

REPRODUCTIVE BIOLOGY OF MISTLETOE
Loranthus odoratus Wall. (LORANTHACEAE)

A THESIS
SUBMITTED FOR THE
PARTIAL FULFILMENT OF THE REQUIREMENTS FOR
THE MASTER'S DEGREE IN BOTANY

BY

PRAKASH DHUNGANA

Symbol Number: BOT. 568/074

TU Registration Number: 5-2-48-1482-2013

Batch: 2074



DEPARTMENT OF BOTANY
AMRIT CAMPUS
TRIBHUVAN UNIVERSITY
KATHMANDU, NEPAL,

MAY 2023

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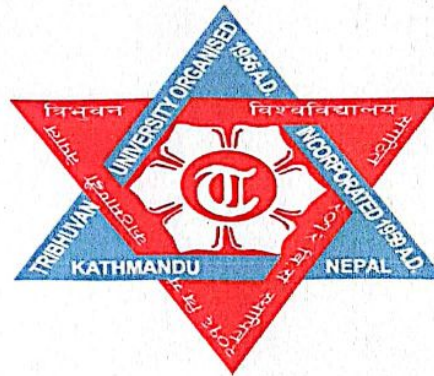
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DECLARATION

I, Prakash Dhungana, hereby declare that the work enclosed here is entirely my own, except were stated by reference or acknowledgement, and has not been published or submitted elsewhere, in whole or in part, for the requirement for any other degree or professional qualification. Any literature, data or works done by others and cited within this thesis has been given due acknowledgement and are listed in the reference section.



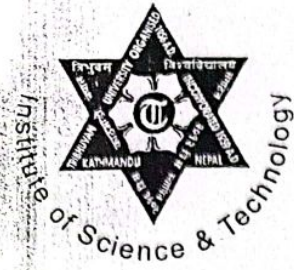
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RECOMMENDATION

This is to recommend that the Master's thesis entitled "**Reproductive Biology of Mistletoe *Loranthus odoratus* Wall. (Loranthaceae)**" is carried out by Prakash Dhungana under our supervision. The entire work is based on original scientific investigations and has not been submitted for any other degree in any institutions. We therefore, recommend this thesis work to be accepted for the partial fulfillment of Master's Degree in Botany.

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The thesis work submitted by Prakash Dhungana entitled "**Reproductive Biology of Mistletoe *Loranthus odoratus* Wall. (Loranthaceae)**" submitted to Department of Botany, Amrit Campus, Tribhuvan University, TU Registration Number: 5-2-48-1482-2013 has been accepted for the partial fulfillment of the requirement for Master's Degree in Botany.

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ABSTRACT

Reproductive floral biology of *Loranthus odoratus* was assessed in this research work in Central Nepal. This study documented the phenology of parasites and hosts, investigated the genetic architecture of flower morphology and inflorescence size, measured fruit set and pollen viability, and flower visitors and pollinators on *L. odoratus*. The pollen viability of *L. odoratus* ranges from 29.79 to 31.01% by using TTZ test. Its flowers show a distinct morphological trait. The presence of calyculus (1mm), inflorescence (1.4-4.9 cm), petals (4.9-5.1 mm), androecium (4.8-4.3 mm), gynoecium (3.7-4.3 mm) and diameter of a mature flower that ranges from 2 to 5 mm. The phenology of *L. odoratus* with its host *Q. glauca* was observed. The results suggest that among host-parasite, the overall phenology extended by one week with an increase in altitude. The fruit set percentage was found as 45-55 %. Honey bees (*Apis mellifera*) and small ant species were observed and detected as pollinators of flowers but no any aggressive interactions were recorded between *A. mellifera* and other flower visitors during the study period. The results of this study indicate that *L. odoratus* functions as a keystone species in its ecosystem. However, the species only accounts for a small percentage of vegetative biomass.

Keywords: Fruit set, *Loranthus odoratus*, Phenology, Pollen viability, Pollinator

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ACRONYMS AND ABBREVIATIONS

<i>A. mellifera</i>	<i>Apis mellifera</i>
CCF	Chalnakhel Community Forest
Cm	Centimeter
<i>et al.</i>	et alia (And others)
gm	Gram
IUCN	International Union for Conservation of Nature
KATH	National Herbarium and Plant Laboratories, Lalitpur
Km	Kilometer
<i>L. odoratus</i>	<i>Loranthus odoratus</i>
Mm	Millimeter
NE	Not Evaluated
<i>Q. glauca</i>	<i>Quercus glauca</i>
TTC	2,3,5-Triphenyl tetrazolium chloride
TTZ Test	Tetrazolium test

CHAPTER 1: INTRODUCTION

1.1 Background

Mistletoes is an English vernacular name for a diverse group of obligate hemi-parasitic plants belonging to several currently recognized genera (e.g., *Viscum*, *Scurrula*, *Helixanthera*, *Phoradendron*, *Loranthus*, etc.). Even families of Santalales (e.g., Viscaceae, Santalaceae, Loranthaceae, Misodendraceae) are parasites of woody plants distributed widely from tropical to boreal physiographic zones (Nickrent *et al.*, 2010). The monophyletic sandalwood order (Santalales) spread worldwide, has 2460 species and 179 genera, making it the most significant order of parasitic plants (Su *et al.*, 2015). Loranthaceae, the most prominent mistletoe family, contains 73 genera and about 900 species. Most aerial hemiparasites are found in the Old and New World tropics. At the same time, some genera also occur in temperate areas. (Vidal-Russell & Nickrent, 2007). Despite of wealthy biodiversity of Nepal, mistletoe contribute very little compared to the world's mistletoe diversity. (Hara, 1982) reported 15 mistletoe species and later Devkota (2005), Devkota and Joshi (2008) and Devkota and Ikeda (2013) added five mistletoe species to the Flora of Nepal, extending the list to 20 mistletoe species for Nepal. The genus *Loranthus* is represented by only one species, *L. odoratus* from Nepal (Hara, 1982).

The genus *Loranthus*, even though it is responsible for the name of the family, is in several ways untypical. Most mistletoes are evergreen and have large, showy, bisexual flowers pollinated by birds. The mistletoes in the genus *Loranthus* are dioecious or trioecious (plants with small, inconspicuous female, male and bisexual flowers that act as males). Generally the haustoria are simple, cone-shaped, and adapted to host trees (mostly members of Fagaceae) with large diameter vessels, and some *Loranthus* species are deciduous (Heide-Jørgensen, 2008).

Although Linnaeus used the name *Loranthus* in 1753, the generic name is credited to the famous chemist and botanist at the University of Vienna, Nicolaus Joseph von Jacquin. In 1762, Jacquin named *L. europaeus* the type for the genus and the family. Initially, nearly all mistletoes in Loranthaceae were classified as a species of *Loranthus*, but subsequently, 33 new genera were detected. Of the more than 1400 species listed under "*Loranthus*" on the International Plant Names Index, only nine are currently recognized; all others are synonyms. There are just two dioecious genera: *Tupeia* and *Struthanthus*, having exclusively unisexual flowers. Only two genera,

Baratranthus, and *Loranthus*, of the tribe Loranthaceae, exhibit both bisexual and unisexual flowers. For both of these genera, for certain species of plants, flowers are bisexual, whereas, for others, they are dioecious. *L. europaeus* is considered sub-dioecious, i.e., the presence of female plants, male plants, and some individuals with bisexual and male flowers (Sakai & Weller, 1999). Jacquin initially described sub-dioecy in *L. europaeus* in 1762, referring to plants with female sterile bisexual flowers as "Hermaphroditus sterilis".

Furthermore, understanding the phenology of the mistletoe would help control mistletoe numbers by making it obvious when the plant's fruit and seeds are ready. Studying the phenology of these plants can teach us about possible effects on other hosts because parasites and host plants have such a strong relationship. Phenology has the potential to alter the phenological occurrences of mistletoe populations (Jermy, 1984).

1.2 Genus *Loranthus*

Aerial stem-parasitic shrubs, sometimes with epicortical runners bearing secondary haustoria, leaves opposite, inflorescences a simple spike, and bracts single under each flower. Flowers are probably mostly functionally unisexual but usually with organs of the other gender, either typically developed or vestigial (Nickrent *et al.*, 2021). Fruits are nearly globular, i.e., pseudo berry.

Several species have been distinguished in this genus based on the number of petals and plants being dioecious or hermaphrodite. *Loranthus* is closely related to the larger genus *Helixanthera*, which extends from Africa to southern Asia and northwestern Malesia. *Helixanthera* is relatively polymorphic, with a few species groups that differ strikingly in flower size, relative style length, and presence or absence of articulation in style. It is challenging to discriminate *Loranthus* as an entity with differences more significant than those distinguishing the groups within *Helixanthera*. The presence of unisexual flowers has sometimes been used to determine *Loranthus*, but the character appears to be variable even within the species of the genus. (Danser, 1934) voiced the opinion that *Helixanthera* and *Loranthus* (Hyphear) are congeneric. Further study is required to resolve this issue, as the taxonomic consequence of uniting the genera would be the bigger genus *Helixanthera* merging with the incredibly tiny genus *Loranthus*.

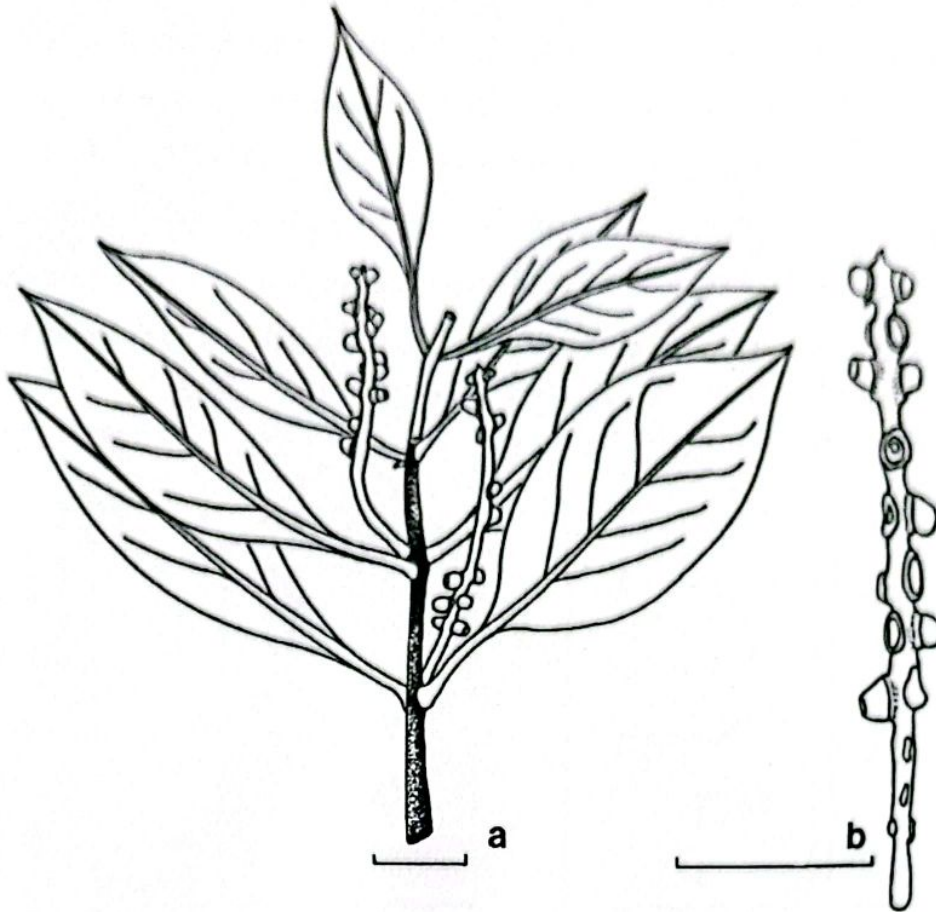


Figure 1. *Loranthus odoratus* a. Twig with fruiting inflorescences; b. fruiting inflorescence Drawing Sandie McIntosh. Scale bars represent 1 cm. (Barlow, 1997)

1.3 Plant Reproductive Biology

A plant is described as "bisexual" if its flowers have both carpels and stamens, whereas a "unisexual" flower only have either of the one sex. Sometimes the flowers may have both sex organs where either the carpels or stamens are vestigial or, missing, or otherwise non-functional. Each flower is either "staminate/ male" (having only functional stamens) or "pistillate/ female" (having only functional carpels). The species is called monoecious if separate staminate and pistillate flowers are found on the same plant. Whereas, a species is called dioecious if separate staminate and pistillate flowers are found on different plant bodies. (Renner & Ricklefs, 1995) stated that angiosperms make up roughly 6% of all known species in the world, and 7% of all the known genera have some dioecious species.

Some individuals, in a usually dioecious population have neither male nor female flowers, a phenomenon known as sub-dioecy. Most of the population produces male or female plants with unisexual flowers, but a few plants may have both male and female flowers, bisexual flowers, or a combination of both, such as female and bisexual flowers. The condition is believed to signify a change between bisexuality and dioecy (Olson & Antonovics, 2000). Sex determination is the method of separation of male and female individuals of a species. The sexual system is the distribution and function of morphological structures that produce gametes (Sakai & Weller, 1999).

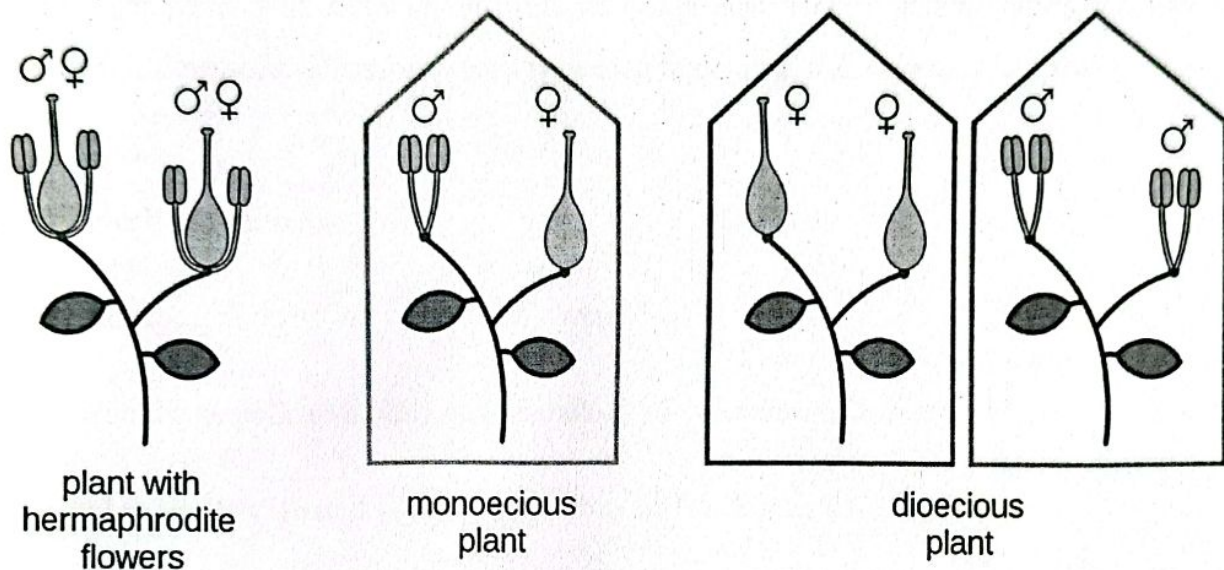


Figure 2. Various types of sexuality reported from flowering plants. (https://en.wikipedia.org/wiki/File:Monoecy_dioecy_en.svg)

1.4 Problem statement

All members of the family Loranthaceae having bisexual flowers are pollinated by birds, whereas all dioecious members are insect pollinated and are small and inconspicuous in color, including the genus *Loranthus* (Kuijt, 1969). Roxburgh reported genus *Loranthus* with a single species *L. odoratus* for the first time from Chandragiri Hills and the Shivpuri area of Kathmandu valley in 1824 but, he was unable to describe the sex of the flowers. (Don, 1834) described the flowers as dioecious within the genus without any further details. (Barlow, 1997) has reported species from Nepal extending to southern China, including Taiwan and Malesia. Barlow further argues that the unisexual flowers are the distinguishing characteristics of the genus *Loranthus*, but the sexuality of the flowers is variable within the genus. (Barlow, 1997) further added that the flowers are most

likely functionally unisexual but often with vestigial organs of the other sex. (Hooker, 1890) does not provide any information regarding the sex of the flower in the Flora of British India Volume 1,2. (Don & Hamilton, 1825) also reported *L. odoratus* from Narayanhiti Palace of Kathmandu without providing details of the flower's sex.

Studies have shown that sub-dioecy is documented for *L. europaeus* and *L. odoratus* (Barlow, 1997). It is difficult to observe small mistletoe flowers that occur high in the tree canopy, it could well be that more or maybe all *Loranthus* species are sub-dioecious but escaped observation. In the fruiting stage, plants with and without fruits are easily seen. Thus, it is tempting to conclude that the species is dioecious. Therefore, work is needed to confirm if *L. odoratus* is actually dioecious or not.

1.5 Research Questions

The central research questions of this study were

- What is the pollen viability and phenology of *Loranthus odoratus*?
- What are the different kinds of pollinators of *L. odoratus*?
- Do pollinators differ in different locations and elevation?

1.6 Research Objectives

The broad objective of this research is to study the reproductive biology of *L. odoratus* with following specific objectives:

- To study the floral morphology of the selected mistletoe species.
- To study the pollen viability of studied mistletoe species.
- To find out various pollinators and visitors of the flowers, and
- To find out the phenology of the mistletoe

1.7 Limitations

The limitations of the study were:

- i. Only two sites within Kathmandu valley were considered for the study.
- ii. Only one method for the pollen viability test was used i.e., TTZ test (Tetrazolium test).

CHAPTER 2: LITERATURE REVIEW

2.1 Mistletoe

Most of the nutrients consumed by parasitic plants come from host (Kuijt, 1969). To acquire nutrients and water, parasitic plants have evolved modified root systems that can penetrate the tissue of their hosts (Kuijt, 1969). With the help of a specialized vascular attachment known as a haustorium, parasitic plants can access the vascular tissue of their host plants, setting them apart from the epiphytic plants that either rely on their hosts for physical support or associate with them through intermediates that are mycorrhizal (Kuijt, 1969).

Trees infested with mistletoe lose strength and experience stunted growth, which may ultimately cause the host plant to die (Aukema, 2003). To get water, mineral nutrients, and, to a lesser extent, carbohydrates and organic solutes from the branches of the host plants, mistletoes, like other parasitic plants, employ the haustorium (Ladley & Kelly, 1996). Mistletoes can obtain a sizable quantity of carbon and other solutes from their hosts, making them hemiparasites because they undergo photosynthesis (Stewart & Press, 1990).

According to (Aukema & Martínez del Rio, 2002), the types of mistletoe and the types of trees affected can affect the degree of tree injury. According to (Adams Jr et al., 1993), when more than half the canopy is parasitized, tree development typically slows down, and tree mortality is frequently three to four times higher in areas with significant infection than in areas without the disease. Smaller trees are less adapted to tolerate intense infestations; thus, when they become infected with mistletoe, they grow less and perish more quickly than giant trees.

A lineage of three Gondwanan plants known as the Loranthaceae evolved in the Southern Hemisphere and spread out between remnants of Gondwana, apparently early. Later, they spread from Africa to Europe and the Americas (Wilson & Calvin, 2006). Except for severely dry or cold areas, mistletoes can be found in boreal temperatures, temperate climates, tropical climates, and arid zones (Norton & Carpenter, 1998). Woodlands and woods contain the widest variety of mistletoes (Kuijt, 1969). However, mistletoes can also be picky; they are like conifers in boreal forests (Wiens *et al.*, 1996), African deserts are habitats for succulent euphorbs (Euphorbiaceae), while South American deserts are habitats for cactus (Cactaceae) (Del Rio *et al.*, 1996).

Mistletoes can have negative consequences that range from mild (Ward, 2005) to severe (Reid *et al.*, 1992). The difference between healthy and infected hosts was little, as demonstrated by (Ward, 2005), who found that a high load of mistletoe caused statistically less foliage in its hosts. Significantly, this research did not find any connection between mistletoe load and dieback. Mistletoes can confine themselves locally to a subset of host species and parasitize various hosts. According to (Clay *et al.*, 1985), host specificity refers to the local adaptation of mistletoe to various host species in different areas within its distribution range.

2.2 Sex Determination

Angiosperms possess two types of reproductive structures, so a plant can produce flowers with both stamens and styles, or one individual can develop both male and female flowers (Renner & Ricklefs, 1995).

In the majority of plants, male and feminine organs are formed and developed simultaneously, but only up to some extent when the expansion of either set of sex organs is inhibited. Most of the flowering plants in the world are hermaphrodite but many of them involve in cross-pollination by means of separation of eggs and pollen (Ainsworth, 2000). A minority of species evolve two distinct and sexually different forms (dioecy). It is evident from research on the relationship between male and female flowers in different species that sex determination mechanisms leading to dioecy have independently evolved time and again throughout evolution (Ainsworth, 2000).

Unisexuality is achieved in dioecious species where both staminate and pistillate flowers begin as perfect by either elimination or suppression of the alternative sexual organ's growth (Wu & Cheung, 2000). Early stages of the androecium or gynoecium's development are delayed or stopped by abolition (Farbos *et al.*, 1997). Ultimately, flowers will be male sterile or female sterile.

In the course of evolution, unisexual flowers have arisen from the bisexual ancestry through mutation either causing male sterility or causing female sterility that results gynodioecious (having female flowers on one plant and hermaphrodite flowers on the other plant of the same species) and androdioecious plant (having male flowers on one plant and hermaphrodite flowers on the other plant of the same species) (Charlesworth & Charlesworth, 1978). Though there are only a few reports in which monoecy appears to be ancestral to dioecy, the comparative study on the evolution of dioecious plants supports the idea that they have evolved from an ancestor gynodioecious

conditions. Hermaphroditism or monoecy have frequently given rise to dioecy, however sexual specialization is sometimes insufficient. Sub-dioecious populations are natural populations that frequently include strictly unisexual individuals as well as individuals that are inadequately differentiated between the sexes (of either or both sexes) (Westergaard, 1958); (Darwin, 1877); (Correns, 1928).

A dioecious plant species differs from the other plant species in terms of its pattern of reproduction, reproductive architecture, and its genetic makeup. These characteristics may have physical or physiological variations with a hereditary basis (Dellaporta & Calderon-Urrea, 1993). Angiosperms are the most varied category of terrestrial plants, and they produce food, seeds for planting, fruits, material for breeding, etc. Most angiosperm species (90%) have hermaphrodite (bisexual) blooms, and the other 10% are either monoecious (male and female flowers are separate but grow on the same plant) or dioecious (male and female flowers grow on distinct plants). Sex determination is the method of separation of male and female individuals of a species. The sexual system is the distribution and function of morphological structures that produce gametes.

2.3 Pollination Biology and Pollen Biology

Studies on plant pollination have evolved through several diverse phases (Cook & Baker, 1983). Most blooming plants require pollination as a critical sustaining ecosystem service; it has been assumed that 87.5 percent of angiosperms need biotic pollination (Ollerton *et al.*, 2011), and 62 percent of these flowering species have restricted reproductive potential. Based on how much pollen they encounter (Burd, 1994).

In the family Loranthaceae, pollination is a purely abiotic process. This dependence is evidenced by their floral morphology distinguished by two primary syndromes: entomophily and ornithophily. Flowers in the first group are usually small (2-10 mm), white or greenish, and choripetalous, whereas, in the second group, the flowers are typically large (30-160 mm) gamopetalous, and brightly colored (Vidal-Russell & Nickrent, 2008).

The mistletoe population in natural plant communities is threatened in Nepal. Some factors are assumed to be responsible for this, such as the loss of pollinators and dispersal agents and the pollen viability, which is further aggravated by the modification of the middle mountain region landscape

due to rapid change in the conversion of natural ecosystems into human-dominated landscapes through forestry, farming, and settlement expansion (Devkota *et al.*, 2015).

Pollination is the migration of pollen grains from the stamens to the pistil, and cross-pollination increases genetic diversity and is usually favored by selection. Flowering plants have developed a wide range of features that affect the success of pollination, such as those that optimize self-pollination, draw in animal pollinators, and efficiently exploit wind pollination (Fattorini & Glover, 2020).

Palynology is the study of pollen grains, which exhibit morphological peculiarities in terms of shape, size, wall ornamentation, and aperture pattern. Variation in pollen morphology and the number has become one of the top research areas in plant systematics and reproductive and pollination biology (Erdtman, 1952). The study of pollen morphology is practical in paleoecology, paleontology, aerobiology, paleobotany, forensic science, and public health (Bryant Jr, 1990). Pollen morphology is described according to its polarity. The pore closer to the center of the tetrad is the proximal pole, and the one away from the center is the distal pole (Faegri *et al.*, 1989). The highly resistant and tough outer pollen wall comprises exine (Edlund *et al.*, 2004). Pollen allergy is caused by pollen, particularly by a biological polymer known as 'sporopollenia', a major component of the exine.

The amount of pollen produced by a plant represents the sexual ability and abundance of that plant in its local habitat (Kearns & Inouye, 1993). The amount of pollen in each flower varies greatly. Counting pollen also becomes essential for observing plant-pollinator interaction (Adler & Irwin, 2006). Pollen production also indicates the type of pollination. Wind and water-pollinated species produce more pollen grains than insect-pollinated species (Shivanna, 2003).

2.4 Phenology

Examining natural, seasonal, and recurrent events that occur during an animal or plant's life cycle and how environmental changes affect them is known as Phenology (Lieth, 1975). However, the most potent ecological processes that might influence a plant's life cycle are not abiotic variables. The interaction between a parasitic plant and its host also can regulate the existence cycle of each flora (Marvier, 1996). Among the many corporations of parasitic flora within the world, the mistletoes deserve special attention for their ecological function in many communities (Watson, 2001). As a parasite, mistletoes have an exploitative interplay with their hosts through stealing

sources together with water and nutrients (Press & Phoenix, 2005). Invasion of the haustoria can also affect the vascular tissue's ability to maintain its proper hydration (ACKROYD & GRAVES, 1997), becoming a greater vulnerability to embolism and collapses (Calvin, 1997), which, therefore, can also have a poor effect on the host growth.

Phenology refers to the recurring biological events in the life of an organism (Lieth, 1974). The phenological event includes the timing of budburst, the length of time required to the entire 'leaf-on' state, and the timing of leaf senescence and abscission. Biological events in some species are triggered by temperature, elevation, moisture, energy fluxes, photosynthesis, and carbon dioxide fluxes. An accessible way to examine the ecological relationships involving these plants is to investigate the phenological events of mistletoes and their hosts (McEwan & McCarthy, 2005).

The main goal of phenological research in public and human health is to predict diseases like malaria or allergic pollen that causes hay fever (Chuine *et al.*, 1999). The distribution of assimilates to reproductive development against vegetative growth and above-ground versus below-ground biomass is significantly influenced by the timing of phenological phases (Mitchell *et al.*, 1996).

Phenological data is utilized to identify places most suited for producing specific crops and fruits. Knowing the timing of phenological phenomena such as fruit ripening or grain maturity was also used to improve coordination in harvesting machinery. New fruit plantations were established in regions where phenological investigations indicated a minimal danger of night frost damage. Knowledge of the timing of phenological events has been and continues to be employed in plant protection, horticulture, and applying pesticides and herbicides (Ruml & Vulić, 2005).

Furthermore, understanding the Phenology of the mistletoe would help control mistletoe numbers by making it obvious when the plant's fruit and seeds are ready. Studying the phenology of these plants can teach us about possible effects on other hosts because parasites and host plants have such a strong relationship. Phenology has the potential to alter the phenological occurrences of mistletoe populations (Jermy, 1984).

In addition to exploiting this host plant, mistletoe has also established a reciprocal relationship with avifauna, which may indirectly benefit other avian species, even as part of the invertebrate fauna (Sekercioglu *et al.*, 2016). Phenological information on parasites in the *Loranthaceae* has also been documented in more considerable research on reproductive plant ecology (Teixeira-Costa *et al.*, 2017). According to (Watson, 2001), this lack of synchrony prolongs the season during which

this species produces flowers and fruit, supplying resources for pollinators and dispersers throughout the year. Elevation impacts phenological characteristics. Therefore, phenological occurrences at lower altitudes occur a few days earlier than at higher elevations. Additionally, the timing of phenological events varied over the years (Hosseini, 2016).

Therefore, even though these authors conducted a rigorous study on pollination and pollinator interactions. The climatic and other environmental factors co-vary with elevation and impose more significant selection pressure on some plant functional traits. Therefore, variations in pollen viability and phenology of *L. odoratus* along elevation gradient have become an exciting area of research to understand reproductive success, past and present assemblages of plants, the pattern of environmental change, and species responses to such change.

In this study, we described the reproductive biology of *L. odoratus*, including flower morphology, Phenology, and pollen viability. We used an observational and experimental approach to examine the effectiveness of *Apis mellifera* as mistletoe pollinators.

2.5 Sex within the genus *Loranthus* and *L. odoratus*

Loranthus odoratus displays functional unisexuality but often with vestigial organs of the other sex or predominantly functionally unisexual (Danser, 1934). *Loranthus odoratus* exhibits remarkable vegetative uniformity and many of the specimens are apparently male or female in function, with variation in the extent to which organs of the other sex are developed. In relatively few cases organs of both sexes appear to be fully developed in one flower (Barlow, 1995).

CHAPTER 3: MATERIALS AND METHOD

3.1 Study Area

The Kathmandu district lies in Bagmati province in the central part of Nepal and falls within 27° 27' E to 27° 49 'E longitude and 85° 10' N to 85° 32' N latitude, at an elevation of 1,324 m above sea level. Bhaktapur and Kavrepalanchok districts border it in the East and Dhading and Nuwakot towards the west. Present study was conducted in the Gokeneshwor Municipality, Mulkharka, at the buffer zone of Shivapuri Nagarjun National Park, about 15 km northeast of Kathmandu City, and in Chalnakhel Community Forest, Dakshinkali Municipality.

Table 1: Study sites.

Feature	Site A (Dakshinkali)	Site B (Gokeneshwor)
Latitude	27° 37' 59" N	27° 46' 32" N
Longitude	85° 16' 45" E	85° 25' 34" E
Elevation	1339 m	1700 m

The study was carried out in two study sites in different places of Kathmandu District, Bagmati Province, Central Nepal. Site A refers to Chalnakhel Community Forest (CCF), located on the valley's southwestern fringes, approximately 10 km from Kathmandu City, now part of Dakshinkali municipality, ward No. 1. The community forest is dominated by mixed broad-leaved forests dominated by *Castanopsis indica*, *C. tribuloides*, *Quercus glauca*, *Schima wallichii*, and *Rhododendron arboreum*. Here mistletoes, commonly *L. odoratus* occurs frequently parasitizing on *Q. glauca* as a common host tree.

Similarly, Site B refers to Mulkharka Area, located in Gokarneshawor municipality of Kathmandu District, at the buffer zone of Shivapuri Nagarjun National Park about 15 km northeast of the Kathmandu City. The area is surrounded by an evergreen natural forest dominated by *Schima wallichii*, *Castanopsis indica*, and *Quercus glauca*. In this site also, mistletoes like *L. odoratus* occur frequently parasitizing on *Q. glauca* as a common host tree.

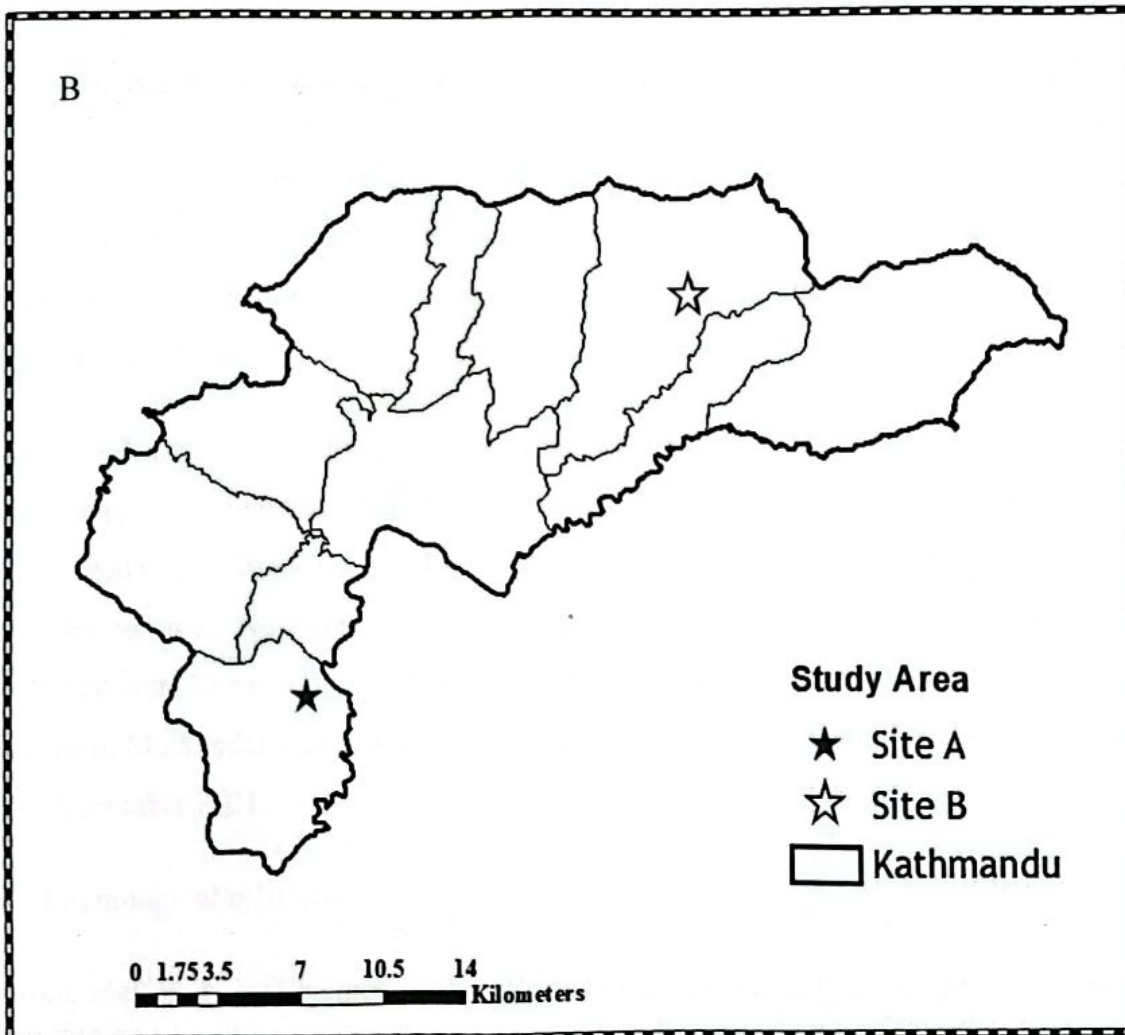
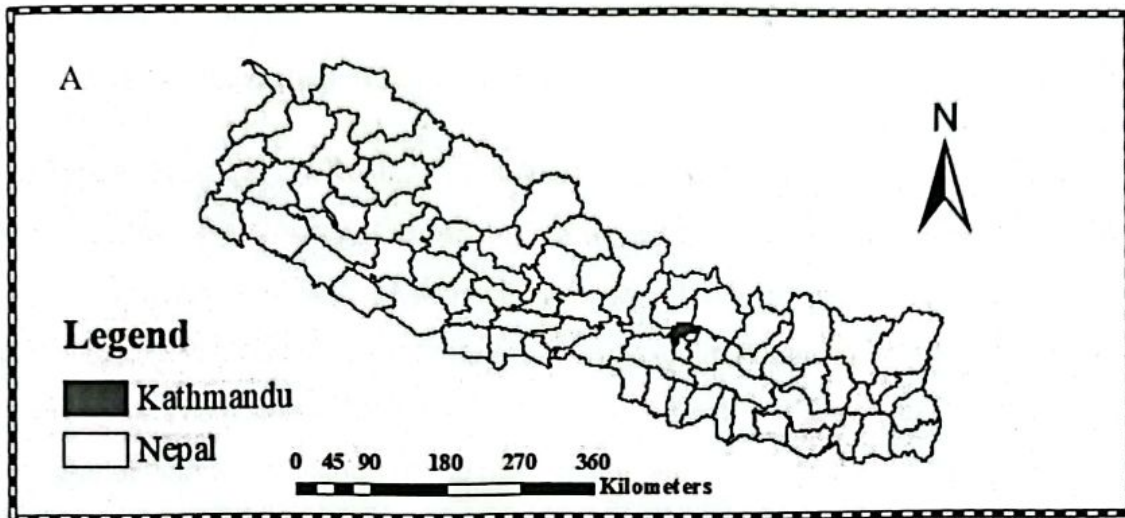


Figure 3. Study area showing (A) map of Nepal and (B) map of the Study area, showing Dakshinkali municipality (Site A) and Gokarneshawor municipality (Site B).

3.2 Methods

3.2.1 Species characters of *Loranthus odoratus*

Loranthus odoratus Wall. in Roxb., Fl. Ind. 2: 215 (1824). (Synonym: *Hyphear odoratum* (Wall.) Danser; Family- Loranthaceae; Genus; *Loranthus*; Specific epithet; *odoratus*; Common name- Mistletoe; Local name- Aaijeru, is a perennial semi parasitic shrub characterized by brittle branches bearing pinnately veined and opposite leaves. Flowers are in racemose inflorescence, and the fruit is pseudo berry. The flower color is white, and they are small and beautiful. During current study, the recognition and identification of the mistletoe in both the study sites were made during field visits in November, 2020.

3.2.2 Selection and Collection of mistletoe and host trees

The selected study sites were visited several times in order to select the host trees infested by *L. odoratus* and the number of infestations on each host tree. Three host trees, that were easy to climb, and have multiple infections of *L. odoratus* were selected in both study sites across a narrow elevation gradient.

Flower samples were carefully collected in small plastic bottles containing 10% Ethyl alcohol solution as a preservative and labeled properly with permanent markers. Location of each host tree were recorded using with Garmin 20 Etrex GPS in both sites. The freshly opened Mistletoe flowers were collected on 29 November 2020 at dakshinkali-1, Chalnakhel about 13:00 PM and pollen viability test were carried out in KATH Xylarium on 1-4 December 2021. Visit and collection of flowers from Mulkharka was done on 7 December 2021 and pollen viability test were carried out on 8-11 December 2021.

3.2.3 Phenology of mistletoe

Flowering phenology was studied in the selected mistletoe individuals on the chosen host trees during the flowering season that starts from October. For each set of mistletoe following phenological events were recorded;

- a. Emergence of inflorescence
- b. Presence of flower bud

- c. Flower opening
- d. Flower maturation
- e. Petals fall
- f. Fruiting
- g. Fruit Ripening

3.2.4 Flower morphology of *L. odoratus*

Freshly opened flowers were collected and preserved in alcohol (10%) for further study. Flowers were dissected to record the length of sepal, petal, stamen, and carpel. From each sample, three flowers were taken to measure the length of calyculus, petal, stamen, and pistil. During the measurement of flower morphology, flowers containing only six petals were chosen because sometimes there is the presence of five petals in the flowers of *L. odoratus*. Three flowers were chosen from each sample for the study of flower morphology in the studied species. All the anatomical studies were carried out in KATH Xylarium and the photographs were taken under HumaScope Premium LED microscope under different magnifications using Nikon Coolpix S2800 digital camera.

3.2.5 Study of pollen viability

The TTZ test, also known as the Tetrazolium test was carried out to assess the pollen viability of the studied species, Tetrazolium can reduce colorless chemicals and convert them into red-colored compounds due to enzyme Dehydrogenase (Norton *et al.*, 1966). The pollen viability test, TTZ test followed was carried out according to (Rathod *et al.*, 2018). The following procedure was carried out at KATH Xylarium to carry out the pollen viability test.

1. Using marker six spots were marked in a glass slide.
2. Two drops of TTZ solution were taken on the slide.
3. Anther sacs from different flower samples were teased with the help of a needle, and covered with a coverslip and temporary slides were prepared.
4. Slides were incubated in a hot air oven at 40° C in dark condition for 40 minutes.
5. The slides were observed under microscope at different magnification and photographs were taken.
6. Each slide was observed above the six spots only, i.e., six microscopic fields.

7. Viable pollen in each microscopic fields were counted by its ability to take stains and appear red, whereas the non-viable pollen did not take color.
8. The pollen viability was calculated (Mosquera et al., 2021)

$$\text{Pollen Viability \%} = \frac{\text{Number of Stained pollen grains}}{\text{Total Number of pollen grains}} \times 100$$

3.2.6 Flower development

The flower development was studied from November, 2021 to January, 2022. In November 2021, the flower development as well as duration of *L. odoratus* were observed by individually tagging flower inflorescences with paper tags. The selected individuals were visited twice a week from the time of bud opening until the flowers fell off. During this period, the changes in color as well as shape of the flowers were also recorded.

3.2.7 Fruit set percentage

A fruit set is the proportion of flowers developed into fruits in each Inflorescence. Flowering twigs of *L. odoratus* were selected in each mistletoe plant in both sites and tagged for the study of the percentage fruit set. The number of inflorescences with flowers was counted to record the number of fruits produced in each inflorescence.

The fruit set percentage is calculated by using this formula, (Freihat *et al.*, 2008)

$$\text{Fruit set (\%)} = \frac{\text{Number of fruits}}{\text{Total no.of flowers}} \times 100 \%$$

3.2.8 Flower visitors and pollinators

Since the flowers of *L. odoratus* are small and insect pollinated, selected mistletoes were observed during the flowering season to determine the flower visitors and their foraging pattern. Mistletoes were observed from the time of flowering for six hours every day.

The selected mistletoe bushes were observed from October, 2021 to August, 2022 during the peak flowering period to record the pollinators and flower visitors. The flowers were observed continuously in 10 minutes intervals in three different periods of the day; between 8 – 10 am, 12 –

2pm, and 3 – 5 pm for three consecutive days by three persons. The total time of observation was 36 hours in both the study sites. Pollinator's behavior was also observed if they interact with anther and stigma. The time (in seconds) each pollinator or visitor spent within mistletoe was recorded, and the number of visits made by pollinators to individual flowers was also recorded. For each visit made by a visitor, the number of flowers visited per foraging bout and the number of probes per flower were recorded. All flower visitors and pollinators were identified using photographs and later identified while they were unknown.

The order of the sample selection was selective in the host but randomized in the bush for unbiased representation and random sample generalize the results to the population. The sampling was done on sunny days with a gentle breeze. It was selected in starting of early flowering, in the middle of flowering, and about the end of flowering.

3.2.9 Data Analysis

All the data was tabulated in Microsoft Excel Office Professional Plus 2019. Statistical tests were done and, necessary plots and diagrams were prepared through SPSS version 25.

CHAPTER 4: RESULTS

4.1 Flower morphology

Being very small and dull colored, *L. odoratus* flowers are insect pollinated and do not confirm the general pattern showy flowers of the family Loranthaceae. *L. odoratus* have profusely branched hemiparasite with terminal simple racemose inflorescences whose flowers develop in an acropetal order and are sessile. The flower is hypogynous and perfect. The calyculus is present, and the six lobed corollas are polypetalous.

The diameter of a mature flower of *L. odoratus* ranges from 2 mm to 5 mm. The total length of inflorescence and number of flowers varied between 1.4 cm to 4.9 cm and 8 to 20 flowers, respectively. Diadelphous anthers; introrse, Gynoecium syncarpous.

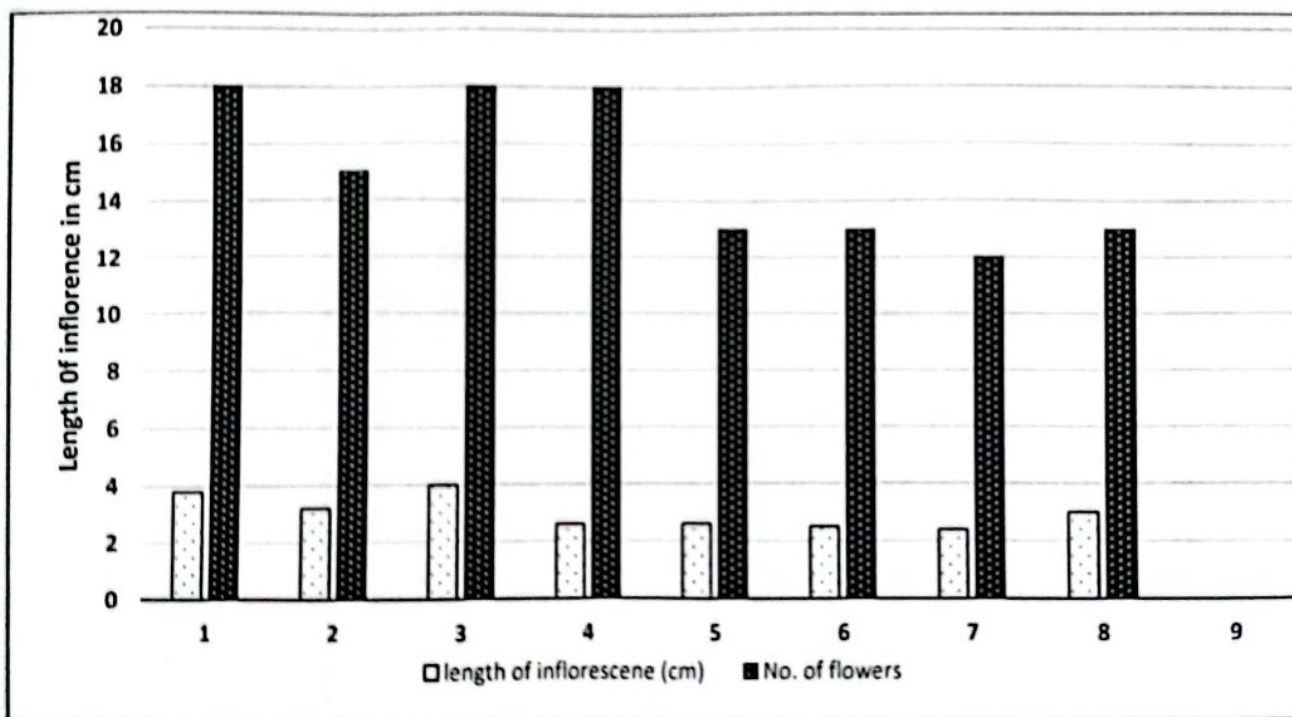


Figure 4: Measurement of length of inflorescence and number of flowers of sample no. 1

Calyculus: Small cup-shaped structure, green in color, present at the top of the inferior ovary, length of calyculus found similar in both sites, i.e., 1mm. The flower is characteristically held by a persistent calyculus; whose principal function is to protect the flower in its bud condition.

Corolla: The corolla has five to six petals enclosed by Calyculus. All individuals showed similar petal color, i.e., white. The arrangement of the petals is valvate. The corolla's primary function is to aid in reproduction by attracting pollinators. The petal length varies from 4.9 mm to 5.1 mm, which is similar in both sites.

Androecium: The Androecium has six stamens; four are bigger, and two are shorter and epipetalous. Anthers were slightly curved, dorsifixed, creamy colored and introse. Filaments were narrow towards their base. The length of longer stamens varies from 4.4 mm to 4.8 mm, and the shorter is from 3.6 mm to 4.3 mm. The base of the filament is narrow, and anther is dorsifixed. Pollen grains have a characteristic triangular shape.

Gynoecium: The stigma reaches 3.7 mm to 4.3 mm in length from the base. There is a ring of non-receptive unicellular hairs surrounding the stigma, which probably prevents contact between a flower's pollen mass and the stigma, which prevents self-pollen germination by holding the secretory products of the stigma. Gynoecium syncarpous, monocarpellary or simple, ovary inferior. Free central placentation.

Fruit: The fruit is a Pseudo berry with a single viscid seed.



Figure 5. Flower of *L. odoratus* under stereomicroscope.

4.2 Flower Development

The flower development starts with the appearance of the flower buds on a simple racemose inflorescence. It started at the beginning of October, continued for around nine weeks, and reached its peak flowering in November. These first buds are green and pale white, about 1mm in length. Then they grow to about 5.1 mm and turn into a beautiful white flower having odor and nectar. The petals are white with a greenish base. In-field floral differentiation was observed in October and November. By the time the flower had bloomed, insects had been attracted to it. As the flower had developed, anthers had changed color. They had gone from yellow-gold to dark yellow and finally brown. The style had been growing from about the height of the anther. The development, from 1mm flower bud to flower in the petal fall stage, was accomplished in less than four weeks. Bees, insects, and ants visit flowers. Flowering progressed more swiftly in Chalnakhel Community Forest (CCF) than in Mulkharka Area.



Figure 6. Development of flower of *L. odoratus* (A) Inflorescence with buds; (B) Flowering inflorescence; (C) Inflorescence with matured flower; (D) Top view of a mature flower; (E) Inflorescence showing petals off; (F) Infructescence of *L. odoratus*.

4.3 Pollen viability evaluation

This study shows two types of pollen: viable (yellow or red) and non-viable (colorless). The assessments of pollen quality for the various samples are contrasted. The pollen viability of three flowers from the same inflorescence on the same date was compared (Figure6). even if statistically distinct differences exist. The viability of the pollen was found to be constant or little variable among the flowers of the same inflorescence. When flowers from the same inflorescence were obtained, the results did not significantly differ across the flower. The amount of each sort of pollen tends to be very consistent among flowers. Pollen viability evaluated on different sites shows low variations, and the pollen viability is somehow different in both locations. The pollen viability percentage is 29.79% in Site A and 31.01% in Site B.

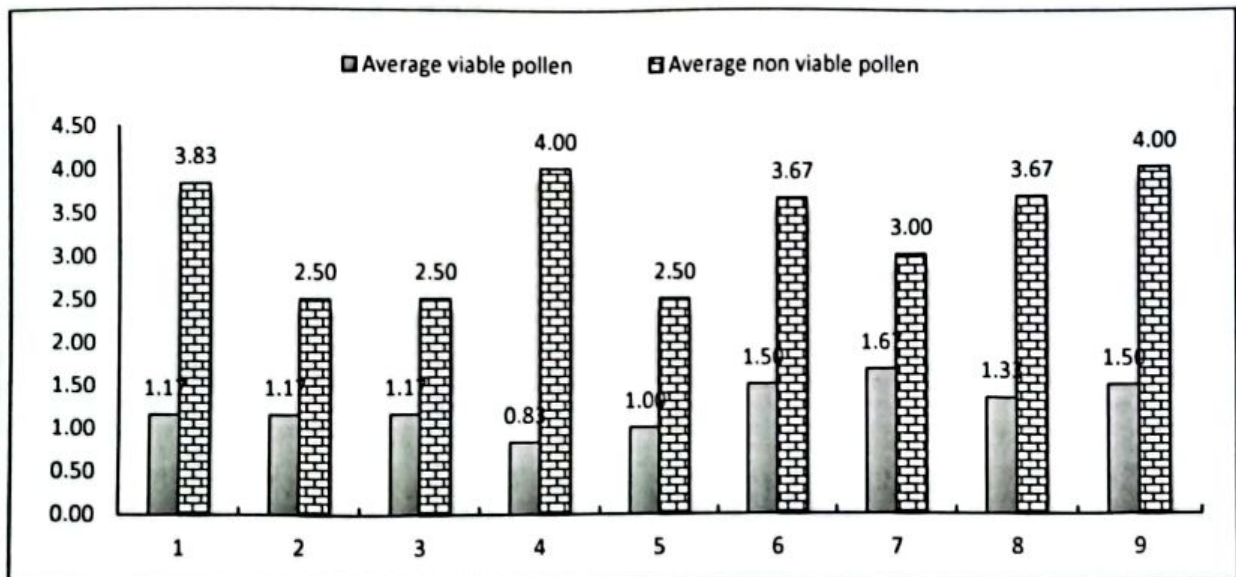


Figure 6. pollen viability of three flowers from the same Inflorescence of site A.

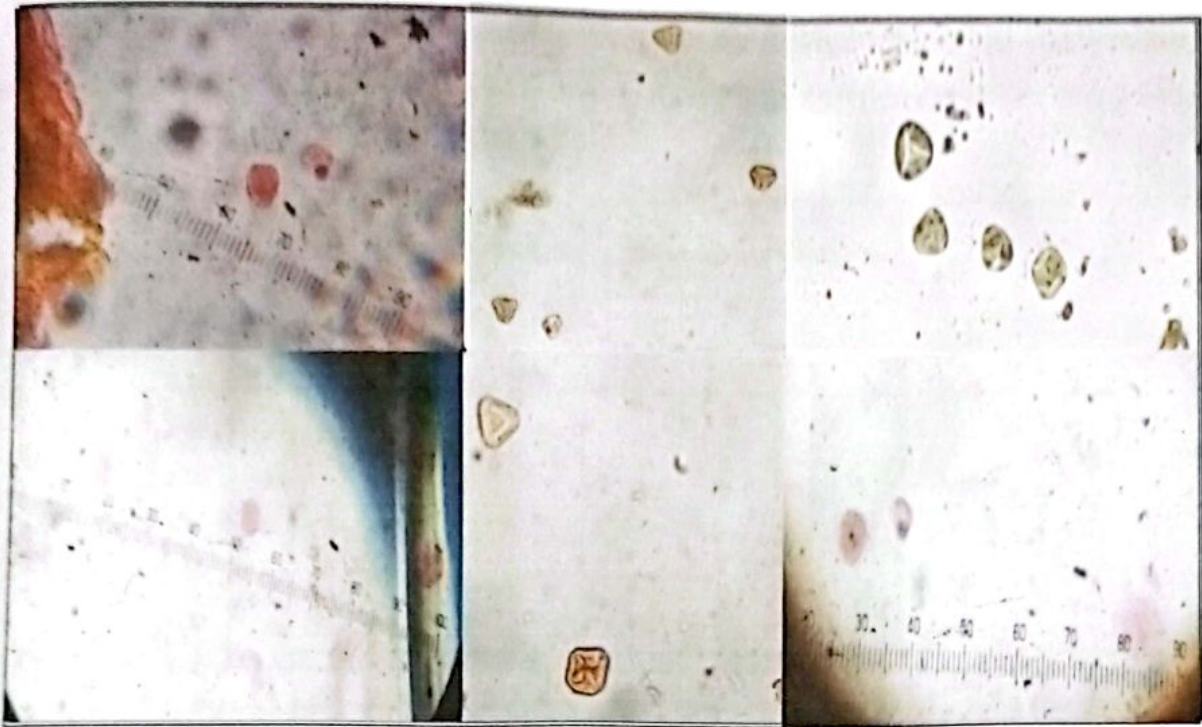


Figure 7. Pollen viability study under microscope, colored are viable pollens and colorless are non-viable pollens.

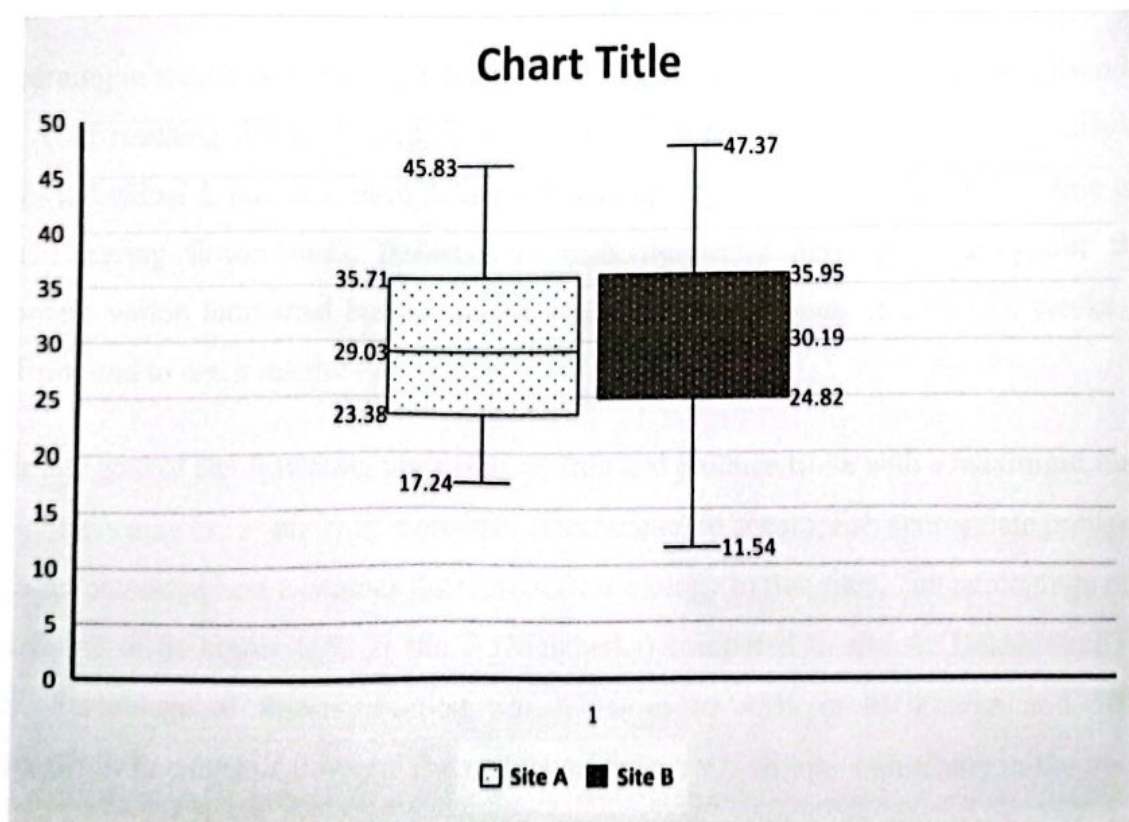


Figure 8. Pollen viability percentage of *L. odoratus* in Site A and Site B.

The average viable and non-viable pollens in site A and site B (Fig.9) shows less viable pollen than non-viable pollen in *L. Odoratus*. There is there is a very little difference between viable and non-viable pollen between flowers from both sites.

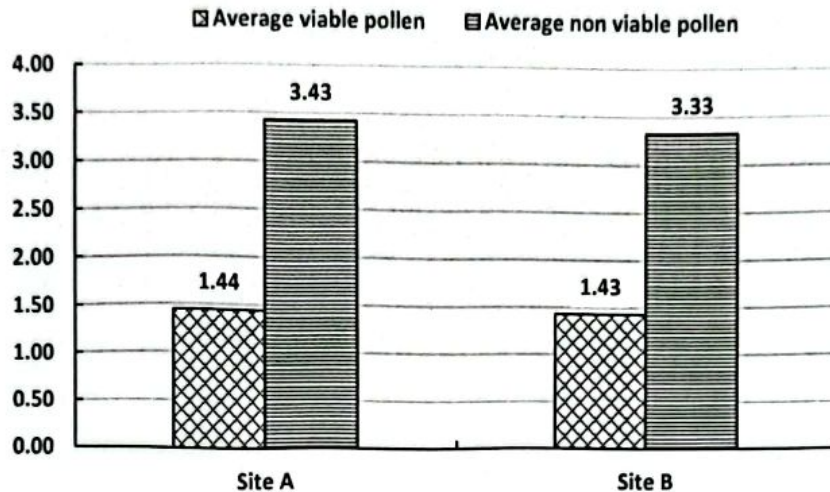


Figure 9. Comparison of viable and non-viable pollen from site A and Site B.

4.4 Fruit set Percentage

The blooming of the *L. odoratus* began at the beginning of October and continued for around nine weeks. After reaching flowering peak in November, most had already started to set early fruit. Different individual *L. odoratus* have different flowering stages; some develop fruits while others were still having flower buds. Branches of buds frequently developed throughout flower development within individual bushes, which was also asynchronous. It took five weeks for a flower from bud to reach mature flower stage.

The ultimate goal of any flowering plant is to set fruit and produce fruits with a maximum number of seeds. Plants may use a variety of reproductive techniques to accomplish appropriate pollination. The data set presented here examines the reproductive biology in two sites. The percentage of fruit set was found to be higher 55% in site B (Mulkharka) compared to site A (Dakshinkali) 45% (Fig.10). Percentage of flower abortion was found to be 45% in MUKharka and 50% in Dakshinkali) in *L. odoratus* flowers. The unknown factors which may constitute in the abortion of flowers may include pests, or the mistletoe itself cannot support the vast number of flowers produced.

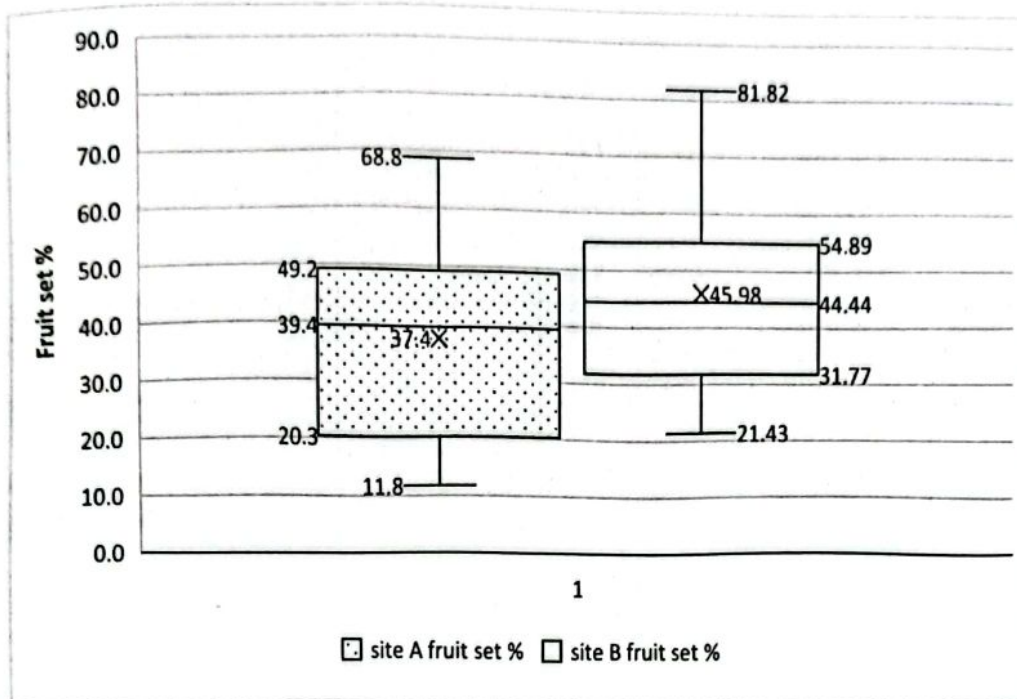


Figure 10. Fruit set %

4.5 Flowering phenology of Mistletoe and Host

Phenology of mistletoe was compared with its host *Quercus glauca* with and without mistletoe. The phenology of the host plant with mistletoe was found to be delayed compared to the host without mistletoe infestations showing the phenology of mistletoe at higher elevation of site B (Mulkharka) have longer phenophase by two weeks.

The flowering period of *L. odoratus* started from October and lasts to early January, its start coinciding with the mid-autumn season and ending at the beginning of the winter season. Flowering was asynchronous within and between individuals, with a flowering peak in mid-November. Table 2 and 3 shows the overall phenology of hemi-parasite *L. odoratus* and Host *Q. glauca* in both sites.

Table 2: Phenological records of *L. odoratus*

Months	Oct				Nov				Dec
	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week	9 th week
Phenology									
Bud emergence	→	→	→	→	→	→			
Flower opening			→	→	→	→	→	→	→
Flower maturation					→	→	→	→	→
Petal fall						→	→	→	→
Start of fruiting							→	→	→
Fruit ripening									→

Table 3: Phenological records for the *Q. glauca*

S. N	Phenophase	Month
1	Emergence of flowering buds	April Third Week
2	Presence of flower bud	May Second week
3	Flower opening	June Third week
4	Petals fall	August Third Week
5	Fruing	May Third Week
6	Fruits ripening	October First Week

4.6 Pollinators and flower visits

Seven different insect species were found visiting flowers of *L. odoratus* during 36 hrs of total observation in each site. Honey bee (*Apis mellifera*), Housefly (*Musca domestica*), dragonfly, butterfly, moth, mosquito, and a small ant species visited the flowers *Apis mellifera* was found to be the most frequent flower visitor which accounted for 53.91% and 56.67% of the total flower visits in site A and site B, respectively, Similarly, ants were the second most frequent visitors, accounting 18.26% and 25.22% of total flower visits in sites A and B, respectively. Flower visits by mosquitos were much less regular only in site A, i.e., 2.61%, and moths in site B, i.e., 6.96%. (Fig.11). *Apis mellifera* and ant species is pollinator.

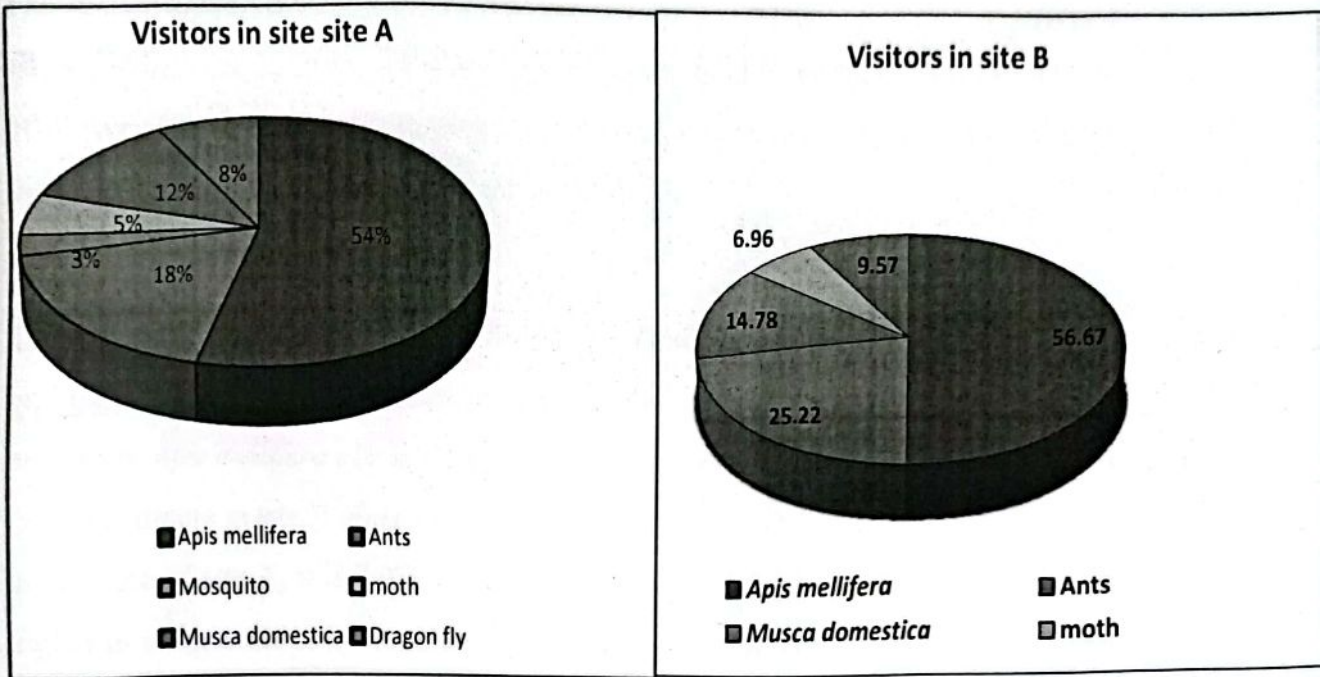


Figure 11. Percentage of flower visitors in sites A and B.

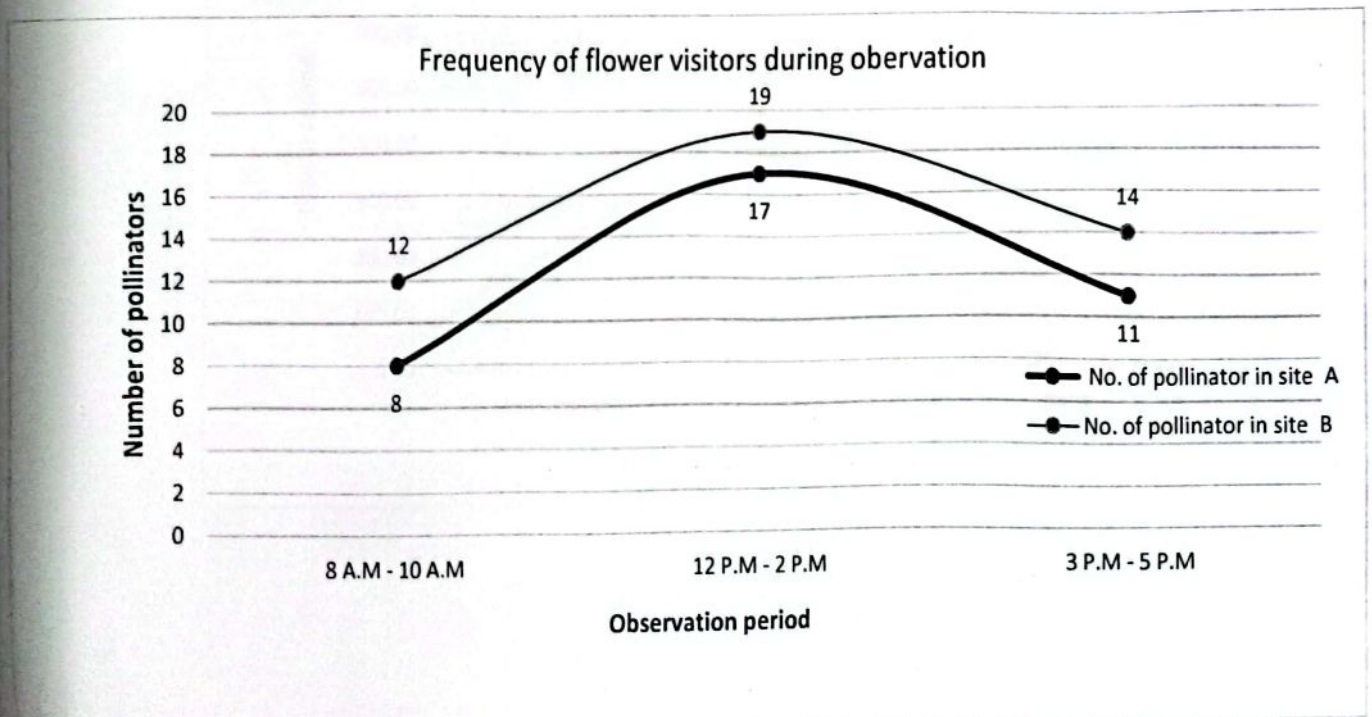
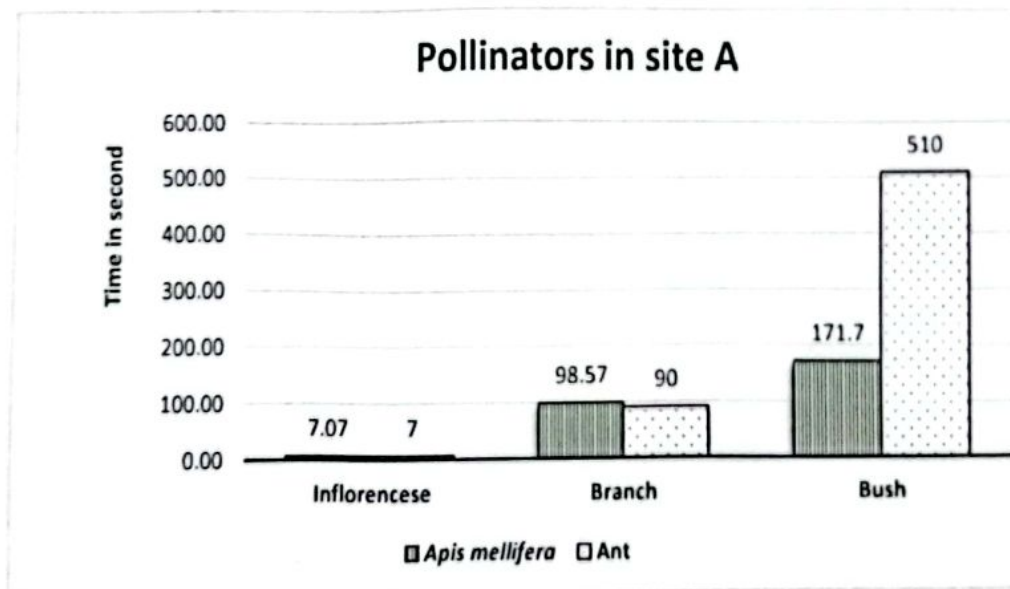


Figure 12. Frequency of pollinators in Both sites.

The above figure 10 clearly shows that the frequency of flower visitors was maximum in the afternoon between 12 PM to 2 PM compared to morning and late afternoon. However, the number of flower visits by different pollinators is more in site B than in site A but the occurrence of flower visitor species is less in site B compared to site A.

The following figures 13 shows that the visits or flow of pollinators is greater in site B and *Apis mellifera* and ant are the dominant pollinators. The number of flower visits by bees is higher in both sites. *Apis mellifera* acts as the main pollinator. The number of visiting pollinators was found to be maximum in site B. *Apis mellifera* stays for 31.63 seconds in an inflorescence in site B, but in the case of site A, it is 7.07 seconds. Though the average time spent by the *Apis mellifera* is higher in inflorescence, whereas, the time spent by ant in the bush is more than two times in both sites. The time spent by the ant in bushes in sites A and B was 510 seconds and 1670 seconds, whereas, for instance, the *Apis mellifera* has 171.7 seconds and 717.13 seconds in sites A and B, respectively.



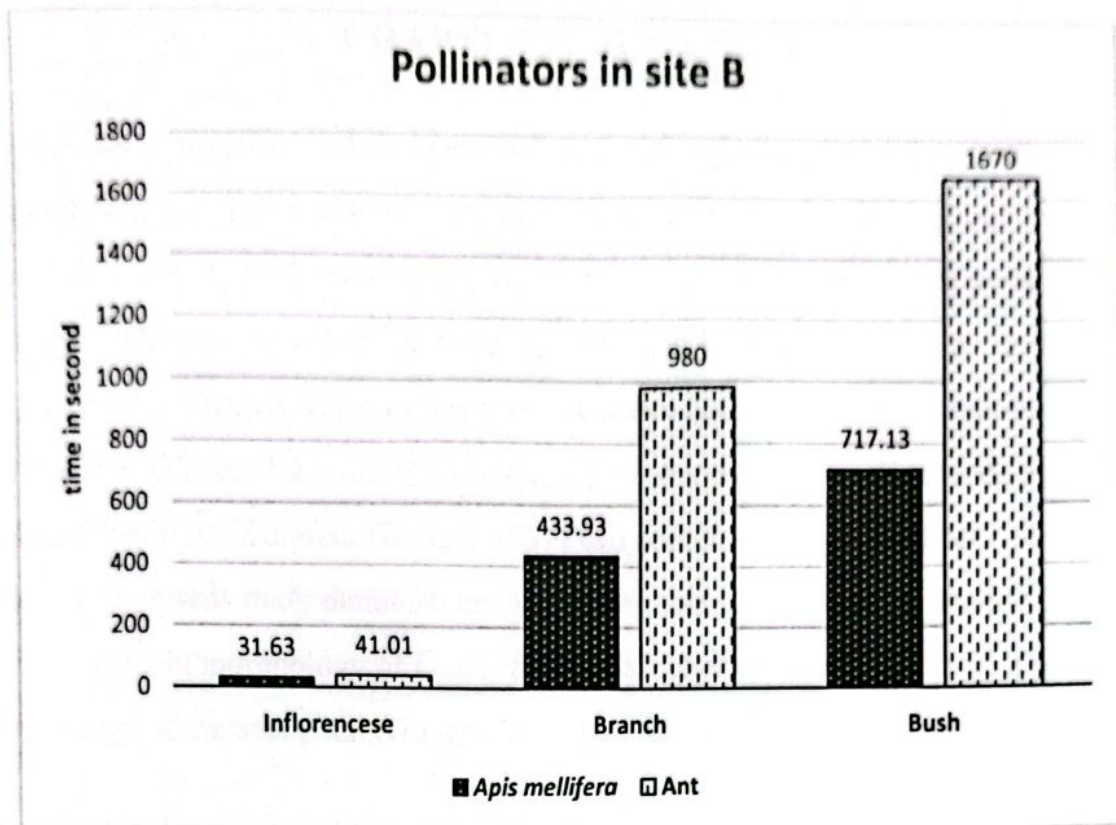


Figure 13. Visiting time of pollinator in site A and site B in average per day.

4.7 Conservation status of *Loranthus odoratus*

According to IUCN Global Red List Category the conservation status of *L. odoratus* is Not Evaluated (NE), (https://www.gbif.org/occurrence/search?taxon_key=5640228 and IUCN Red List Category = NE. [iucn_red_list_category=NE](https://www.iucn.org/redlist)).

CHAPTER 5: DISCUSSION

L. odoratus is a competitive and sophisticated group of angiosperm plants that infests and exploits various plant species. It is a practical and specialized group of flowering plants that invades and influences a variety of hosts including Oaks (*Quercus glauca*), forming an aerial stem parasitic shrub. The conservation area offers a better environment for mistletoe species variety (Devkota et al., 2010). Mistletoe seems to be a semi-parasitic shrub since it often grows on various host trees and shrubs. While it produces its food through photosynthesis, it still depends on each host for water, and mineral nourishment (Griggs, 1991) can sometimes harm the host trees also (Ward et al., 2006). The present study demonstrated the pollen viability, host and parasitic phenology, and flower development morphology of *L. odoratus*. The higher parasitism of the host plant suppresses the morphology of the host plant (Goverse & Smant, 2014).

5.1 Flower development and floral morphology

L. odoratus flowers have a rare and distinct morphological trait. The calyculus is present in all Loranthaceae flowers, but there is debate about its identification and origin (Nickrent et al., 2010). Most contemporary scholars think that the calyculus and the calyx are closely related. (Narayana, 1958) believed that the calyculus was a genuine calyx since his anatomical research on the *Nuytsia floribunda* plant revealed that the calyculus was linked with vascular bundles. (Johri & Dixit, 1957) who investigated the Loranthaceae floral morphology and embryology likewise maintained that opinion. (Wanntorp & De Craene, 2009) did floral ontogenetic research on two Loranthaceae species (*Struthanthus* and *Phthirusa*), the calyculus began as two lateral primordia that quickly united on the adaxial side to form a continuous ring around the flower. The calyculus primordia did not develop under the typical calyx development; instead, they were in the location of prophylls. This research showed that a calyculus of bracteolate origin had replaced the calyx. The source of the calyculus is still a debate among researchers, although there is no significant disagreement across studies. The authors advise staying with the word "calyculus" until new data from fields other than morphology or anatomy can be gathered.

The stamens of *L. odoratus* are numerically equivalent to the petals and probably initiate in the same manner as two series with four and two stamens in each due to their arrangement and different sizes. The petals and stamens of *L. odoratus* flowers seem to be trimerous (Nickrent et al., 2010).

The fundamental floral pattern is preserved in this family despite the different morphologies. A calyculus, also known as a circular rim outside the petals, always covers *Lesanthaceae* flowers, in addition to the outer foliar structures like the bract, bracteoles, and cupular pedicel. The stamen and carpels are contained within the two whorls of the calyculus and petals, and the number of stamens and petals is equal. Other *Santalaceae* species also have unusual calyculus, but there is still debate regarding its precise identity and origin (Lin *et al.*, 2019).

5.2 Fruit Set Percentage

The length of mature inflorescence on the *L. odoratus* plant ranged from 1.4 to 4.9 cm and contained 8 to 20 flowers during the flowering season. The fruit set is most in Site B (Mulkharka), i.e., 55% and 45% in Site A (Dakshinkali). This result shows that the amount of fruit set is higher in site A, where there is an excess flow of pollinators and more sunlight. The amount of fruit produced does not seem to be related to how appealing each display is to pollinators. The proportion of flowers that set fruit remained essentially consistent regardless of the total number of flowers per plant, the total number of inflorescences per plant, or the total number of flowers per plant; therefore, increases in floral display size had little effect on fruit set (Abe, 2001). The period of flowering in dioecious plants typically varies among males and females, with males starting to bloom sooner than females (Lloyd & Webb, 1977).

The parasite gains an adaptive advantage over the host plant as a result. The percentage of flower abortion in *L. odoratus* is 58.31%. Infections and pests, or the parasite cannot survive an excess number of flowers. The natural pests and diseases might act as mistletoe's biological control agents if found.

5.3 Pollen viability Evaluation

The rate of pollen viability in *L. odoratus* was found to be significantly similar in both sites. The highest pollen viability of *L. odoratus* was obtained in site B. Viability of pollen has been defined as having the capacity to live, grow, germinate or develop. The pollen viability of the examined *L. odoratus* might be affected by the temperature, slope, aspect, altitude, and humidity.

Pollen viability may decline with rising temperatures expected by climate change, potentially limiting reproduction in *P. edulis*, (Kellomäki *et al.*, 1997). Our result shows less pollen viability in lower elevations. Though the increase in altitude decreases the pollen viability, our impact is somehow different; this may be due to the aspect and slope.

5.4 Phenology of *L. odoratus* and host phenology

The host-parasite interactions are significant. Mistletoe dispersion is recognized to be regulated by host specialization, host periodicity, and associated environmental conditions (Garcia-Franco & Rico-Gray, 1996). *L. odoratus* especially requires *Quercus* species for their growth and development. All *L. odoratus* were attached to the *Quercus gluca* plant in our study. These study findings imply that the distributions of *L. odoratus* might vary depending on the host's traits and the surrounding environment.

Phenological research can disclose the phenological patterns of the species under investigation and offer significant insights into the biology of the plants in question. This study would also be very beneficial for comparison over a long period to check if the phenological patterns of the same plant species alter over the following ten or so years. There is currently no literature available for this region that would make a comparative analysis feasible.

a. Phenology of the hemi-parasite (Loranthus odoratus)

Phenological information about parasites in the Loranthaceae family has been documented in border research on reproductive plant ecology (Leck, 1972). These authors and others who have studied mistletoe species have demonstrated that individuals of some species, such as *Amyema quandang*, display delayed blooming or fruiting phenology (Barea & Watson, 2007, 2013). As per (Watson, 2001), this lack of synchronization extends the time this species' flowers or fruits are available, giving pollinators and dispersing resources throughout the year.

In the present study, *Loranthus odoratus* demonstrated a remarkable synchronization across all reproductive phenophase, which would shorten the length of time these phenological events last throughout the year. This finding could be connected to the phenological patterns of other nearby mistletoe species. It shows that the phenology of mistletoe *L. odoratus* in upper altitude or site B

seems to have an increment in phenophase length nearby two weeks. The increment or longevity of phenology might be due to the height increment.

b. Phenology of the host (*Quercus glauca*) due to influence of mistletoe

Parasite's morphological and physiological characteristics differ depending on the infected (Marvier & Smith, 1997). The host-parasite connection may be viewed as a two-way street. In *Quercus glauca*, the nutrients from the host species might be absorbed by the mistletoe, which extends the overall phenology of the host species. The mistletoe growth stopped during the dry season while it was infesting its host. The host's water deficit during its deciduous stage may be connected to this restriction on mistletoe development by lowering the water flow and slowing its growth.

When we compared the phenology of the examined mistletoes with their hosts, we discovered that *L. odoratus* reproductive events do not match its host tree, *Quercus glauca*. Since the flower opening of *Quercus* remains for a long time. While comparing the non-infected and infected hosts, the overall phenology is drastically changed. We found that the mistletoe phenology can vary depending on the connection formed with various hosts. The peculiarity of each host-mistletoe interaction is brought forth by this finding (Teixeira-Costa *et al.*, 2017).

5.5 Pollinator and flower visitation observations

The White flowers of *L. odoratus* were visited by a spectrum of pollinators, i.e., insects, mainly bees. Honey bees (*Apis mellifera*), Houseflies (*Musca domestica*), dragonflies, Butterfly, Mosquitos, and a small ant species were flower visitors. *Apis mellifera* and ants were observed as pollinators since they interact with both anther and stigma. *L. odoratus* had the highest rate of *Apis mellifera* visits in pollinator activity. The frequency of visits is not a reliable predictor of reproductive success; a link between achievement (McDade & Davidar, 1984) concluded that the effectiveness of floral alteration was connected to fruit yield. Small-flowered taxa seem more likely to exhibit entomophily (Ladley *et al.*, 1997) which supports our result as *A. mellifera* and other insects as pollen visitors. In both agricultural and natural ecosystems, bees the *Apis* species play a vital role in pollination (Corlett, 2004); (Potts *et al.*, 2010); (Hung *et al.*, 2018).

Apis mellifera visits flowers with various pollination conditions as a generalist forager (Roubik, 1980), where the *A. mellifera* were found to be excellent and effective pollinators of *L. odoratus*. *Apis mellifera* are frequent visitors and are not chased away by competitors, and ants were also regarded as regular visitors. Pollinators that are less effective but more numerous might make up for the absence of the primary pollinators. (Waser & Price, 1982).

Our study shows that the slope shift in blooming features and pollinator activity accounts for the increased reproductive success of *L. odoratus* in site B compared to site A. Pollination system with several pollinators may eventually be preferred if changes in pollinator numbers are substantial at this site. More research is required between *Loranthus odoratus* and its pollinators and their long-term role in the floral evolution of this plant species. No aggressive interactions were detected between *A. mellifera* and other flower visitors. The frequency of the pollinator peak during the afternoon period, which might be due to the intensity of light, and also because most insects are diurnal (Steen, 2017).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study was carried out to find the pollen viability, Phenology, fruit set, floral morphology, and various pollinators and visitors of the flower of *Loranthus odoratus* from two sites in Kathmandu District. Since the TZ test has proven to be suitable for determining pollen viability, the pollen viability of *L. odoratus* ranges from 29.79% - 31.01% by using 2, 3, 5 Triphenyl tetrazolium chloride. The conservation status of this species is Not Evaluated (NE), according to IUCN Global Red List Category and conservation should be done in order to minimize the threats of disappearing. *L. odoratus* flowers shows rare and distinct morphological trait. The presence of calyculus (1mm), inflorescence (1.4-4.9 cm), petals (4.9-5.1 mm), androecium (4.8-4.3mm), and gynoecium (3.7-4.3 mm). The overall phenology of *L. odoratus* with its host *Q. glauca* is observed with host-parasite interaction. The fruit set percentage found similar. Honey bees (*Apis mellifera*) and small ant species were detected as pollinators, and Houseflies (*Musca domestica*), dragonflies, butterflies, and mosquitos are observed and noticed as flower visitors of *L. odoratus* and peak time is mid of day due to available of nectar is more in day time and most pollinators are diurnal.

6.2 Recommendations

From the finding of this study, the following recommendations are made.

- At present and future *L. odoratus* locations, host species protection must be considered to maintain the species' existence at these places.
- It is essential to raise awareness among the general public about challenges to conservation, biology, biogeography, and the presence of rare, endemic mistletoes. Working with neighborhood organizations, educational institutions, colleges, social media, and internet reporting are the best ways to do this.
- The exact amount of viable pollen may be determined in vitro by pollen germination, so this method can be applied in future studies.
- Phytochemical screening of this species can give clear picture about the medicinal and use value which may also help to conserve it better.
- Future studies that can incorporate longer elevation gradient to see the variation in reproductive behavior of the species with elevation.

CHAPTER 7: REFERENCES

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https://en.wikipedia.org/wiki/File:Monoecy_dioecy_en.svg

APPENDIX I: Photographs of field visit and Lab Test.

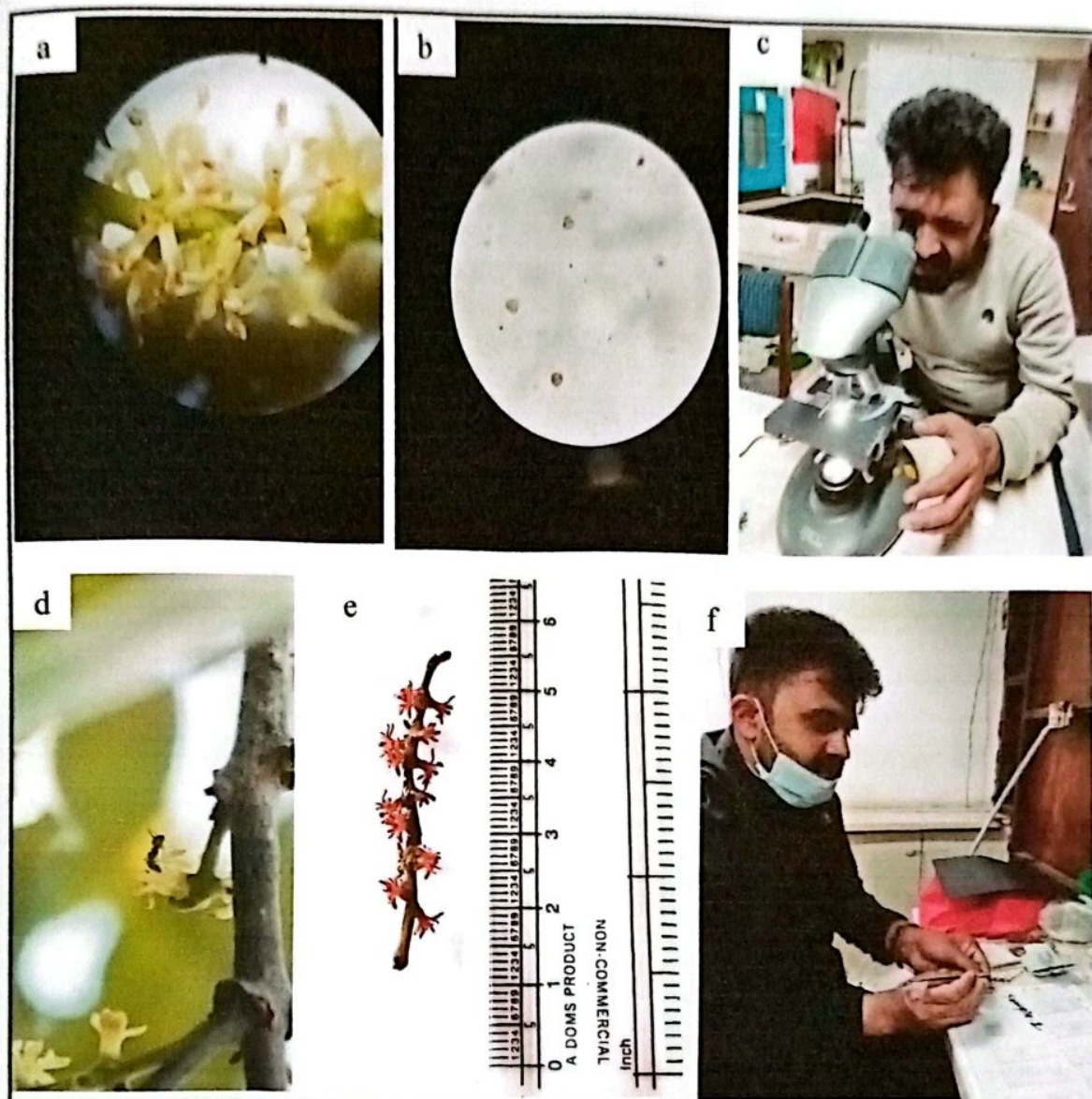


Figure 14. Photographs of field visit and Lab Test, (a) Showing *L. odoratus* flower, (b) Shows pollen in microscopic field, (c) Observing pollen status, (d) Shows ant in flower for pollination, (e) Represents measuring length of flower, and, (f) Represents preparing slide for pollen viability test.

APPENDIX II: Details about study site

S.N.	Study location	Altitude	Latitude	Longitude	Remarks
1.	Mulkharka	1700 m	27° 46' 32" N	85° 25' 34" E	
2.	Mulkharka	1665 m	27° 46' 32" N	85° 25' 33" E	
3.	Mulkharka	1680 m	27° 46' 32" N	85° 25' 33" E	
4.	Chalnakhel	1349 m	27° 37' 59" N	85° 16' 45" E	
5.	Chalnakhel	1349 m	27° 37' 59" N	85° 16' 45" E	
6.	Chalnakhel	1349 m	27° 37' 59" N	85° 16' 45" E	

Flower Measurement (random 3 big flowers from each sample)

Sample	S. N	calyculus(mm)	Corolla(mm)	Stamen (mm)	Pistil (mm)
1	1	1	5	4*4.6, 2*3.7	3.7
	2	1	5.1	4*4.5, 2*3.6	3.6
	3	1	5	4*4.6, 2*4.3	4.3
2	1	1	5	4*4.6, 2*3.7	3.7
	2	1	5.1	4*4.5, 2*3.6	3.6
	3	1	5	4*4.6, 2*4.3	4.3
3	1	1	5	4*4.6, 2*3.7	3.7
	2	1	5.1	4*4.4, 2*3.8	3.8
	3	1	5.1	4*4.5, 2*3.6	3.6
4	1	1	5	4*4.6, 2*3.7	3.7
	2	1	4.9	4*4.5, 2*3.6	3.6
	3	1	5	4*4.6, 2*3.8	3.8
5	1	1	5	4*4.5, 2*3.6	3.6
	2	1	5.1	4*4.6, 2*3.7	3.7
	3	1	5	4*4.6, 2*3.6	3.6
6	1	1	5	4*4.6, 2*3.8	3.8
	2	1	5.1	4*4.4, 2*3.6	3.6
	3	1	4.9	4*4.8, 2*3.7	3.7

4*4.6, 2*3.7 it refers 4 stamens of 4.6mm and 2 stamens of 3.7mm

APPENDIX III: Tests

Test of Homogeneity of Variances pollen viability

		Levene Statistic	df1	df2	Sig.
Pollen Viability	Based on Mean	3.019	1	52	.088
	Based on Median	2.906	1	52	.094
	Based on Median and with adjusted df	2.906	1	45.695	.095
	Based on trimmed mean	3.058	1	52	.086

ANOVA

Pollen Viability

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.984	1	19.984	.253	.617
Within Groups	4104.784	52	78.938		
Total	4124.768	53			

