# LORANTHUS IGUSTRINUS IS AN EMERGING PARASITE CAUSING DECLINING THE YIELD OF KHASI MANDARIN ORANGE IN ARUNACHAL PRADESH AND ITS MANAGEMENT METHODS

**A thesis submitted to**
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**In partial fulfillment of the requirements for the award of the degree of**
**Master of Science in BOTANY**

**Submitted by**

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## CERTIFICATE

**This is to certify that this thesis entitled "Studies on the *Loranthus ligustrinus* is an emerging parasite causing declining the yield of khasi mandarin orange in Arunachal Pradesh and its management methods" submitted to the Assam Science & Technology University, Guwahati, for the award of the degree of Master of Science in Botany is a bonafide research work carried out by the student Mr HIRAK JYOTI BHUYAN (Roll No-202820047008) under my guidance and supervision during the period between April 2022 to August 2022. I further certify that no part of this thesis has been submitted anywhere else for the award of any Degree, Diploma, Associateship, Fellowship or other similar titles.**

19/09/2022.

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# DECLARATION

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**I hereby declare that the work embodied in this thesis entitled "Studies on the *Loranthus ligustrinus* is an emerging parasite causing declining the yield of khasi mandarin orange in Arunachal Pradesh and its management methods", is a research work done by me under the supervision and guidance of Dr. M. MATHIYAZHAGAN Associate Professor of Botany, Silapathar Science College, Silapathar. I further declare that this work has not been submitted earlier in full or in parts to any other university for the award of any other Degree, Diploma, Associateship, Fellowship or other similar titles.**

**Date :** 19/9/2022

**HIRAK JYOTI BHUYAN**

**Place : Silapathar**

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## ABSTRACT

Cultivation of Khasi Mandarin forming is the only source of income for the farmers in Arunachal Pradesh. The production and productivity of Khasi Mandarin are mainly affected by the *Loranthus* stem parasite weed. It is lowering its yield and productivity and finally killed the host plant within 4-5 years after infestation. Loranthus leaves are light green and arranged oppositely. Leaf patterns are small simple leaves, elliptical shape, and entire margin and reticulate venation. It is ranged from 4.00 to 8.5 cm in length and leaf breadth varies from 1.60 to 3.00 cm. *Loranthus* has single haustoria and the stem length varies from 30 to 42 cm and has 6 to 8 branches at the tip. Spraying of sixty percent diesel was found to be more effective followed by 2, 4-D 5%, and 30% diesel. The per cent reduction over control for the treatment of 60% diesel, 2, 4-D 5% and 30% diesel are 97.5%, 90% and 87.5% respectively. The average yield per tree was 37.5 kg. Fifty percent reduction in yield was noticed in control. We have also observed that after mechanical treatment the *Loranthus* are emerging out only if the cutting stem is more than 10 cm.

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## LIST OF ABBREVIATIONS AND SYMBOLS USE

| | |
|---|---|
| **sp, spp** | **: Species (singular and plural)** |
| **Viz** | **: Namely** |
| **et al.** | **: and other co workers** |
| **Sl. No.** | **: Serial number** |
| **PROC** | **: Percentage of reduction over control** |
| **PDI** | **: Percent disease index** |
| **%** | **: Per cent** |
| **SEd** | **: Standard error deviation** |
| **CD** | **: Critical difference** |
| **CV** | **: Coefficient of variation** |
| **cm** | **: Centimetre** |
| **Fig** | **: Figure (s)** |
| **0 c** | **: Degree Celsius** |
| **kg** | **: Kilogram (s)** |
| **ha** | **: Hector (s)** |
| **mg** | **: Milligram (s)** |
| **gm** | **: Gram (s)** |
| **ml** | **: Millilitre** |
| **mm** | **: Millimetre** |
| **MSL** | **: Mean Sea Level** |
| **No** | **: Numbers** |
| **Trt** | **: Treatment** |

# CHAPTER-I

## INTRODUCTION

*Citrus* spp is largely grown in the north east region viz. Arunachal Pradesh, Mizoram, Tripura, etc. Among the citrus species, Khasi Mandarin is the most common one. Cultivation of Khasi Mandarin forming is the only source of income for the farmers who live in a hilly region with altitude ranging from 150 to 500 MSL. The production and productivity of Khasi Mandarin are mainly affected by unscientific way of cultivation, lack of budded seedlings, lack of quality planting material, citrus decline, citrus stem borer, and *Loranthus* stem parasite weed (Hazarika and Singh, 2013). *Loranthus* parasite is contributing to the citrus decline in the Khasi Mandarin growing region of Arunachal Pradesh (Hazarika and Singh, 2013). It is mainly transmitted by birds viz. Plain flowerpecker (*Dicaeum concolor*) and Fire-breasted flowerpecker (*Dicaeum ignipectus*) (Singh *et al*. 2013). *Loranthus* absorb water and minerals which affect the fruiting and yield. The infected tree will die within 3-4 years. Hence, we have aimed to study the occurrence of *Loranthus* in a region with following objectives.

**Objectives:**

1. Survey and collection of loranthus sp from various orchards of khasi mandarin orange.
2. Study of host parasite relationship and morphology of loranthus.
3. Devise the suitable effective management methods to eliminate loranthus.

# CHAPTER-II

## REVIEW OF LITERATURE

Parasitism is a highly successful life strategy and a theme that bridges all kingdoms of life. The degree of host dependence varies among parasitic genera. Parasitic plants are common in many natural and semi-natural ecosystems, ranging from tropical rain forests to the high Arctic (Press, 1998), accounting for approximately 1% of angiosperm species (34000) spread across c. 270 genera and more than 20 families (Nickrent et al., 1998; Press, 1998). They occur in many life forms, including annual and perennial herbs (Rhinanthus spp.), veins (Cuscuta), shrubs (Mistletoes) and trees (Sandalwoods), which grow up to 40 m tall (Veenendaal et al., 1996). Grierson and Long (1983) reported that 15 mistletoe species belong to the Loranthanceae and six species of Viscaceae in Butan. Singh (1962) expanded the host list of Dendrophthoe falcata from 153 (Fischer, 1926) to 319. Rao and Ravindranath (1964) added 24 new hosts along with the host list of Singh (1962), bringing the total to 343. Johri and Bhatnagar (1972) gave a partial host list modified from Singh (1962). Hawksworth (1974) listed the mistletoe on 38 introduced trees in India and Sri Lanka.

### 2.1 Host Range of Hemiparasite

Loranthaceace, with 73 genera and 900 species, comprises mostly aerial hemiparasites, but three monotypic genera are entirely root parasitic. The family occurs mainly in tropical areas worldwide, although they are also found in temperate habitats in Europe, Asia, South America, Australia, and New Zealand (Vidal-Russell, 2007). Most parasitic plants can potentially attack a large number of different co-occurring species (i.e., they have a broad host range), often simultaneously (Gibson and Watkinson, 1989; Nilsson and Sevensson, 1997; Pennings and Callaway, 2002; Westbury, 2004). In this respect, most parasitic plants can be considered generalists. Examples of a wide host range are documented for shoot parasites. Among the parasitic plants, the tropical rain forest mistletoe, Dendrophthoe falcate, Dendrophthoe falcate Desr, is a climbing woody parasitic plant (Dashora et al., 2011). It is indigenous to tropical regions, especially in India.

Sri Lanka, Thailand, China, Australia, Bangladesh, Malaysia, and Myanmar. In India, it is widely distributed throughout the country, up to 900 m (Pattanayak et al., 2008). It has 400 known host species (Narasimha and Rabindranath, 1964; Narayanasamy and Sampathkumar, 1981; Joshi and Kothyari, 1985). The world wide distribution of Loranthaceae was divided into seven geographical areas; Africa, South America, Asia, Malaysia, east of Wallace's line, Malaysia west of Wallace's line Australia and New Zealand A matrix for 36 genera with one multistate character was built and optimised on a phylogenetic tree using MacClade (Maddison and Maddison, 2000). Keeble (1896) gives a beautiful account of the Loranthaceae of Ceylon, describing in detail the fertilisation of the flowers, the mode of distribution of seeds, etc., but does not mention the hosts. Cooke (1903–1908) states that Loranthus is common on mango trees in Bombay and throughout the Konkan. In addition, Diospyros melanoxylon and Bassia latifolia are recorded as hosts of Dendrophthoe falcata by Partridge (1911) and Duthie (1915), who reported that the partial parasite, Loranthus, commonly grows in the upper Gangetic plain on a number of host plants belonging to different families of the Dicotyledonae. They are especially found on trees or shrubs, with the commonest hosts being Mangifera indica and Bassia latifolia.

In Poona, Patwardhan (1924) noted Eucalyptus rotra, a non-indigenous plant, as a host of Dendrophthoe falcata in Poona. Fischer (1926) listed 153 hosts of Denrophthoe falcate in southern India. The parasite, Dendrophthoe falcata, was growing on a guava (Psidiumgujava) tree, which was an unfamiliar sight with no other plant in the vicinity. A number of guava trees in an orchard had Loranthus on them. In Hyderabad, the occurrence of Dendrophthoe falcata on Psidium guajava, Melia azadirachta, Cordia myra, Annona squamosa, Punica granatum, Tamarindus indica, Citrus aurantium, and Millingtonia hortensis was described by Srivastava (1935). Sayeed-Ud-Din and Salam (1935) reported that although this parasite is becoming somewhat cosmopolitan, it is worthwhile recording, from time to time in different localities, on what new hosts it spreads. Evidently, there does not seem to be any specialisation of hosts in this parasite. host plants belonging to different families of the Dicotyledonae. They are especially found on trees or shrubs, with the commonest hosts being Mangifera indica and Bassia latifolia. In Poona, Patwardhan (1924) noted Eucalyptus rotra, a non-indigenous plant, as a host of Dendrophthoe falcata in Poona. Fischer (1926) listed 153 hosts of Denrophthoe falcate in southern India.

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Hawksworth et al. (1993) reported that Dendrophthoe falcate is generally considered to have the broadest host range and it attacks a greater number of native as well as introduced trees. It is particularly damaging to several fruit and timber trees, notably citrus, mango, rosaceous fruits, and sal. The mistletoe species of Dendrophthoe falcata (L. longiflorus) has 401 host plants which belong to 227 genera and 77 families (Hawksworth, 1983; Devkota, 2005). Osadebe and Ukwueze (2006) reported that the common host trees of African mistletoe (Loranthus micranthus) are Kola acuminate, Baphia nitida, Citrus limon, Jatropha curcas, Pentaclethra macrophylla, Azadirachta indica, and Persea americana. Swant et al. (2008) reported about 60 hosts of Loranthus. All the host plants are perennial, woody, and dicotyledonous. According to them, forest trees predominate in the host range, recording a maximum of 35 hosts, followed by 10 hosts of fruit trees, 8 hosts of forest shrubs, and 7 hosts of ornamental plants. Out of these 60 hosts, 19 are reported as hosts for the first time in the world. The infestation of Loranthus was more on wild trees than on the trees in managed orchards, like that of mango (Swant et al., 2008).

## 2.2 Haustorial system

The haustorial system in Loranthaceae shows considerable diversity. The primitive state, found in a few genera,is terrestrial root-parasitism with no primary haustorium, which is common in the related families Olacaceae and Santalaceae (Calder & Bernhardt 1983). Among aerial Loranthaceae, the apparently most primitive haustorial system involves basal epicortical roots (referred to as' runners'), which grow in a vine-like fashion along the host stems, producing haustoria at regular intervals. They can produce new leafy shoots and sometimes inflorescences along their length. The epicortical manners may represent an early stage in evolution from terrestrial root-parasitism to aerial stem-parasitism, in which root-like structures producing numerous haustoria are still present(Hamilton & Barlow 1963). Species with epicortical runners are most common in humid tropical forests, and in Malesia, the great majority of loranths exhibit this character. The more advanced haustorial systems are those in which epicortical runners are not produced, and only the primary haustorium developed from the embryo is present. This may be very complex, especially in internal structure; some produce long strands which extend for metres inside the host, either in the cambial zone or in the cortex (Hamilton & Barlow 1963).

In Malesia, such haustoria are rare, generally being found in species with close phyletic links to Australia, where such haustoria are common. produced from stem-borne roots, as in the neotropical genus Struthanthus, probably represent a derived state, but do not occur in any Malesian species. The haustoria in most mistletoe species are xylem-tapping, although some species also tap the phloem and therefore have easier access to host photosynthate. Xylem tapping mistletoes nevertheless divert and concentrate nutrients such as nitrogen and phosphorus from the host's sap. On average, about 15% of the total carbon gain of xylem-tapping mistletoes is from photosynthate diverted from the xylem stream of the host(Moore 1994; Marshall et al. 1994).

### 2.3 Inflorescences

There are several parallel trends in inflorescence structure in Loranthaceae. The most primitive inflorescences appear to be simple cymes, and a common inflorescence unit widespread in the family is the simple dichasium (triad). The triads are often aggregated into larger compound inflorescences, which are usually racemose or umbellate. A secondary trend in inflorescence evolution appears to be reduction from this compound state, and simple racemes or umbels, for example, are probably derived through the reduction of oftriads to single flowers. This is clearly evident in Macrosolen, where each flower in the raceme is subtended by three bracts. In Malesia, reduction in inflorescence structure reaches its extreme in some species of Amyema and in Sogerianthe, where the inflorescence is a single flower but its articulate pedicel is evidence of its derivation.

Another trend in inflorescence evolution is the contraction of the flowers into heads, often subtended by enlarged bracts which form an involucre. Such inflorescences are more common in loranths in Malesia than in any other region, and parallel evolution of capitate inflorescences has occurred in a number of lineages. The basic racemose or umbellate nature of the inflorescence is still usually evident, as well as the presence of triads or single flowers as the basic inflorescence unit. In many capitate inflorescences, the young flowers are tightly enclosed during development, possibly for protection. However, at anthesis, the flowers may be exserted from the involucre, which is often brightly coloured and probably contributes to the presentation of the flowers to pollinators. This interpretation of inflorescence evolution agrees in its broadest principles with the conclusions of Kuijt (1981), but differsin many details.

**2.4 Flowers**

Whilst the external form of the flowers does not appear exceptional, there are some very unusual features in ovary structure and embryology (Maheshwari et al. 1957; Johri& Bhatnagar 1960). There are no normal ovules. In many species, there is a central mound or column, the mamelon, which occupies most of the ovarian cavity and which may be homologous with an axile placenta. In some cases, the base of the mamelon is lobed, and these lobes may be homologous withovules. Bands of tissue radiating between the lobes to the ovary wall may form 3 or 4 obscure cells in the ovarian cavity, and they are possibly homologous with septa. In other cases, the mamelonis simple or completely absent, and the ovarian cavity is hardly more than a small dilation of the base of the stylar canal. The sporogenous tissue is massive, located either in 3 to 4 blocks in the mamelon lobes or in a single block at the base of the ovarian cavity.

These structures may represent progressive reduction of a syncarpous ovary, and the extreme of the reduction sequence, for example in Amyema, is an almost completely undifferentiated ovary with a single group of sporogenous cells at the base of the stylar canal. There are several embryo sacs which elongate the stylar canal to various levels, so that fertilisation occurs in the style, sometimes close to the base of the stigma (Helixanthera, Dendrophthoe). Rapid development of a long suspensor pushes the proembryo down into the ovary before the style is shed. Normally, only one embryo develops in the seed, and the viscous layer develops from a zone in the ovary wall outside the vascular bundles. These features of embryology are so exceptional in angiosperms that Van Tieghem (1896) treated loranths and related groups as a subclass of the angiosperms with a rank equivalent to the monocotyledons and dicotyledons.

**2.5 Seeds and seedlings**

Seed and seedling features may provide useful diagnostic characters at a tribal or subfamilial level in Loranthaceae. The seeds are primarily endospermic, although in the neotropical genus Psittacanthus the endosperm appears to be entirely absorbed in mature seeds. The embryo is normally green. In many genera, the cotyledons emerge as the first photosynthetic leaves after germination. In others, the slender cotyledons remain in the endospermic seed, elongating to push out the plumule, which produces the first leafy shoot. These two basic germination patterns are generally correlated with other characters of taxonomic importance at higher ranks, especially basic chromosome number and ovary structure.

### *2.6* Properties of *Dendrophthoe falcate*

Previous reports (Chandel et al., Biodiversity in Medicinal and Aromatic Plants in India, p 45-46) indicate the general information of Dendrophthoe falcata as follows: The plant has a large, bushy, evergreen, parasitic plant with smooth grey bark. Young parts are glabrous or nearly so. Leaves are sup-opposite, coriaceous, and highly variable in size and shape, most often ovate-oblong, 7.5 to 20 cm long and 2 to 10 cm wide, with an obtuse apex and base and minutely white margins; petioles are 0 to 13 mm long. Flowers are borne in short, spreading axillary racemes; pedicels are short; bracts are 1.6mm long, broadly ovate, and concave; calyx is 4 cm long, tomentose, tube cylindrical, limb copular, truncate, or briefly toothed.

The fruit is a fleshy, ovoid or oblong berry 0.8 to 1.7cm long and 0.06cm wide, bright pink when mature. Chandrakasan (2013) described the general characteristics of Loranthus longiflorus (Dendrophthoe falcata) collected from two host trees as follows: Habit parasitic shrub on the branches of dicot plants; Leaves-Opposite & alternate, entire, estipulate, thick, elliptic or oblong, linear, prominent midrib, wavy margin, and reticulate venation, varying in length and breadth (length ranges from 4.7cm to 18.3cm with an average of 9.94cm and breadth ranges from 1.0cm to 2.7cm with an average of 1.82cm in leaves obtained from Casuarina equisetifolia host tree;. In Ficus religiosa host tree leaves, length ranges from 3.6 cm to 18.3 cm with an average of 12.2 cm and breadth ranges from 1.0 cm to 5.3 cm (with an average of 1.87 cm). Inflorescence-racemose, cluster, 1-4 inches long; flowers varying in length (ranging from 2.9cm to 6.1cm with an average of 5cm in Casuarina equisetifolia host tree flowers; regular, actinomorphic, and bisexual flowers ranging from 2.5cm to 6.3cm with an average of 5.5cm in Ficus religiosa host tree flowers);

The perianth is usually upcurved, biseriate, valvate, and adnate to the ovary, forming urceolode calycules with a coloured inner whorl. calyx-green, entire, adnate to the ovary, copular, and entire; Corolla-five united petals from a tube; the base of the tube is pink in colour, but the tip is free (split) and green in colour; Androecium-stamens-5, antiphyllous, epiphyllus, anthers basifixed, dehiscence longitudinal; Gynoecium-ovary inferior, unilocular, basal placentation, stigma simple (Chandrakasan, 2013).

### *2.7* Medicinal properties of the Hemiparasite - *Dendrophthoe falcate*

Dendrophthoe falcata has many medicinal properties. It is neutral in property and helps in the treatment of liver and kidney channels; used as an abortifacient (stem); narcotic, wounds, and asthma (bark); increases blood pressure and myocardial effect (leaf extract); the lukewarm leaf juice is used as an eardrop to cure earaches; anti-viral and lowers blood pressure (5% EtOH plant extract, excluding root). Besides, the following conditions are treated by using D. falcate: Abscesses, acne, amoebiasis, bacillary dysentery, bacterial tracheitis, bacterial vaginosis, boils, bronchitis, campylobacteriosis, carbuncles, cellulitis, cholera, ear infection, enteric fevers, erysipelas, furuncles, giardiasis, infectious mononucleosis, laryngitis, measis, otitis media, pharyngitis, shigellosis, sinus infection, tonsillitis, typhoid, uti, vaginal infection, whooping cough, etc. The leaf paste of Dendrophthoe falcata is used for skin diseases and is taken for abortion (Bhattarai, 1991; Siwakoti and Siwakoti, 2000). Kusumoto et al. (1992) reported that methanol and water extracts of 30 Indonesian medicinal plants were tested for inhibitory activity on avian myeloblastosis virus (AMV) –reverse transcriptase (RT). Of these, the most potential inhibition was shown by extracts of Loranthus parasiticus (whole plant).

The extracts showed no appreciable cytotoxicity at concentrations where over 90% of RT activity was inhibited. Extracts of botanically identified plant materials of Dendrophthoe species from 15 different host trees have been tested for cytotoxicity and only two of them were found to be positively cytotoxic by Mary et al. (1992). Bark juice/decoction is employed for menstrual problems and asthma (Bohora, 1998; Sapkota, 2000; Pandey, 2001; Bhattarai, 2002). Its paste is applied for boils, setting dislocated bones, and extracting pus (Manandhar, 2002). Fruit is taken as a flavour and is edible (HMGN, 1982; Panthi and Chaudhary, 2002; Shrestha and Kunwar, 2003), astringent, narcotic, and for curing wounds (Siwakoti and Varma, 1996; 1999), and its paste is applied to fractures for setting bones (Manandhar, 1990) and other medicinal purposes (Sah et al., 2002). Nectar is food for hair-crested Drungo and sunbirds (BPP, 1995).

Leaf, along with Urtica doica (Sisnu), is made into a paste and used to treat bone fractures (Bhattarai, 1993). Kunwar et al. (2005) reported that the local people of Nepal were found to use Dendrophthoe falcata for edible and medicinal purposes. Bark juice/decoction is employed for menstrual problems and asthma (Bohora, 1998; Sapkota, 2000; Pandey, 2001; Bhattarai, 2002). Its paste is applied for boils, setting dislocated bones, and extracting pus (Manandhar, 2002). Fruit is taken as a flavour and is edible (HMGN, 1982; Panthi and Chaudhary, 2002; Shrestha and Kunwar, 2003), astringent, narcotic, and for curing wounds (Siwakoti and Varma, 1996; 1999), and its paste is applied to fractures for setting bones (Manandhar, 1990) and other medicinal purposes (Sah et al., 2002). Nectar is food for hair-crested Drungo and sunbirds (BPP, 1995). Leaf, along with Urtica doica (Sisnu), is made into a paste and used to treat bone fractures (Bhattarai, 1993). Kunwar et al.

(2005) reported that the local people of Nepal were found to use Dendrophthoe falcata for edible and medicinal purposes.

## 2.8 Phytochemical Studies of the hemi-parasites

Medicinal plants that have been proven to be active in-vitro should be taken through the stages of phytochemical analysis to assess their potential as lead compounds (McRae et al., 2006). While purely synthetic compounds have produced many beneficial medicines, it is now being realised that the comparatively complex structures of natural compounds are needed to combat diseases (Lesney, 2004). Several factors play important roles in the phytochemical composition and pharmacological activities of the mistletoe plant, such as the host, species of mistletoe used, season of harvest, etc. (Osadebe and Ukwueze, 2004). Medicinal plants contain various phytochemicals that are very important for human life for the treatment of various diseases. Knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents but also for disclosing new sources of economic material information such as tannins, oil, gums, and precursors for the synthesis of complex chemical substances. In addition, knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Monjab et al., 2003).

Phytochemistry is a rapidly expanding area for the analysis of organic compounds and quality assessment of plants, such as preliminary phytochemical screening, chemo-profiling, and marker compound analysis using modern analytical techniques. Phytochemical tests were used to detect the presence of steroids, alkaloids, tannins, glycosides, reducing sugars, etc. Medicinal plant research is directed towards verifying ethano-medical claims by herbalists with the ultimate aim of isolating active compounds and standardising the crude extracts used in traditional medicines (Sofawora, 1986; 1993). Phytochemicals are natural bioactive compounds found in plant parts such as vegetables, fruits, flowers, leaves, stems, and roots.

They act as a defence system against diseases, more accurately to protect against disease-causing microbes. Uppuluri Venkata Mallavadhani et al. (2006) isolated three new triterpenes from fruit extracts of the Indian Ayurvedic plant, Dendrophthoe falcata: 3-acetoxy-1-(2-hydroxy-2propoxy)-11-hydroxy-olean-12-ene (1); 3-acetoxy-11-ethoxy-olean- 12-ene (6); 30-nor-lup-3-acetoxy-20-one (8); (20 Many antioxidants are plant-based and play an important role in protecting plants as well as human health that are exposed to sunlight and live under severe oxygen stress. According to recent investigations, activated oxygen is thought to be a major factor in ageing, hardening of the arteries, diabetes, cancer, and tissue injury in the skin. Indeed, approximately 90% of age-related diseases are linked with activated oxygen. When the human skin is exposed to UV rays, active oxygen (free radicals) is generated, which is scavenged by excess melanin pigmentation. This excess melanin pigmentation can cause spots and freckles on the skin (Tominaga et al., 2005). Antioxidants are considered as possible protective agents, reducing oxidative damage to the human body (Yam et al., 2008). A bioassay is the estimation of the activity or potency of a drug or other substance (e.g., plant extract) by comparing its efficiency on a test organism with that of a standard preparation. It is a type of scientific experiment conducted to measure the effect of a substance on a living organism and is essential in the development of new drugs and other scientific monitoring.

The driving force behind much phytochemical production is the discovery of new biological active compounds for medicinal or agricultural uses. Biological assays must be carried out in order to identify and evaluate the lead compounds in the promising plant extracts by various separation and isolation processes. Identification of natural products from plants that may serve as valuable sources of bioactive agents for medicinal and agricultural uses largely depends on bioactivity-directed isolation (Cseke et al., 2006). 18 The modern methods describing the identification and quantification of active constituents in plant material may be useful for proper standardisation of herbals and their formulations. WHO also emphasised the need to ensure the quality of medicinal plants using modern controlled techniques and applying suitable standards (Chaudhay Ranjit et al., 1992). Modern extraction methods are based on chemical polarity and solubility.

Thus, an aqueous extract or organic extract is prepared and further analysis is required to clean and purify the compounds by bio-separation using the elucidated methods. Finally, the selected sample is subjected to mass spectroscopy or nuclear magnetic resonance analysis in order to avoid ambiguous identification of interested compounds (Kaufmann, 1999). Antioxidants found in plants play an important role in protecting cells and tissues from damage caused by reactive oxygen species. Plants containing flavanoids have been reported to possess strong antioxidant properties (Ara and Nur, 2009). The leaves, shoots, pseudoberries, and flower buds of four different mistletoe plants have been examined by Saheer et al. (1992) based on their seasonal development for dry weight, lipids, protein, polysaccharides, lectins, and viscotoxins. Furthermore, vesicles derived from the extraction of mistletoe leaves were analysed.

Saheer et al. (1992) observed that the maximum values of proteins, polysaccharides, and vesicles were detected in vegetative organs in summer, whereas lipids and lectins were most enriched in generative organs in winter. Therefore, two times is ingenious. The vegetative mistletoe in summer and the generative mistletoe in winter, combined in an appropriate quantity, lead to a more complete mistletoe. The chemical constitutions and biological activities of Loranthus species depend to a large extent on the host plant and the season of harvest (Mortan, 1977; Tuquet and Salle, 1996). Information on mistletoes of different regions (European, Korean, Australian, etc.) is widely documented (Kuttan et al., 1990; Fernandez et al., 1998; Yoon et al., 1998). But differences in the metabolic pathway, characteristic protein pattern, and immunogenic capacity between the Australian and European species have been documented (Joller et al., 1996).

Such differences may also exist between the European and the African mistletoe because of obvious climatic and host tree variations. There have been reports (Osadebe and Ukwueze, 2004) on the phytochemical and antimicrobial properties of African mistletoe (Loranthus micranthus). A comparative study of the phytochemical properties of leaves of Loranthus micranthus harvested from six host trees, namely, Irvingia gabonensis, Pentaclethra macrophylla, Kola acuminate, Baphia nitida, Persea americana, and Azadirachta indica, was carried out by Osadebe and Ukwueze (2004). The result showed marked variations in phytochemical constituents of the extracts from the samples of different host trees, both in kind and in degree (Osadebe and Ukwueze, 2004). Many medicinal plants contain large amounts of phytochemicals that possess significant antioxidant capacities associated with lower occurrence of several human diseases and lower mortality rates (Anderson et al., 2001; Djerdane et al., 2006).

The presence of avicularin (aglycosylated flavonoids) and quercetin in the hemiparasitic plant Loranthus was reported by Wang et al. (2006). The presence of tannins, flovanoids, saponins, phenols, alkaloids, and anthocyanins were reported by Orji et al. (2013) in the ethnol leaf extract of L. micranthus. Chandrakasan (2013) carried out preliminary phytochemical analysis and noted the presence of many phytochemicals in the bark and leaf samples of Loranthus longiflorus collected from Casuarina equisetifolia and Ficus religiosa host trees. The total phenol content (335 2.3) of L. europeas recorded by Ahmed and Rocha (2009) was within the same range as the value reported by Chopra et al. (1997) with more antioxidant activity at a higher total phenol content. They found the antioxidant activity of L. europeas water extract ranged from 25-73% at 3.5-16.9mg/ml at various concentrations. Some authors (Katsub et al., 2004; Djeridane et al., 2006; Katalin et al., 2006) have demonstrated a linear correlation between the amount of total phenolic compounds and their antioxidant capacity. High performance liquid chromatography (HPLC) is an invaluable quality assessment tool for the evaluation of botanical materials. The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines. Chemical fingerprints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines since they might represent the "chemical integrities" of the herbal medicines appropriately and therefore be used for authentication and identification of the herbal products.

Based on the concept of phytoequivalence, the chromatographic fingerprints of herbal medicines could be utilised to address the problem of quality control of herbal medicines. By definition, a chromatographic fingerprint of herbal medicine is, in practice, a chromatographic pattern of pharmacologically active and or chemically characteristic constituents present in the extract. This chromatographic profile should be characterised by the fundamental attributions of "integrity" and "fuzziness" or "sameness" and "differences" so as to chemically represent the herbal medicines investigated. This suggests that chromatographic fingerprint can successfully demonstrate both "sameness" and "differences" between various samples, and the authentication and identification of herbal medicines can be accurately conducted even if the number and/or concentration of chemically characteristic constituents are not very similar in different samples of herbal medicine. Thus, chromatographic fingerprints should be considered to evaluate the quality of herbal medicines globally, considering multiple constituents present in the herbal medicines. Herbal medicines have a long therapeutic history and are still serving many of the health needs of a large population of the world. But, quality control and quality assurance still remain a challenge because of the high variability of chemical components involved.

Herbal drugs, singularly and in combinations, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards for raw materials and standardisation of finished herbal drugs. Traditionally, only a few markers of pharmacologically active constituents were employed to assess the quality and authenticity of complex herbal medicines. However, the therapeutic effects of herbal medicines are based on the complex interaction of numerous ingredients in combination, which is totally different from those of chemical drugs. Thus, many kinds of chemical fingerprint analysis methods to control the quality of herbal drugs have gradually come into being, such as thin layer chromatography, gas chromatography, high performance liquid chromatography, etc. Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality, and ensuring the consistency and stability of herbal drugs and their related products.

The entire pattern of compounds can then be evaluated to determine not only the presence or absence of desired markers or active constituents but also the complete set of ratios of all detectable analytics. The chemical fingerprints obtained by chromatographic and electrophoresis techniques, especially by hyphenated chromatography, are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the "chemical integrities" of herbal medicines and therefore be used for authentication and identification of the herbal products. Chandrakasan (2013) carried out HPTLC and GC-MS analysis on the leaf and bark samples of Loranthus longiflorus infested on Casuarina equisetifolia and Ficus religiosa host trees. The HPTLC analysis of methanolic extracts of L. Longiflorus leaf/bark samples collected from C. equisetifolia and F. religiosa host trees confirmed the presence of alkaloids (9/5 & 5/4 compounds, respectively), carotenoids (9/8 & 8/8 compounds, respectively), coumarin (10/9 & 9/4 compounds, respectively), flavonoids (10/3 & 2/10 compounds, respectively), glycosides (7/17 & 6/14 compounds, respectively), phenolic acids (13/9 & 8/9 compounds, respectively), phenolic acids (13/9 & 8/9 compounds, respectively), phenolic acids (13/9 & 8/9 compounds, respectively), phenolic acids (13/9 & 8/9 compounds, respectively), phenolic acids (13/9 & 8/14 compounds, respectively). Chandrakasan (2013) indicated that the GC-MS analysis of leaf/bark ethanolic extracts of Loranthus longiflorus collected from Casuarina equisetifolia and Ficus religiosa host trees shows the presence of 5/6 cmpounds and 6/9 compounds, respectively (Chandrakasan and Neelamegam, 2013).

## 2.9 Bioactive Studies in Hemi-parasites

Some plants draw special attention for their low lethal and other environmentally friendly activities (Talukder and Howse, 1995). Epiphytes, or air plants that do not normally root in the soil but grow upon other living plants, draw attention as a source of bioactive potential. Peroxidase enzymes are widespread in the plant kingdom and apparently play an important role in plant infection (Ebermann and Lickl, 2006). Frequently, increased peroxidase levels are found in the host as well as the invading organism (Stahmann and Demorest, 1972; Uritani, 1978). It is known that peroxidase activity is enhanced during infection in many cases (Uritani, 1978). Stichk and Ebermann (1984) reported the course of activity of peroxidase enyme during the infection of oak by L. europaeus. Loranthus species as semiparasitic plants are known to produce a variety of bioactive compounds, i.e., sesquiterpene lactones from L. parasiticus for the treatment of schizophrenia (Okuda et al.,

1987) and (+)-catechin, 3, 4-dimethoxycinnamyl alcohol, and 3,4,5-trimethoxycinnamyl alcohol from L. globosus for their antimicrobial and antifungal properties (Sadik et al., 2003). Many other chemical components such as triterpenoids from L. grewinkii (Rahman et al., 1973) and L. falcatus (Anjaneyulu et al., 1977), flavonoids from the leaves of L. kaoi (Lin and Lin, 1999) and from L. europaeus (Harvala et al., 1984), a cytotoxin from L. parasiticus (Zhou et al., 1993), and phenolics from Dendrophthoe falcata (Indrani et al., 1980) were also reported. D. falcate contains biologically active substances such as phenolic content (Khanna et al., 1968), flavonoids and quercetin (Ramchandran and Krishnakumary, 1999), 24 tannins, -sitosterol, -amyrin, oleanolic acid (Kacharu and Krishnan, 1979; Rastogi and Mehotra, 1993), and Indrani et al., (1980) found (+)-catechin and leucocyanidin from the bark of D. falcate. Chandrakasan (2013) carried out studies on phytochemical constituents of Loranthus longiflorus collected from Casuarina equisetifolia and Ficus religiosa host trees and noted the presence of various phytocompounds in different solvent extracts of leaf and bark samples. Members of the genus Denrophthoe are reported to have antioxidant, antimicrobial, anticancer, antidiabetic (Osadebe et al., 2004), antilithiatic, and anthypertensive (Balaram et al., 1981) qualities. Mallavadhani et al. (2006) mentioned that D. falcate has oestrogen receptor binding activity that, on the other hand, offers male anti-fertility agents and properties for the inhibition of spermatogenesis. Warrier (1993) reported that the entire plant of Dendrophthoe falcate is used extensively in the traditional system of medicine as an astringent, narcotic, bitter, diuretic, and is used in the treatment of tuberculosis, asthma, and mania.

It is used for menstrual disorders, wounds, prevention of stones in the kidney and bladder, hemorrhage, miscarriage, and abortion during pregnancy. It is used as an anti-ulcer, an anti-helmintic agent, and as a blood purifier. It is also used in foetal development and blood pressure management. A plant grown on a Ficus fistula host is used for foetus development in Ayurveda. It is used in vatta, kapha, and pitta (balancing/equilibrium). This plant is also used to avoid abortion, which occurs during the 3rd month of pregnancy (Nadkarni, 1927; 2000; Tenpe et al., 2008). The whole plant is used in the indigenous system of medicine as cooling, bitter, astringent, aphrodisiac, narcotic, and diuretic (agent/base) and is useful in pulmonary tuberculosis, asthma, menstrual disorders, swelling wounds, ulcers, real and vesicle calculi, and vitiated conditions of kapha and pitta, and also decoction of the plant is used by women as an antifertility agent and also has anticancer activity (Nadkarni, 2008 in Anarthe et al., -oxidation.

Yusuf et al. (2013) discovered that mistletoe leaf, which is widely used in the treatment of hypertension, diabetes, and rheumatism in arid north Nigeria, has antimicrobial and antioxidant properties. Other biological activities identified are antihypertensive effects (Obatomi et al., 1996), antiviral activity of L. parasiticus (Kusumoto et al., 1992), anti-diabetic properties of L. bengwensis (Obatomi et al., 1994), smooth muscle contracting activity (Chantarasomboon et al., 1974), and antioxidant and anti-inflammatory activity of Loranthus longiflorus infested on Casuarina equisetifolia and Ficus religiosa host trees (Chandrakasan, 2013). Chandrakasan and Neelamegam (2011 and 2012) reported the antioxidant activity of Loranthus longiflorus leaf and bark samples on Casuarina equisetifolia and Ficus religiosa host trees. Young-Kyoon Kim et al. (2004) reported on L. tanakae (Loranthaceae) (Joe, 1992), a kind of mistletoe in the Loranthus genus that was exclusively found in Korea and Japan, which grew on the branches of Quercus and Betila species as host trees, and found that the MeOH extract of the species exhibited moderate cytotoxicity against human tumour cell lines in vitro. Medicinal plants are an important source of antioxidants, and natural antioxidants increase the antioxidant capacity of plasma and reduce the risk of certain diseases such as cancer, heart diseases, and stroke (Prior and Cao, 2000). The secondary metabolites like phenolics and flavanoids from plants have been reported to be potent free radical scavengers.

They are found in all parts, such as leaves, fruits, seeds, roots, and bark (Mathew and Abraham, 2006). There are many synthetic 26 antioxidants in use and they have several side effects, such as risk of liver damage and carcinogenesis in laboratory animals (Gao et al., 1999). Antioxidants from natural sources play a paramount role in helping endogenous antioxidants neutralise oxidative stress. Several epidermal, clinical, and experimental studies indicate that plant-based antioxidants can help prevent chronic diseases (Halliwell, 1994; Soler-Rivas and Wichers, 2000). Under some conditions, oxygen involved in the respiratory process can be transformed under some conditions into superoxide anion, hydroxyl radical singlet oxygen, and hydrogen peroxidase (Cimanga et al., 2001).

These reactive oxygen species are implicated in some diseases such as inflammation, cancer, ageing, anemia, degenerative diseases, and atherosclerosis (Gilbanananda and Hussain, 2002). Raphael et al. (2002) have shown that oxidative stress has a role in the elevation of diabetes and related problems. In diabetes, protein glycation and glucose auto-oxidation may generate free radicals in the body, which in turn catalyse lipid peroxidation (Jakus, 2000). Andlauer and Furst (1998) reported that antioxidants are more important in the prevention of human diseases than Antioxidants are often used in oils and fatty foods to retard their auto-oxidation. Hydroxyanisole (BHA) is restricted in foods as it is suspected to be carcinogenic. Therefore, the importance of the search for natural antioxidants has greatly increased in recent years (Jayaprakash et al., 2003). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Burlon and Ingold, 1984). Oxidative stress has been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury, and neurodegenerative disease (Palozza, 1998). The common free radicals are oxygen reactive species (ROS), namely superoxide radical, hydroxyl radical, and peroxyl radical, which can be internally produced by cellular metabolism, inflammation by immune cells, and externally by radiation, pharmaceuticals, hydrogen peroxide, toxic chemicals, smoke, alcohol, oxidised polyunsaturated fats, and cooked foods. Free radicals can cause damage to parts of cells such as proteins, DNA, and cell membrances by stealing their electrons through a process called oxidation. Free radicals may cause heart damage, cancer, and a weak immune system (Feinman, 1988; Esterbauer et al., 1991; Maharaj et al., 2006; Puntel et al., 2006).

Further, a strong relationship between atherosclerosis and acetaldehyde formed from lipid peroxidatin has been reported (Glavind et al., 1992). Most living organisms possess enzymatic defence systems against excess production of reactive oxygen species (Willet, 1994; Olalye and Rocha, 2007). However, different external factors such as smoke, diet, alcohol, drugs, and ageing could decrease the capability of such protective systems, resulting in disturbances of the redox equilibrium that is established in healthy conditions. Therefore, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and/or the propagation of oxidative systems, resulting in the disturbances of redox-equilibrium that are established in healthy conditions.

Therefore, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and or propagation of oxidative diseases. Besides the traditional resources used for antioxidants, many plant species have been investigated in the search for natural antioxidants (Banias et al., 1992). The discovery of "taxol" in the bark of the Pacific Yew tree stimulated interest in antioxidants from woody plants and other medicinal plants as anticancer agents. Compared with wood or leaves, bark is the most economical and convenient resource for the extraction of possible antioxidant compounds. Previous studies have focused on the isolation and identification of chemical compounds from bark and have found polyphenol compounds. The medicinal effects of plants are often attributed to the antioxidant activities of the phytochemical constituents, mostly the phenolics (Adimiluyi and Oboh, 2008).

Antioxidants are also compounds that scavenge reactive oxygen species, which may be of great value in preventing the onset and or propagation of oxidizing chain reactions. Of late, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have more antioxidant activity than vitamins-C, E, and beta-carotene (Vinson et al., 1998; Haslam, 2006). The antioxidative effects of natural phenolic compounds in pure forms or in their extracts from different model systems of antioxidation have been reported (Gassani et al., 1998). Therefore, antioxidants, which can neutralise free radicals, may be of central importance in the prevention of carcinogenicity and cardiovascular and neurodegenerative changes associated with ageing (Halliwell, 1995; Yu, 1994; Felter and Lloyd, 2008).

Epidemiological studies show that the consumption of plants can protect human beings against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames et al., 1993; Chu et al., 2002; Materska and Perucka, 2005). 29 The free radical species contain unpaired electrons. The oxygen radicals,including superoxide radical (O2-), hydroxyl radical (OH-) and non-free radical species, such as H2O2 and singlet oxygen (O2), are various forms of activated oxygen (Gulcin et al., 2002; 2003; Yildirim et al., 2001), generated in many redox processes. These radicals are trapped and destroyed by specific enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase. Overproduction of free radicals, together with A, C, and E avitaminosis and a reduced level of the above mentioned enzymes, is considered to be the main contributor to oxidative stress (Ellnain-Wojtaszek et al., 2003). Besides, excessive generation of ROS, induced by various stimuli and which exceeds the antioxidant capacity of the organism, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity, and cancer (Kourounakis et al., 1999; Gulcin et al., 2002; 2003). The increase of antioxidant activity for each plant related to increasing plant extract concentration could be attributed to the presence of antioxidants, especially phenols (Matusfujii et al., 1998; Chu et al., 2002).

Numerous studies have conclusively shown that the majority of the antioxidant activity may be from compounds such as flavonoids, catechins, and isocatechins (Martin et al., 2004; Materska and Perucka, 2005). The total phenolic (1.031%w/w) and flavonid (0.024mg/g) content of D. falcate plays a major role in controlling antioxidants (Yerra et al., 2005; Anarthe et al., 2008). The methanol extract (200 m/ml) showed good antioxidant activity as compared to the aqueous extract of Dendrophthoe falcata (Anarthe et al., 2008). The Dendrophthoe falcata ethanol extract (DFEE) possesses potent antioxidant activity by inhibiting lipid peroxidation, reducing glutathione, superoxide dismutase levels and increasing catalase activity (Pattanayak and Sunitha, 2008). Dendrophthoe falcata stem extracts exhibit potent free radical scavenging and antioxidant activity that might be attributed to their polyphenolic content and other phytochemical constituents. The stem of Dendrophthoe falcata stem could be a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing or slowing the progress of ageing and age-associated oxidative stress-related degenerative diseases. It is also suggested that the presence of phytoconstituents like quercetin can be attributed to the antioxidant property of the plant (Dashora et al., 2011).

Over the last two decades, an abundance of research evidence has accumulated on the causes and consequences of oxidative stress caused by the excessive production of free radicals in biological systems (Aruoma, 2003). Free radicals having one or more unpaired electrons are produced in normal or pathological cell metabolism. Reactive oxygen species react easily with free radicals to become radicals themselves (Halliwell, 1995; Squadriato and Peyor, 1998; Yildrim et al., 2001; Gulcin et al., 2002; 2003). Excessive generation of ROS leads to a variety of pathophysiological processes. As a result of this, much attention has been focused on the use of antioxidants, especially natural antioxidants, to inhibit free radicals and protect cellular damage. The mechanism of action of most of the phytochemical components like phenolics, ascorbic acid, and carotenoids in overcoming oxidative stress has been ascribed to their radical scavenging potential (Yan et al., 1999; Nakatani et al., 2000; Baderschneider and Winterhalter, 2001; McCarthy, 2004; Franke et al., 2004; Garcia-Alonso et al., 2004; Kuti, 2004; Zhang and Hamauzu, 2004).

Natural antioxidants are primarily found in plant phenolics, which can be found in all parts of the plant (Osawa, 1999; Mathew and Abraham, 2006). Recently, many attempts have been made to review different in vitro models for estimating the antioxidant properties of natural products from medicinal plants. To test the hypothesis that the higher antioxidant potential of hemiparasitic plants is due to the sequestration of phenolic compounds from the host plants, samples of Dendrophthoe falcata, a hemiparasite collected from different hosts, were investigated by Ramachandra et al. (2005) for total phenolics, total flavonoids, and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity. According to Ramachandra et al. (2005), the hosts significantly influenced the phenolic content of the hemiparasite.

However, similar influences were not detected on radical scavenging activity, and no correlation was found between phenolics and free radical scavenging activity. Furthermore, they reported that the host had significantly affected total phenolics and total flavonoids in a hemiparasite. The DPPH radical scavenging and nitric oxide scavenging activities of D. falcate methanolic extract have an IC50 value of 179.67 and 33.43, respectively (Anarthe et al., 2008). Ahmed and Rocha (2009) reported that the DPPH radical scavenging activity of the L. europaeus plant water extract ranged from 10–61.8% at a concentration of 2.5 to 50 ug/ml.

Free radical scavenging activity of Loranthus longiflorus infested with Casuarina equisetifolia and Ficus religiosa host trees (Chandrakasan and Neelamegam, 2011; Chandrakasan, 2013). A high correlation between free radical scavenging and the phenolic content has been reported in cereals (Peterson, 2001) and fruits (Gao, 2000). Halliwell et al. (1981) and Ahmed and Rocha (2009) suggested that the extracts by hydrogen and/or electron donation might prevent reactive radical species from reaching biomolecules such as lipoproteins, poly-unsaturated fatty acids (PUFA), DNA, amino acids, proteins, and food systems.

### 2.10 Cytotoxicity Studies

Plants have long been known to be beneficial in the treatment of various types of diseases in humans. As per WHO, about 80% of the world's population problems should be treated by medicinal herbal drugs for their primary health care (Etkin, 1981; WHO, 2003). Plants have a long history of being used in the treatment of cancer. Active constituents of various medicinal plants have been used in the treatment of advanced stages of various malignancies (Eva et al., 2006).

There are various medicinal plants reported to have anti-cancer as well as anti-inflammatory activity in the Ayurvedic system of medicine. Researchers and pharmaceutical companies use cytotoxicity studies to investigate the toxicity of compounds or extracts on cellular systems. Cytotoxicity assays provide a rapid, sensitive, and validated approach to quantify harmful dose ranges of compounds and to analyse the biological effects of toxicity on living plant or animal cellular systems. The toxicity of each extract was determined in both prokaryotic and eukaryotic cells. Various toxicity assays were reported both in plant and animal cells as microbial assays and allelopathic assays.

### 2.11 Antimicrobial activity study

Osadebe and Akabogu (2006) reported that various solvent extracts of Loranthus micranthus exhibited varying degrees of antimicrobial activity. Only the petroleum ether extract showed antifungal activity. The methanol extract showed the best antimicrobial activity against Escherichia coli and Bacillus subtilis. Dashora et al. (2010) report the presence of phytochemicals like carbohydrates, phytosterols, flavonoids, glycosides, and phenolic compounds in the Dendrophthoe falcata. The antioxidant activities of Dendrophthoe falcata methanol and aqueous stem extracts were assessed by Dashora et al. (2011). According to them, the Dendrophthoe falcata stem extracts exhibit potent free radical scavenging and antioxidant activity.

They suggested that the overall antioxidant activity might be attributed to its phytochemical constituents and that the plant could be a potential source of natural antioxidants that could have great importance as therapeutic agents in preventing or sowing the progress of ageing and age-associated oxidative stress-related degenerative diseases. Staphylococcus aureus, a gram-positive cocci responsible for infections of the skin and respiratory tract, food poisoning, and toxic shock; Salmonella choleraesuis, a gram-negative facultative aerobe responsible for food poisoning; Pseudomonas aeruginosa, a gram-negative rod that causes infections in wounds, sores, and boils (Orji et al., 2013); and Escherichia coli, the common causative agent of travelers' diarrhoea infections in man (Cowan, 1999a; 1999b). Eukaryotic cells like Aspergillus species and Penicillium species are causative agents of infectious diseases such as candidiasis, respiratory mycosis, vaginosis, pelvic inflammatory diseases, etc. (Cowan, 1999a; 1999b).

The plant powder and some of its solvent fraction have established some significant antibacterial properties by Loranthus micranthus, though with negligible anti-fungal activity (Osadebe et al., 2008; Ukwueze et al., 2012). Ukwueze et al. (2013) tested the methanol leaf extracts of Loranthus micranthus harvested from Persea americana host pant against Staphyllococcus, Pseudomonas, and E. coli and noted higher antibacterial activity with higher zones of inhibition of their growth. Antibacterial activity in Loranthus micranthus may be due to synergy among such constituents as tannins, flavanoids, terpinoides, and saponin (Ukwueze et al., 2013).

The hydroxyl groups in phenol are thought to be responsible for its use as an antimicrobial agent (Ademiluyi and Oboh, 2008; Ogundare and Onifade, 2009). Yusuf (2013) reported that a parasitic plant might have absorbed pharmacological active compounds into their system through their haustorium and the presence of phytochemicals like phenols, alkaloids, saponins, flavanoides, terpinoids, and phylates has been found to confer antimicrobial properties on the parasitic plant. Allelopathic studies

## 2.12 Allelopathic Studies

The term allelopathy is from the Greek-derived compounds allelo and pathy (meaning "mutual harm" or "suffering") and was first used in 1937 by Austrian scientist Hans Molisch in the book Der Einfluss einer Pflanze auf die andere-Allelopathie (The Effect of Plants on Each Other) (Willis, 2010). First widely studied in forestry systems, allelopathy can affect many aspects of plant ecology, including occurrence, growth, and plant succession; the structure of plant communities; dominance, diversity, and plant productivity. Initially, many of the forestry species evaluated had negative allelopathic effects on food and fodder crops, but in the 1980s, research began to identify species that had beneficial, neutral, or selective effects on companion crop plants.

Early research grew out of observations of poor regeneration of forest species, crop damage, yield reductions, replanting problems for tree crops, the occurrence of weed-free zones, and other related changes in vegetation patterns. Commonly cited effects of allelopathy include reduced seed germination and seedling growth. Like synthetic herbicides, there is no common mode of action or physiological target site for all allelochemicals. However, known sites of action for some allelochemicals include cell division, pollen germination, nutrient uptake, photosynthesis, and specific enzyme function.

For example, one study that examined the effect of an allelochemical known in velvetbean, 3-(3',4'-dihydroxyphenyl)-lalanine (l-DOPA), indicated that the inhibition by this compound is due to adverse effects on amino acid metabolism and iron concentration equilibrium. Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals, such as phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone.

Furthermore, physiological and environmental stresses, pests and diseases, solar radiation, herbicides, and less than optimal nutrient, moisture, and temperature levels can also affect allelopathic weed suppression. Different plant parts, including flowers, leaves, leaf litter, and leaf mulch, stems, bark, roots, soil, and soil leachates and their derived compounds, can have allelopathic activity that varies over a growing season. Allelopathic chemicals, or allelochemicals, can also persist in soil, affecting both neighbouring plants as well as those planted in succession. Although derived from plants, allelochemicals may be more biodegradable than traditional herbicides, but allelochemicals may also have undesirable effects on non-target species, necessitating ecological studies before widespread use. The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds. To demonstrate allelopathy, plant origin, production, and identification of allelochemicals must be established, as well as persistence in the environment over time in concentrations sufficient to affect plant species.

In the laboratory, plant extracts and leachates are commonly screened for their effects on seed germination, with further isolation and identification of allelochemicals from greenhouse tests and field soil, confirming laboratory results. Interactions among allelopathic plants, host crops, and other non-target organisms must also be considered. Furthermore, allelochemistry may provide basic structures or templates for developing new synthetic chemicals. From the above literaterature collected, it is conformed that there is no work reported on phytochemical and bioactive properties of the hemiparasitic mistletoe plant, Dendrophthoe falcata, infested on Artocarpus heterophyllus host tree.

**2.13 Control methods to control parasite *Loranthus* weed**

The recommended management strategy for mistletoes is the manual cutting of infested branches or complete removal of the severely infested parts as cutting suffers from the practical difficulty of removing the penetrated subepidermal haustoria from the host and the ability of the parasite to re-emerge from any left-over portion (Perry 1995; Torngren et al. 1980). Alternate strategies include foliar spray of herbicides such as 40% diesel emulsion or 2,4-D (Baillon and Frochot 1987; Vidhyasekaran 2004) or glyphosate (Baillon et al. 1988) and a practically difficult procedure of basal injection with a mixture having seven parts of $CuSO_4$ and one part of 2,4-D (Prakash 2004). Hawksworth and Wiens (1996) review a series of tests from the 1970s to early 1990s with a number of herbicides and growth regulators, including Dacamine, MCPA, Butyrac, Goal, Thistrol, D-40, Weedone, Emulsamine, DPX, Prime and 2,4,5-T. Although these chemicals cause shoot mortality with minimal host injury, they fail to kill the endophytic system.

Experiments to date with systemic chemicals are inconclusive (Shamoun and DeWald 2002). Ethephon is a ripening hormone that acts by releasing ethylene that, in general, leads to early abscission of flowers, fruits, and shoots; complete defoliation at concentrations above 100 ppm; and still higher concentrations question the viability of plants (Schott and Walter 1991). Previous efforts to contain the mistletoes with ethephon or ethephon-releasing chemicals such as Florel (Robbins et al. 1989; Perry 1995; Zhiwei et al. 1995; Begho et al. 2007) have resulted in regeneration within variable periods. Mechanical cutting and foliar spray with either 40% diesel or 1% ethephon are the prevailing farmers' practises in India. However, all these procedures fail to cause total mortality of the parasite, leading to re-emergence within 6–12 months.

Although the over-or under-abundance of mistletoe is addressed with short-term solutions such as pruning, this requires an ecosystem approach that involves both direct and indirect causes of the current status (Norton and Reid 1997). Feasibility for genetic manipulation for incorporating the host resistance is also being explored. Because trees and mistletoes have coevolved for 25 million years, we can expect trees to have developed genetic resistance (Roth 1978).Resistance for D. Mathew and P.V. Habeeburrahman  Broadleaf mistletoe is observed in pear, Chinese pistachio, crapemyrtle, ginkgo, sycamore, and conifers such as redwood and cedar (Perry 1995), and heritability of this resistance is also successfully demonstrated (Scharpf et al. 1992). Furthermore, the mistletoe resistance in poplars is understood to be offered by the higher status of flavonoids (Harari et al. 1999). Though not under practice, the possibility of Loranthus management through biological methods such as release of oligophagous insects (Mushtaque and Baloch 1979) or fungi (Hawksworth and Geils 1996) has also been detailed.

However, none of these mechanical, biological, chemical, or genetic measures under practise at present offers complete eradication of the infestation but merely assists in temporary growth suppression or decreased biomass (Aly 2007). The initial experiments on mistletoe management were carried out during 2005–2007 in the mango progeny orchard maintained at Krishi Vigyan Kendra (Kerala Agricultural University, India) to test the hypotheses that higher and repeated application of known herbicides and growth retardants can induce parasite mortality. Overcoming the factors that hinder the systemic action of these agents should be identified and alternatives should be formulated. Further confirmatory On Farm Tests (OFTs) were performed in the mango orchards of 15 selected farmers in Malappuram District, Kerala State, India.

The effects of various mechanical and chemical treatments on the eradication of mistletoes from mango trees, in terms of time taken for defoliation, extent, and time taken for regeneration, are presented in Table 1. The conventional practise of mechanical removal by cutting from the base has led to the re-emergence of 76.8% of mistletoes within 3.2 months of the treatment. This condition necessitates a reliable intervention leading to the complete eradication of the parasite with no negative effects on the host. It is already shown that mere hand pulling or cutting of mistletoes has only temporarily suppressed the exterior parasitic biomass, but the competition for the synthates remained unaltered, leading to a possible more intensified absorption by parasites to compensate for the necessitated regenerative growth (Glatzel 1983).

As an alternate, removal of infested branches is recommended, but when the infestation is limited, removal of the entire branch may be less cost-effective (Torngren et al. 1980). Foliar application of 1% ethephon has resulted in complete defoliation of mistletoes. On doubling the concentration, even the death of immature and greenish stems was observed. But in both cases, the parasite re-emerged within 5.5 months of hormonal application with no difference in re-emergence levels. Though these methods failed to eradicate the pest completely, they helped in inducing a temporary dormancy, as reported by Perry (1995). Drying of immature stems with 2% ethephon has hinted that a higher concentration, if continuously used, may kill the parasite completely.

This assumption was supported when foliar spray with 2% ethephon followed by a second spray on twigs at a one-month interval delayed the reemergence further by 5.8 months and the percent re-emergence has come down to 42.1%. The latter, upon the initiation of reemergence, was proved to be promising to ensure complete mortality of the pest and hence is recommended for commercial practice. The complete eradication of mistletoes by two consecutive ethephon foliar sprays shows that the timing of the spray is much more important than the concentration. Furthermore, the organ of perception of ethephon is leaves, not twigs. A second spray at a critical stage, when the parasite is all set for second growth, results in complete mortality. Studies on this are published in Archives of Phytopathology and Plant Protection 5Downloaded by [Kerala Agricultural University] at 21:04 on October 16, 2012 Numerous mistletoe-host combinations (Livingston and Brenner 1983; Livingston et al. 1985; Nicholls et al. 1987; Frankel and Adams 1989; Hawksworth and Johnson 1989; Robbins et al. 1989; Parks and Hoffman 1991; Johnson 1992; Adams et al. 1993) clearly show that its foliar application is not effective in bringing the complete mortality of the parasite and re-emergence happens after a definite period. With good control, mistletoe seed production is delayed by 2–4 years, but not a permanent cure. However, none of the previous workers reported the effect of two consecutive sprays leading to complete parasitic mortality. Foliar application with 40% diesel emulsion has caused only a partial defoliation (60%) in mistletoes.

A higher concentration (60%) of diesel was useful in complete defoliation. But both these treatments were incapable of complete eradication of the parasite since definite re-emergence was recorded, even though the higher concentration delayed the re-emergence from 4.8 to 7.5 months and the time taken for defoliation was reduced from 4.5 to 2.3 days. Though cheaper than ethephon, diesel has yielded similar results and two foliar applications at 60% level, followed by re-emergence, have resulted in the complete death of the parasite.

The plant killing power of diesel emulsion is commercially exploited for the removal of early emerged suckers in banana (Mathew and Habeeburrahman 2010) and this principle is used here also.

The complete death of mistletoes on repetition of foliar spray at reemergence has confirmed the previous observation that the time of application is more important than the concentration of the herbicide, and re-emergence is a critical stage, and leaves are better receptors for diesel than twigs. Foliar application of 2,4-D at 1, 2 and 5% concentrations has resulted in a proportionately higher percentage of leaf drying (20–30, 50–60, and 80–100), but the growth was unaffected. An increase in concentration was found to result in proportionately quicker leaf drying, and with 5% 2,4-D, leaf drying was completed in just 5 days. 2,4-D is an accepted strong herbicide which is commercially used worldwide (Audus 1964). The speed and level of drying have shown that doses as high as 5% are toxic for the parasite and suggest the formulation of better administration.

Foliar sprays with several herbicides including ethephon, glyphosate, paraquat dichloride, and polyborate exhibited little or no long-term efficacy in mistletoe management on pecan trees, but 2,4-D gave temporary growth suppression, as we have seen in this experiment (Wood and Reilly 2004). The dimethylamine salt of dicamba killed mistletoe but exhibited a potential for harming host trees. They also suggested that the dormant season treatment of mistletoe clusters with 2,4-D reduced photosynthesis by about one-third soon after the treatment, and spot treatment of mistletoe clusters with 2,4-D (0.24%) 2 weeks before bud break gave effective long-term (not permanent) control of mistletoe. However, treatment before bud break is not applicable under Indian tropical conditions since there is no winter dormancy and plants are evergreen.

Foliar application with 5 ppm ABA has only caused leaf yellowing and defoliation. Re-emergence was almost complete (86.7%) just within 3.4 months. On doubling the concentration, ABA resulted in quicker defoliation but no better than 5 ppm with respect to re-emergence. Abscisic acid is a proven plant growth regulator that results in cellular abscission in plants even at very low in vivo concentrations (Addicot et al. 1968). The quicker re-emergence coupled with high cost and non-availability in the market ruled out the possibility of suggesting ABA for commercial use. In normal cases, plant hormones used as herbicides act systematically in accelerating the essential plant physiological processes, leading to sudden senescence 6 D. Mathew and P.V. Habeeburrahman [Kerala Agricultural University] downloaded at 21:04 on October 16, 2012, and died as a result.A microscopic examination of mistletoe stems revealed the reason behind the inefficiency of foliar sprays in effecting the complete parasitic mortality even at high concentrations. In ordinary dicots or monocots, the foliar applied hormones or chemicals will be absorbed through stomata and transported to the entire plant system through phloem. In this parasite that absorbs food from the host and rarely translocates the meagre synthates from the leaf, evolution has resulted in the weakening of phloem vessels, only into discrete bundles as seen in the species under study.

Keeping this fact in mind, in the present study, xylem vessels were successfully used in the management of Loranthus by adopting the base banding technique. Complete leaf drying followed by mortality of mistletoes was achieved within 15–20 days of treatment. Banding done at the middle of the parasite has shown that only the portion above the band has died and portions below the band were in no way affected, showing that there is no backflow of the herbicide (Figure 1). This suggests that the banding should be as close as possible to the point of attachment to the host. Enhancing the concentration of 2,4-D to 2% has resulted in the quicker drying and death of the parasite within 10 days. Among the chemicals used in this study, only diesel was cheaper than 2,4-D, and hence the feasibility of parasite killing by diesel base banding was also attempted. Banding with 60% diesel has resulted only in 20% leaf drying, even after 15–20 days of treatment, and hence was not promising.

The absence of phloem in the stems of mistletoes was first reported by Calvin (1967). Menzies (1954) also observed that the haustorial strands of Loranthus develop a few indistinct phloem elements but that these are crushed before they reach maturity. Exploitation of this feature by base banding has saved the trouble of re-emergence and opened a reliable but simple path for quick and complete eradication of mistletoe parasitism from mango and other perennial trees. In comparison with the mechanical measures, this methodology is much less labour intensive, and in comparison with the most appropriate chemical measures, the cost of management is at least halved, making it efficient on a commercial scale. Banding the stem base with 1% 2,4-D is an efficient single-step methodology for the complete eradication of mistletoe from perennial fruit trees.

Furthermore, this strategy is more practical at the farmer's level since 2,4-D is a cheaper herbicide and no costly equipment such as sprayers is required. Furthermore, the removal of bark and winding with herbicide soaked cloth are less labour intensive and, above all, offer total mortality with a single treatment. Two consecutive foliar sprays with either 1% ethephon or 60% diesel with the second spray on the re-emergence of fresh leaves is also effective but demands two seasons’ treatment.

# CHAPTER-III

## MATERIALS AND METHODS

### 3.1 Survey and Collection

We have surveyed the incidence of *Loranthus* in the citrus growing region of East Siang District, Arunachal Pradesh. The citrus growing regions range from 150 MSL to 500 MSL. The location of the study site represents a subtropical, hot and humid climate.

### 3.2 Weather data collection

We have collected the weather data for the location during the study period. During the summer season (June, July, and August), temperatures usually rise to about 30 °C in the lower valleys. In general, temperatures in winter (December, January, and February) generally fall to 13°C. Between April and September, annual rainfall in the center of the state averages about 130 inches (3300mm). The weather data is as follows –

**Table: 3.1 Weather data**

| | Jan-22 | Temperature | | **Relative humidity (%)** | | Rainfall (mm) |
|---|---|---|---|---|---|---|
| | **Date** | MAX(°C) | MIN(°C) | 6:00 AM | 01:00 | |
| | | 06:00 | 06:00 | | Pm | |
| 1 | 1 | 13 | 17 | 69 | 70 | 0 |
| | 2 | 15 | 19 | 86 | 80 | 0 |
| | 3 | 15 | 19 | 84 | 80 | 0 |
| | 4 | 16 | 15 | 69 | 71 | 0 |
| | 5 | 15 | 14 | 84 | 79 | 0 |
| | 6 | 16 | 15 | 59 | 61 | 0 |
| | 7 | 17 | 16 | 61 | 61 | 0 |
| 2 | 8 | 17 | 14 | 61 | 61 | 0 |
| | 9 | 16 | 15 | 59 | 61 | 0 |
| | 10 | 15 | 17 | 89 | 89 | 0 |
| | 11 | 17 | 16 | 70 | 70 | 0 |
| | 12 | 15 | 16 | 89 | 89 | 14.3 |
| | 13 | 15 | 16 | 89 | 89 | 2 |
| | 14 | 17 | 16 | 70 | 70 | 0 |
| 3 | 15 | 15 | 16 | 70 | 70 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 16 | 17 | 16 | 70 | 53 | 0 |
| | 17 | 16 | 15 | 69 | 53 | 0 |
| | 18 | 16 | 15 | 69 | 53 | 0 |
| | 19 | 17 | 16 | 52 | 53 | 0 |
| | 20 | 15 | 14 | 58 | 70 | 0 |
| | 21 | 15 | 11 | 68 | 70 | 0 |
| 4 | 22 | 15 | 16 | 69 | 69 | 18 |
| | 23 | 19 | 17 | 63 | 69 | 0 |
| | 24 | 17 | 16 | 80 | 61 | 0 |
| | 25 | 19 | 17 | 81 | 89 | 0 |
| | 26 | 15 | 13 | 68 | 68 | 7.6 |
| | 27 | 15 | 14 | 89 | 57 | 7.7 |
| | 28 | 15 | 14 | 68 | 64 | 0 |
| 5 | 29 | 14 | 13 | 57 | 54 | 0 |
| | 30 | 15 | 16 | 69 | 69 | 0 |
| | 31 | 16 | 15 | 59 | 43 | 0 |
| | Total | | | | | **49.6** |
| | AVERAGE | **15.93** | **15.33** | **70.4** | **67.2** | **1.6** |
| | | | | | | |
| | Feb-22 | Temperature(°C) | Temperature(°C) | **Relative humidity(%)** | | Rainfall(mm) |
| | Date | MAX | MIN | 06:00 | 01:00 | |
| | | 06:00 | 06:00 | | | 06:00 |
| | 1 | 16 | 15 | 69 | 89 | 3.07 |
| | 2 | 15 | 14 | 78 | 61 | 3.07 |
| | 3 | 16 | 15 | 78 | 42 | 0 |
| | 4 | 17 | 16 | 61 | 71 | 0 |
| 6 | 5 | 14 | 12 | 88 | 89 | 7.69 |
| | 6 | 15 | 14 | 78 | 85 | 3.64 |
| | 7 | 17 | 14 | 78 | 71 | 12.3 |
| | 8 | 16 | 15 | 69 | 66 | 0 |
| | 9 | 17 | 16 | 61 | 62 | 0 |
| | 10 | 17 | 15 | 61 | 62 | 0 |
| | 11 | 16 | 15 | 69 | 70 | 3.07 |
| | 12 | 14 | 12 | 88 | 80 | 6.8 |
| 7 | 13 | 16 | 15 | 78 | 73 | 0 |
| | 14 | 17 | 15 | 65 | 33 | 0 |
| | 15 | 15 | 14 | 68 | 39 | 0 |
| | 16 | 18 | 17 | 53 | 35 | 0 |
| | 17 | 20 | 19 | 57 | 65 | 0 |
| | 18 | 19 | 18 | 72 | 57 | 0 |
| | 19 | 20 | 19 | 64 | 57 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 20 | 20.00 | 19.00 | 64.00 | 57.00 | 0.00 |
| | 21 | 15 | 14 | 78 | 59 | 7.69 |
| | 22 | 18 | 15 | 53 | 59 | 0 |
| | 23 | 15 | 13 | 78 | 64 | 0 |
| | 24 | 17 | 16 | 73 | 47 | 0 |
| | 25 | 17 | 16 | 65 | 47 | 0 |
| | 26 | 20 | 20 | 63 | 41 | 0 |
| | 27 | 20 | 19 | 57 | 65 | 0 |
| 9 | 28 | 18 | 16 | 61 | 58 | 5.8 |
| | Total | 455 | 419 | 1863 | 1647 | 53.13 |
| | Average | 16.85 | 15.52 | 69 | 61 | |
| | | | | | | |
| | | | | | | |
| | Mar-22 | Temperature(°C) | Temperature(°C) | **Relative humidity (%)** | **Relative humidity (%)** | Rainfall(mm) |
| | **date** | MAX | MIN | 06:00 | 01:00 | |
| | | 06:00 | 06:00 | | | 06:00 |
| | 1 | 22 | 20 | 81 | 46 | 0 |
| | 2 | 21 | 20 | 81 | 73 | 0 |
| | 3 | 24 | 20 | 48 | 47 | 0 |
| | 4 | 23 | 20 | 73 | 44 | 0 |
| | 5 | 23 | 23 | 73 | 46 | 0 |
| 10 | 6 | 22 | 19 | 73 | 44 | 0 |
| | 7 | 24 | 23 | 68 | 45 | 0 |
| | 8 | 22 | 19 | 43 | 24 | 0 |
| | 9 | 22 | 19 | 91 | 24 | 0 |
| | 10 | 23 | 21 | 45 | 39 | 0 |
| | 11 | 24 | 22 | 83 | 39 | 0 |
| | 12 | 23 | 22 | 91 | 34 | 0 |
| 11 | 13 | 23.00 | 22.00 | 91.00 | 34.00 | 0.00 |
| | 14 | 23 | 22 | 91 | 32 | 0 |
| | 15 | 24 | 23 | 83 | 24 | 0 |
| | 16 | 24 | 22 | 91 | 39 | 0 |
| | 17 | 24 | 23 | 75 | 39 | 0 |
| | 18 | 24 | 22 | 91 | 39 | 0 |
| | 19 | 25 | 24 | 76 | 36 | 0 |
| 12 | 20 | 25.00 | 24.00 | 76.00 | 36.00 | 0.00 |
| | 21 | 20 | 19 | 91 | 60 | 0 |
| | 22 | 24 | 23 | 68 | 60 | 0 |
| | 23 | 22 | 21 | 91 | 74 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 24 | 22 | 21 | 82 | 75 | 0 |
| | 25 | 20 | 18 | 81 | 75 | 7.7 |
| | 26 | 19 | 18 | 90 | 91 | 9.2 |
| 13 | 27 | 20 | 19 | 73 | 74 | 30.8 |
| | 28 | 23 | 22 | 67 | 74 | 0 |
| | 29 | 22 | 21 | 82 | 60 | 0 |
| | 30 | 23 | 21 | 83 | 75 | 0 |
| | 31 | 21 | 19 | 91 | 83 | 9.7 |
| | | | | | | |
| | Apr-22 | Temperature(°C) | Temperature(°C) | **Relative humidity(%)** | | **Rainfall (mm)** |
| | **date** | MAX | MIN | 06:00 | 01:00 | |
| | | 06:00 | 06:00 | | | |
| | 1 | 20 | 19 | 91 | 91 | 37.4 |
| | 2 | 22 | 21 | 91 | 82 | 18.5 |
| 14 | 3 | 21 | 19 | 81 | 90 | 35.4 |
| | 4 | 20 | 19 | 91 | 91 | 67.54 |
| | 5 | 20 | 19 | 91 | 91 | 25.2 |
| | 6 | 20 | 19 | 91 | 90 | 13.84 |
| | 7 | 21 | 18 | 91 | 91 | 4.1 |
| | 8 | 21 | 20 | 82 | 65 | 9.2 |
| | 9 | 21 | 20 | 82 | 57 | 22 |
| 15 | 10 | 21 | 21 | 82 | 60 | 23 |
| | 11 | 23 | 22 | 75 | 59 | 21 |
| | 12 | 25 | 24 | 68 | 69 | 0 |
| | 13 | 27 | 26 | 70 | 58 | 0 |
| | 14 | 21 | 19 | 91 | 58 | 21.54 |
| | 15 | 22 | 21 | 91 | 60 | 2.46 |
| | 16 | 22 | 21 | 91 | 69 | 18.5 |
| | 17 | 21 | 21 | 91 | 60 | 2.46 |
| 16 | 18 | 24 | 23 | 68 | 49 | 13.9 |
| | 19 | 27 | 26 | 62 | 47 | 0 |
| | 20 | 27 | 26 | 62 | 47 | 0 |
| | 21 | 27 | 26 | 59 | 49 | 0 |
| | 22 | 27 | 25 | 76 | 76 | 0 |
| | 23 | 24 | 23 | 91 | 84 | 10.1 |
| | 24 | 21 | 20 | 91 | 84 | 9.33 |
| 17 | 25 | 21 | 20 | 91 | 83 | 49.23 |
| | 26 | 23 | 21 | 83 | 83 | 116.9 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | 27 | 21 | 20 | 91 | 71 | 13.84 |
| | 28 | 27 | 26 | 63 | 50 | 0 |
| | 29 | 28 | 27 | 57 | 50 | 0 |
| | 30 | 23 | 19 | 83 | 59 | 21 |
| | | 688 | 651 | 2427 | 2073 | 556.44 |
| | | 22.93 | 21.7 | 80.9 | 69.1 | |

### 3.3 Sample collection and botanical research

We have selected 20 trees for our study on three farmers' fields.

### 3.4 Management Techniques

#### 3.4.1 Mechanical strategies:

A. Pruning of infested branches:

i. This is the best & easy method to prevent the spread of *Loranthus* plants.

ii. The affected branches cut before flowering & fruiting of *Loranthus* weed to prevent the spread of seeds from one plant to another by birds..

B. Mechanical removal by hand when there is less infestation before flowering of plant.

We have devised the management strategies and the treatments are mechanical removal with a pruner, spraying with 30% diesel and 60% diesel, and spraying with 2,4-D 1% and 2,4-D 5%. We have selected 40 *loranthus* for each treatment. OPSTAT and SPSS (25) software were used to analyse the data.

**Table: 3.2 Lay out of experiments**

**The each tremaents are replicated thrice in khasi mandarin oranges**

| **Sl.No** | **Treatment** | **Subject** |
|---|---|---|
| **1** | **T1** | Mechanical (removal with pruner) |
| **2** | **T2** | 30% Diesel |
| **3** | **T3** | 60% Diesel |
| **4** | **T4** | 2-4,D 1% |
| **5** | **T5** | 2-4,D 5% |
| **6** | **T6** | Control water |


**Fig: 3.1 Preparation of chemicals**

### 3.4.2. Chemical strategies:

**We have selected the following chemical strategies and the results are presented in table**

A. Spraying of 30 percent diesel.

B. Spraying of 60 percent diesel. Spray of high-speed diesel is also helpful to kill the foliage of this parasitic weed.

*C.* Injecting 1 percent 2, 4-D solution to the *loranthus*

D Injecting 5 percent 2. 4-D solution to the *loranthus.*

**<u>Preparation of 30% per cent diesel</u>**

- Collect water, surf and diesel.
- Take a beaker .
- In 100ml beaker add 70 ml

  water and 30 percent diesel i.e the 30 per cent diesel.
- Add washing powder to the 30 per cent diesel so that the water and diesel mixed up properly.

**<u>Preparation of 60% percent diesel</u>**

- Collect water, surf and diesel.
- Take a beaker .
- In 100 ml beaker add 40 ml

  water and 60 percent diesel i.e the 60 percent diesel.

**<u>Preparation of 1% (percent) 2, 4-D</u>**

- Take 2-4-D, water, and 100 ml beaker.
- Add 1g of 2, 4-D to 100 ml water i.e the 1% (percent) 2, 4-D.

**<u>Preparation of 5% (percent) 2, 4-D</u>**

- Take 2-4-D, water, and 100 ml beaker.
- Add 5g of 2, 4-D to 100 ml water i.e the 5% (percent) 2, 4-D.

# CHAPTER-IV

## RESULTS AND DISCUSSION

### 4.1 Survey and Collection

We have recorded the incidence of Loranthus at five villages and collected the Loranthus partial stem parasite in Khasi mandarin growing region of Arunachal Pradesh. The incidences varied from 2.5 to 30 (Fig 4.1 and Table 4.1).

### 4.2 Weather data collection

After weather data collection, we have noticed that the partial stem parasite loranthus seeds dispersing birds come in the months of June, July and August because in this season loranthus flowers and seeds are more attractive.

### 4.3 Morphological studies of Loranthus

The partial stem parasite, viz., Loranthus, was collected from the infected plot and its morphology was studied. The results are presented in Table 4.1 and Fig 4.1.

**Table: 4.1 *Loranthus ligustrinus* incidence in the Khasi mandarin growing region in each village per tree.**

| Tree number | Village 1 | Village 2 | Village 3 | Village 4 | Village 5 |
|---|---|---|---|---|---|
| 1 | 8 | 7 | 12 | 9 | 13 |
| 2 | 9 | 10 | 10 | 10 | 10 |
| 3 | 10 | 8 | 9 | 8 | 8 |
| 4 | 7 | 9 | 7 | 9 | 10 |
| 5 | 9 | 9 | 9 | 7 | 8 |
| 6 | 8 | 10 | 8 | 11 | 11 |
| 7 | 7 | 8 | 11 | 9 | 8 |
| 8 | 10 | 7 | 9 | 7 | 9 |
| 9 | 9 | 9 | 7 | 9 | 10 |
| 10 | 8 | 7 | 9 | 8 | 8 |
| Total | 85 | 84 | 91 | 87 | 95 |
| Average no of | 8.5 | 8.4 | 9.1 | 8.7 | 9.5 |

| | | | | | |
|---|---|---|---|---|---|
| *Loranthus ligustrinus* per tree | | | | | |

The highest parasitic incidence is noticed in village 5 (9.5/tree) and followed by village 3 (9.1/tree).

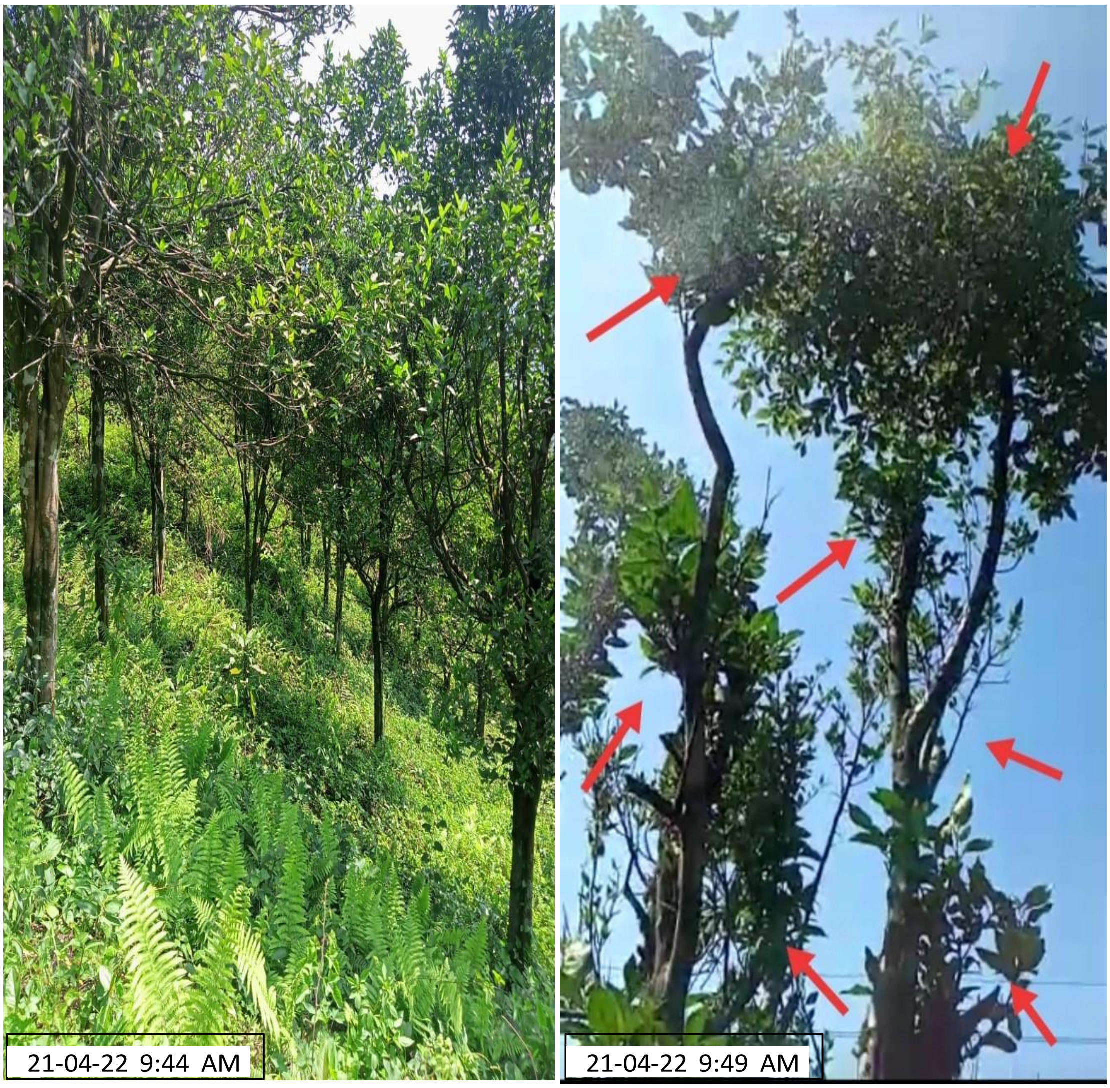


**Fig: 4.1 Incidence of *Loranthus ligustrinus* parasitic weed on Khasi mandarin orange tree.**


**Fig: 4.2 *Loranthus ligustrinus* leaf morphology**

The collected *Loranthus sp is* studied for its morphological characters, which are presented in Table 4.2 and Fig 4.2.

**Table: 4.2 *Loranthus ligustrinus* leaf characters**

| Place of collection site | Leaf colour | Leaf length in(cm) | Leaf breadth in(cm) | Leaf pattern | Leaf arrangement |
|---|---|---|---|---|---|
| 1 | Light green | 8.50 | 1.60 | Small simple leaves, elliptical shape, margin entire, reticulate venation. | opposite |
| 2 | Light green | 4.00 | 1.80 | Small simple leaves, elliptical shape, margin entire, reticulate venation. | opposite |
| 3 | Light green | 5.70 | 3.00 | Small simple leaves, elliptical shape, margin entire, reticulate venation. | opposite |
| 4 | Light green | 4.30 | 2.20 | Small simple leaves, elliptical shape, margin entire,reticulate venation. | opposite |
| 5 | Light green | 5.50 | 2.50 | Small simple leaves, elliptical shape, margin entire, reticulate venation. | opposite |

From the leaf morphological description, we have found that all the leaves are light green in colour and arranged oppositely. Leaf patterns are small simple leaves, elliptical shape, and entire margin with reticulate venation. It ranges from 4.00 to 8.5 cm in length, and leaf breadth varies from 1.60 to 3.00 cm (Table: 4.2).

attenuate at the base to a petiole 1-6 mm long, acuminate and acute or short rounded at the apex; thin, dull on both sides but slightly darker above; venation pinnate with the midrib and the main laterals distinct above and the midrib prominent below.


**Fig: 4.3 *Loranthus ligustrinus* flower morphology.**

**Table: 4.3 *Loranthus ligustrinus* flower characters**

| No of collection | Flower color | Flower size (Length) in cm | Flower Biology | Types of Flower | No. of Androecium | No. of Gynoecium |
|---|---|---|---|---|---|---|
| 1 | Red | 1.3 | Bisexual or unisexual | Actinomorphic | 4 | 1 |
| 2 | Red | 1.2 | Bisexual or unisexual | Actinomorphic | 4 | 1 |
| 3 | Red | 1.4 | Bisexual or unisexual | Actinomorphic | 4 | 1 |
| 4 | Red | 1.3 | Bisexual or unisexual | Actinomorphic | 4 | 1 |
| 5 | Red | 1.5 | Bisexual or unisexual | Actinomorphic | 4 | 1 |

From the flower description, we have found that all the flowers are red, bisexual or unisexual, and actinomorphic. The number of androecium and gynoecium is found to be 4 and 1, respectively (Table 4.3 and Fig.4.3).

raceme; axis 5–20 mm long, flattened upwards; pedicels 0.2-2 mm long, subumbellately crowded near the tip of the axis; bracts triangular, acute, c. 1 mm long. 1—1.5 mm long urceolate ovary; slightly curved calyx limb

spread, entire, c. 0.2 mm long. Corolla in full bloom 4-merous, 5-8 mm long, slightly keeled in the lower part, narrowed above, weakly clavate and obtuse at the apex, red or purple. Anthers are 2-2.5 mm long, obtuse, and 2 to 3 times as long as the free part of the filament. Style 5-7 mm long, reaching to the top of the anthers, conical at the base, uniformly slenderabove, lacking constriction; stigma capitate, about twice as wide as the style..


**Fig: 4.4 *Loranthus ligustrinus* stem morphology**

**Table: 4.4 *Loranthus ligustrinus* stem characters**

| Collection no | No of Haustoria | *Loranthus* stem length in( cm) | No the branch at the tip |
|---|---|---|---|
| 1 | 1 (SINGLE) | 30.00 | 7.00 |
| 2 | 1 (SINGLE) | 32.00 | 6.00 |
| 3 | 1 (SINGLE) | 40.00 | 8.00 |
| 4 | 1 (SINGLE) | 42.00 | 6.00 |
| 5 | 1 (SINGLE) | 35.00 | 7.00 |

We have found that most of the *Loranthus* have single haustoria and the stem length varies from 30 to 42 cm and has 6 to 8 branches at the tip (Table 4.4, fig 4.4).

**4.4 Management methods**

We have formulated several treatment methods to manage the disease. The different methodology is described here. From the management studies, we have found that the treatment results are found to be highly significant. Spraying of sixty percent diesel was found to be more effective followed by 2, 4-D 5%, and 30% diesel. The percent reduction over control for the treatment of 60% diesel, 2, 4-D 5% and 30% diesel is 97.5%, 90% and 87.5% respectively. The average yield per tree was 37.5 kg. Fifty percent reduction in yield was noticed in control. We have also observed that after mechanical treatment the Loranthus are emerging out only if the cutting stem is more than 10 cm. We have observed that in 60% diesel spray, 39 *Loranthus* are dead out of 40. Where as in 5% 2, 4-D stem injection 36 *Loranthus* are died. We have observed that the treated *Loranthus* died completely after 3 weeks and the sum of them survived. We have noticed that 2,4-D 1% stem injection killed only 28 *Loranthus* and 12 survived. Following this, by mechanical method, 31 died and 9 survived (Fig 4.6 to 4.10, Table 4.5).


**Fig: 4.5 Mechanical management practices in *Loranthus ligustrinus***


**Fig: 4.6 30% diesel management practices in *Loranthus ligustrinus***

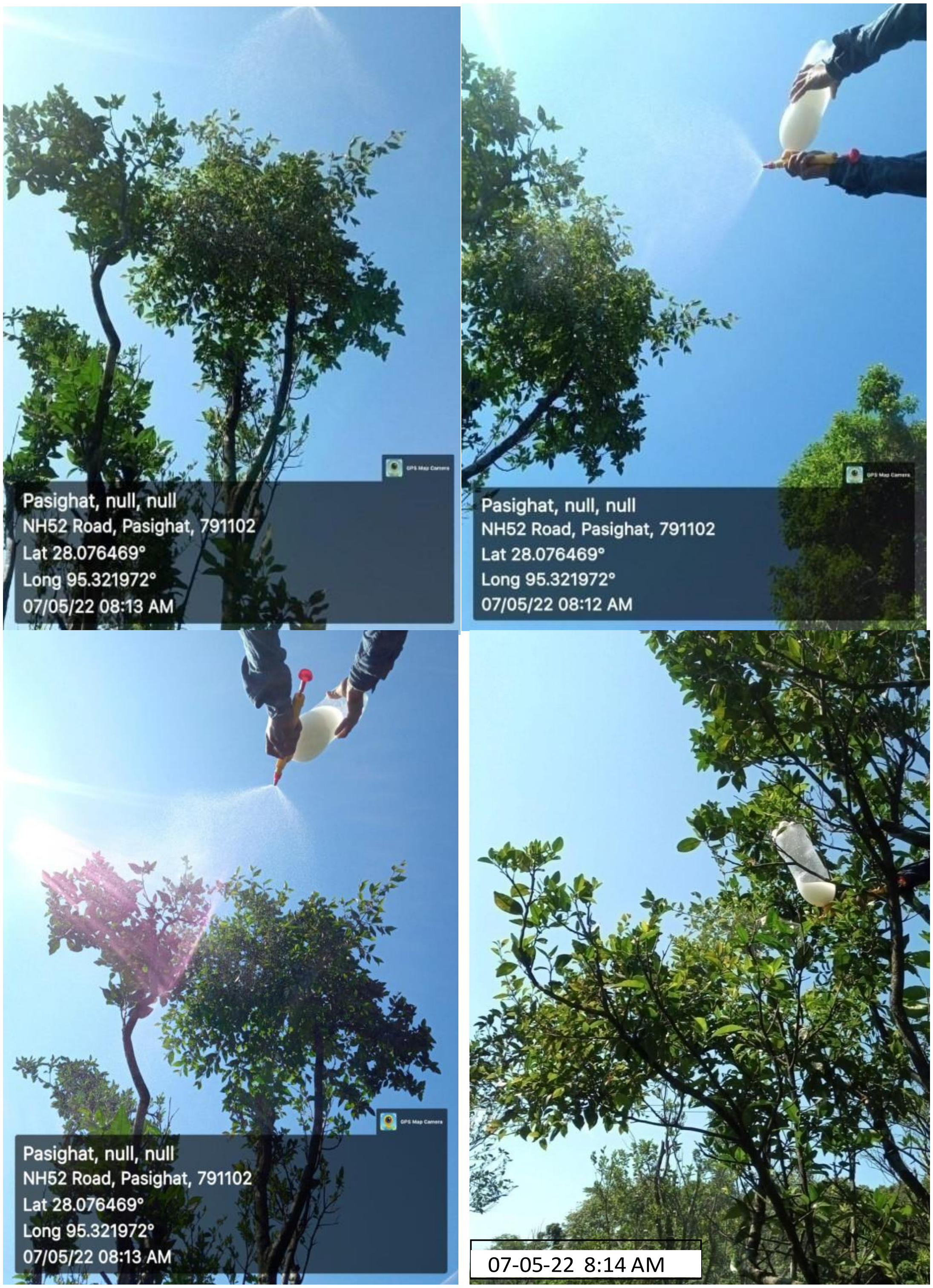


**Fig: 4.7 60% diesel management practices in *Loranthus ligustrinus***


**Fig: 4.8 2-4,D 1% management practices in *Loranthus ligustrinus***


**Fig: 4.9 2-4,D 5% management practices in *Loranthus ligustrinus***

**Table: 4.5 Management of *Loranthus ligustrinus* with various treatments**

| Sl. no | Treatments | No of plants Trt. | Died | Live *Loranthus* | Percentage Disease incidence (after treatment) | PROC | Average yield (kg/ha) |
|---|---|---|---|---|---|---|---|
| 1 | Mechanical | 40 | 31 | 9 | 22.5 | 77.5% | 30.5 |
| 2 | 30% Diesel | 40 | 35 | 5 | 12.5 | 87.5% | 33.8 |
| 3 | 60% Diesel | 40 | 39 | 1 | 2.5 | 97.5% | 36.2 |
| 4 | 2-4,D 1% | 40 | 28 | 12 | 30 | 70% | 38.2 |
| 5 | 2-4,D 5% | 40 | 36 | 4 | 10 | 90% | 34.2 |
| 6 | Control water | 40 | 0 | 40 | 100 | 0 | 15.2 |
| | SEd | CD(P=0.05) | | | CD(P=0.01) | | |
| Treatment | 8.32 | 24.11 | | | 21.34 | | |
| CV | 26.53 | | | | | | |

*We have found that significant diference among the treatment*

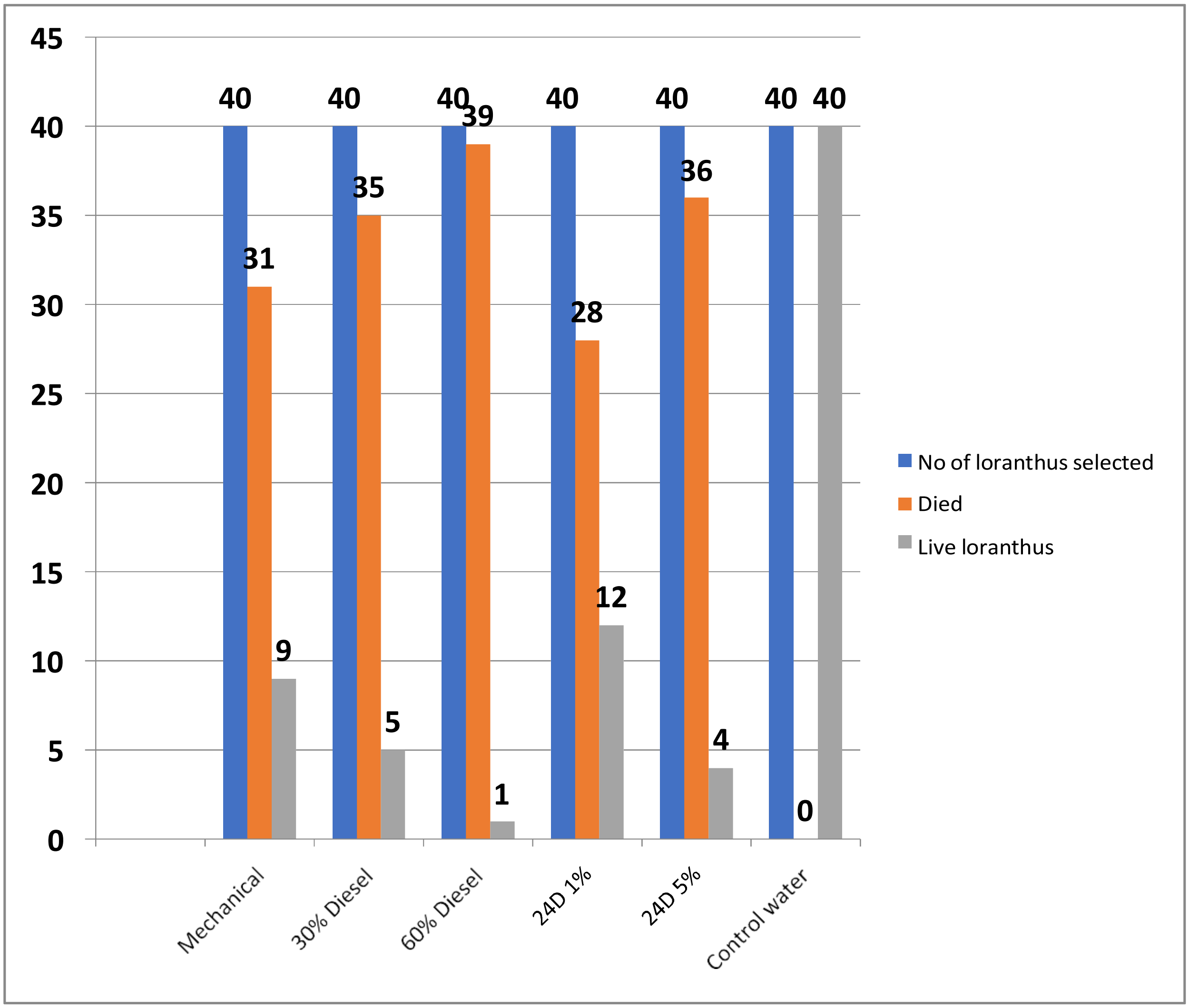


**Fig: 4.10 Column charts of various management practices of *Loranthus ligustrinus* in Khasi mandarin oranges.**

# CHAPTER-V

## SUMMARY AND CONCLUSION

From the survey, we have noticed that village five has a higher incidence of Loranthus. It is mainly due to unhygienic practises in the farmer's field. From the morphological studies, we could confirm that it is Loranthus ligustrinus. There is a significant variation in the two partial stem parasites. The flowering will be observed from May to July. Fruiting will be there in August. The birds are actively involved in the transmission of seeds from August to September.

From the leaf morphological description, we have found that all the leaves are light green in colour and arranged oppositely. Leaf patterns are small simple leaves, elliptical shape, and entire margin with reticulate venation. It ranges from 4.00 to 8.5 cm in length, and its leaf breadth varies from 1.60 to 3.00 cm. From the flower description, we have found that all the flowers are red, bisexual or unisexual, and actinomorphic. The number of androecium and gynoecium is found to be 4 and 1, respectively. We have found that most of the Loranthus have single haustoria and the stem length varies from 30 to 42 cm and has 6 to 8 branches at the tip.

From the management studies, we have found that spraying of sixty per cent diesel was found to be more effective, followed by 2,4-D 5%, and 30% diesel. The percent reduction over control for the treatment of 60% diesel, 2, 4-D 5% and 30% diesel is 97.5%, 90% and 87.5% respectively. The average yield per tree was 37.5 kg. A fifty-percent reduction in yield was noticed in control. We have also observed that after mechanical treatment, the Loranthus emerges only if the cutting stem is more than 10 cm. We have observed that in 60% diesel spray, 39 Loranthus are dead out of 40. In the 5% 2, 4-D stem injection group, 36 Loranthus died. We have observed that the treated Loranthus died completely after 3 weeks and the sum of them survived. We have noticed that 2,4-D 1% stem injection killed only 28 Loranthus and 12 survived. The results are confirmed with the findings of Hazarika, B.N. and Singh, S.R.(2013), Singh, S.R., et al (2016), and Singh, K.M., et al (2013). Loranthus stem injection with 2,4-D 5% is superior in killing the loranthus in 3 weeks. When the Loranthus was sprayed with 60% diesel, 39 plants died, representing a 97.5% reduction over the control. Similar studies were conducted by Baillon and Frochot 1987; Vidhyasekaran 2004; and Audus 1964; a 50% reduction in yield was noticed if there was management. Hence, it is recommended to manage the loranthus by 60%. Here is the conclusion of our studies for managing the loranthus partial stem parasite. Using diesel spray or 5% 2,4-D loranthus stem injection is the best method to manage the disease.

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