis* able to improve degraded and landslide prone land. Thus this tree species is a potential soil improver and also added the considerable quantities of nutrients through the litter. This tree is multipurpose tree species in subtropical and temperate regions of western Himalaya. The value of *Alnus nepalensis* has increased substantially in recent years and interest in the management of the species has increased accordingly, which in turn has led to an increased need for detailed information on the reproductive biology of the species.

The reproductive phenology study of *A. nepalensis* was carried out in five randomly selected tree containing clusters of male catkins and female cones of *A. nepalensis* at on the way of Dandachali to Ranchauri, Tehri Garhwal, Uttarakhand from August 2019 to December 2019. Various parameters of reproductive phenology i.e., Floral morphology structure and development, Flowering phenology, Pollen release in relation to diurnal temperature, Pollen production, Total ovule production, Pollen ovule ratio, Pollen viability, Pollen germination was observed. Also in adjacent site of this patch, the pollen mediated flow was recorded in a representative patch of 13 trees which was selected as pollen source (focal trees) for downhill directions from the source trees, and in downhill upto 10 m due to physiological barrier and uphill directions upto 80m the pollen mediated gene flow was carried out for five isolated trees.

The observed parameter in present study i.e., reproductive structure, pollen ovule ratio supported that *A. nepalensis* is xenogamous species. The inflorescence structure and floral morphology it is highly compact and inconspicuous in *A. nepalensis*. It was observed that flowering in male phase was started in the last week of September and continued till November. Time between onset and peak flowering was 2 weeks 4 days and the total duration of flowering period was 24.8 days. Pollen release in relation to temperature indicated that maximum pollen release was occurred at 30^0 C at 1300 hrs. Since *A. nepalensis* is an anemophilous tree. It produces large amount of pollen, to ensure successful pollination in long range distance. The amount of ovule production leads to the high pollen: ovule ratio, and high pollen : ovule ratio leads to the higher seed set in trees. Pollen: ovule ratio observed in *A. nepalensis* which falls within the reported range of xenogamy. Pollen viability and germination percentage of pollen grains under *in vitro* in *A. nepalensis* have showed that 100 percent viability but very poor germination percentage. Which indicated that availability of large number of functional pollen but pollen tube formation needs some atmospheric conditions, such as relative humidity for germination.

Pollen-mediated gene flow reveals the migration of gene and the degree of mode of pollination. which depends on the spatial distance between the individuals of species. It is well known fact that gene flow is a key factor in the evolution of species, influencing effective population size, hybridisation and local adaptation. Results of pollen mediated flow shows that the frequency of pollen flow were increased with increasing distance upto 10 m from source trees in uphill and downhill direction. Maximum average pollen grains was found in 10m distance from source trees i.e. 44.00±6.00 per slide in uphill direction and for downhill side maximum pollen grains per slide was found at 10 m distance from source tree, i.e. 139±17.2 per slide for isolated source of trees as a source in downhill direction per slide was 22.±4.04. Pollen grains can travel upto 80 m distance from source tree towards downward directions, (1.3±0.6 pollen grains per slide). However for uphill directions it can travel upto 40m only.

So present study was carried out to know flowering phenology, Extension of the catkins and anther dehiscence in relation to time and temperature of the day, Estimation of variation in pollen output per anther, catkin and per tree, Estimation of pollen viability and *in-vitro* pollen germination following hormones media composition. And lastly pollen mediated gene flow by considering a patch of trees as a pollen source.

An elaborated study on reproductive structure, pollen germination, anthesis, anther dehiscence, seed development and germination are required to understand the eco-physiology of *A. nepalensis*. The finding of present study indicates that being a multipurpose tree species over exploitation may leads to population reduction. Which should taken to consideration for further improvement and breeding program for conservation as well as sustainable use of tree species.

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VITAE

The author Anchal Bisht was born on March 6, 1993 at Nainital Distt. Uttarakhand. She graduated her High school and Intermediate from St Mary's Convent High School Nainital in the year 2008 and 2010. She received B.Sc. (Hons.) Forestry from VCSG University of Horticulture and Forestry, Ranichauri in 2018. She joined the VCSG University of Horticulture and Forestry, Ranichauri in 2018 for persuing the degree of M.Sc. (Forestry) Tree Improvement.

Permanent address

Anchal Bisht

D/o Mr. L. K. Bisht

Sudarshan Hotel Compound, Zoo road

Tallital, Nainital.

anchalbisht987@gmail.com