PHYSIOLOGICAL AND MOLECULAR STUDIES ON GENERA OF LORANTHACEAE AND THEIR MANAGEMENT

by

Garggi G.

(2015-21-011)

DEPARTMENT OF PLANT PHYSIOLOGY
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KERALA, INDIA

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THESIS

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DEPARTMENT OF PLANT PHYSIOLOGY
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VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled "***Physiological and molecular studies on genera of Loranthaceae and their management***" is a bonafide record of research work done by me during the course of research and that this thesis has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "***Physiological and molecular studies on genera of Loranthaceae and their management***" is a record of research work done independently by Mrs. Garggi G. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Garggi G.

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ABBREVIATIONS

%	Percentage
@	At the rate
>	Greater than
^{o}C	Degree Celsius
ΔCt	Threshold cycle
µg	Microgram
µl	Microlitre
µM	Micromole
mgL^{-1}	Milligram perlitre
2,4-D	2,4-dichloro phenoxy acetic acid
^{32}P	Radio labelled Phosphorous
A	Assimilation
ANOVA	Analysis of Variance
APG	Angiosperm Phylogeny Group
BLAST	Basic Local Alignment Search Tool
bp	Basepair
$cpmg^{-1}$	Counts per minute per gram
cDNA	complementary DNA
C_i	Interstitial carbon

Ci	Curie
CO_2	Carbon dioxide
CPBMB	Centre for Plant Biotechnology and Molecular Biology
CRD	Completely Randomised Design
CRS	Cocoa Research Station
CTAB	Cetyl trimethyl ammonium bromide
D	*Dendropthoe falcata*
DEPC	Diethylpyrocarbonate
DPX	Dibutylphthalate Polysterene Xylene
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyribo NucleosideTriphosphates
DW	Dry weight
eATP	extracellular Adenosine Thio Phosphate
E	East
ER	Epicortical root
EDTA	Ethylene Diamine Tetra Acetic acid
EtBr	Ethidium Bromide
FAA	Formalin- Acetic acid -Alcohol solution
Fig	Figure
FW	Fresh Weight
G	Gram

H	*Helicanthus elastica*
HCl	Hydrochloric acid
ICAR	Indian Council of Agricultural Research
IRGA	Infra-Red Gas Analyser
ISSR	Inter Simple Sequence Repeat
KAU	Kerala Agricultural University
kb	kilobase
L	Litre
M	Molar
MEGA	Molecular Evolutionary Genetics Analysis
Mg	Milligram
min	Minutes
ml	Millilitre
mM	MilliMole
N	North
Ng	Nanogram
NaCl	Sodium Chloride
NCBI	National Centre for Biotechnology Information
nm	Nanometre
NPE	Nonyl Phenoxy ethoxylate
NS	Not significant
OD	Optical Density

OS	Organosilicone
PAR	Photosynthetically Active Radiation
PCR	Polymerase Chain Reaction
pH	Hydrogen ion Concentration
ppm	parts per million
PVC	Poly Vinyl Chloride
PVP	Poly Vinyl Pyrrolidone
RLS	Radial Longitudinal Section
RNase	Ribonuclease
rpm	Revolutions per minute
ROB	Rail Over Bridge
RT	Room Temperature
RWC	Relative Water Content
s	Seconds
sp.	Species
SLW	Specific Leaf Weight
TAE	Tris Acetate EDTA
Taq	*Thermus aquaticus*
TE	TrisEDTA
TS	Transverse Section
TLS	Transverse Longitudinal Section

UPGMA	Unweighted Pair Group Arithmetic Mean Method
viz.	Namely
WASP	Web Agri Stat Package
WUE	Water Use Efficiency

Dedicated to the Almighty God

Introduction

1. INTRODUCTION

Plant parasitic species constitute 1% of flowering plants belongs to 20 families with 3000 - 5000 species;40 per cent of these species parasitize on above ground parts of their host plant (Norton and Carpenter, 1998). They may be either shoot or stem parasites. Mistletoes are predominant group of stem-tree parasites, damaging the wood quality of conifers and hardwood timber trees (Mathiasen *et al*., 2008). Current predictions indicate that due to global climate change, the hemiparasitic species are bound to expand their geographical range (Rigling *et al*., 2010). Hemiparasites of Loranthaceae family show wide genetic diversity. They are a major pest of tropical tree species. In Kerala 29 tree parasites belonging to the families Loranthaceae and Viscaceae have been identified (Gamble, 1936). Among these, *Dendrophthoe falcata* has the widest host range. *Helicanthus elastica* is a common species widely seen on commercially important fruit crops like mango, sapota, cashew and cocoa especially in the lowlands. *Macrosolen sp.* is a stem parasite infecting tropical trees with very limited host range (Lim *et al*., 2017). *Scurulla sp*., *Taxillus sp*., *Helixanthera sp*. are genera of Loranthaceae commonly observed in the high ranges of Kerala mainly in Idukki, Waynad and Nelliyampathi areas. The members of Loranthaceae family exhibit wide range of morphological and phonological innovations (Rodriguez *et al*., 2018). Hence qualitative and quantitative study of the cross relationship between the host and the parasite is a novel area which need further elucidation.

In recent years there has been a significant increase in the application of molecular genetic methods for assessing the conservation and use of plant genetic resources. Molecular techniques have critical roles in studies of phylogeny and species evolution. They have been applied to increase our understanding of the distribution and extent of genetic variation within and between species (Mondini *et al.*, 2009). Inter Specific Sequence Repeats (ISSR) are semi arbitary markers amplified by PCR in the presence of one primer complimentary to a target microsatellite. ISSRs have been successfully used in the molecular

characterization of members of related species of fruit and, vegetable crops. (Zeitkiewicz *et al*., 1994; Fang and Roose, 1997: Pharmawati *et al*., 2005).

Anatomical studies have indicated that, parasites derive water and nutrients from the vascular system of their host through a specialized transfer organ called haustorium (Irving and Cameron, 2009). The mechanism of resource acquisition (Tesitel *et al*., 2010) and the relative efficiency of parasites in mobilizing nutrients and water from the host varies from species to species and is a measure of its competitiveness (Girija *et al*., 2013). Apart from water, mineral nutrients and metabolites, the host parasitic interactions also result in the uptake of secondary metabolites from the host (Marko and Stermitz, 1997). Translocation and partitioning patterns of mineral nutrients between host and parasite is a topic yet to be unravelled. Thus, an exhaustive investigation on the mechanism of xylem feeding and phloem feeding of the hemiparasites need to be undertaken.

Globally members of Loranthaceae family have been identified as one of the most troublesome parasitic weeds in perennial fruit crops like mango, jack citrus, sapota etc. (Mathew and Duraimurugan, 2002). Since they compete for water and minerals taken up by the host plant, they reduce the productivity of the crop bringing ultimately death to the host plant. Out of the total crop loss due to mistletoes in Kerala, highest loss is reported to be due to *Dendrophthoe falcate* (Girija *et al*., 2009). In order to control the parasite, the earlier recommended management practise includes manual cutting of the infested branches of trees or complete removal of the infected trees (Perry 1995; Torngren *et al*., 1980). However, all these practices failed to provide a complete management of the parasite since the re-emergence of the parasite was observed within a period of 6-12 months. Thus, an efficient and non-regenerative management practice for the control of mistletoes has to be standardized. Application of ethephon @ 25ml/L was reported to be effective in controlling the parasite without harming the host plant (Girija. 2015). Knoche *et al*., (1991) reported an increased efficacy of ethrel when the growth regulator was applied in combination with surfactants. An improved management strategy for complete eradication of mistletoes without re-

emergence is an important need of the hour. Hence the current study was initiated with the following objectives:-

(i) to identify the genetic diversity of common Loranthus genera in Kerala,

(ii) to understand the dynamics of host parasite interaction and

(iii) to modify and improve the prevailing management strategies.

Review of Literature

2. REVIEW OF LITERATURE

Parasitism by various species of Loranthus on different angiosperms as well as gymnosperms, including numerous economic and horticultural taxa has been recorded from time to time from several states in India (Ghosh, 1968, 1971). Even though they cause negative impact in terms of economy they do have important ecological functions in maintaining the ecosystem as they provide food and shelter for a variety of birds, mammals and insects (Watson, 2001). This chapter reviews the morphological, physiological and molecular aspects of the genera Loranthaceae and its management.

2.1 General aspects of genera Loranthaceae

In Kerala 29 tree parasites belonging to the families Loranthaceae and Viscaceae have been identified (Gamble, 1936). Common vernacular names used for these parasitic species are *Banda* or *Bandba*, *Panda* (Hindi), *Ithikanni* (Malayalam), *Manda* (Bengali), *Banje*, *Banduka* (Kannada), *Othu*(Tamil), *Bajinike* (Telugu) (Vijayan *et al*., 2015). The extent of host specificity in these parasites depends on their ability to adapt with the hosts (Norton and Carpenter, 1998).

2.2 Structural interactions between host and parasite in Loranthaceae

Mistletoe is a common term used to denote obligate aerial hemiparasitic plant of the order Santalales (Good,1974). The order Santales has five families of which Loranthaceae and Viscaceae constitute 98% of mistletoe species (Nickerent and Malecot, 2001). The centre of origin is the Southern hemisphere from where they are reported to have dispersed between the fragments of Gondwana (Wilson and Calvin 2006). An intimate physiological connection called haustorium connects the host and parasite tissues through which the photosynthates and nutrients transport occurs (Combes, 2001).

In hemiparasites, as suggested by Lamont and Southhall (1982) accumulation of many mineral elements is observed due to the active uptake by the

parenchymatous cells at the host- parasite interface. According to Glatzel, (1983) the haustorium lacks an endodermis to regulate the solute flux in it and thus the mineral content in mistletoes were proposed to be a function of xylem sap. Transfer of solutes from host to parasite occurs either across host xylem pit membranes or through 'oscula'- a tube –like cell from the endophyte penetrating through the pit membrane of the host xylem (Dorr, 1997). The abstraction of solutes through haustoria can be based on a combination of the mechanisms including luminal continuity, across cell wall, and across parenchyma cells (Hibberd and Jeschke, 2001). Their seeds must firmly attach to the host branch after overcoming all possible defences by the host (Rodl and Ward, 2002) and after successful establishment between the host and parasite, they utilise and compete for water and mineral nutrition from the host. They can perform photosynthesis utilising the water and solutes obtained from the host (Boussim, 2002). Efficient abstraction of solutes from host xylem is possible by hemiparasites by direct luminal continuity between the host and parasite vessels (Dorr, 1997 and Cameron *et al*., 2006) According to certain studies, solute transfer in haustoria is proposed to be a combination of all possible mechanisms *viz*. luminal continuity across a cell wall or across parenchyma cells (Finncran and Calvin, 2000; Heide-Jorgensen, 2008).

2.3 Genetic diversity and molecular characterisation of Loranthaceae

In India 70 species of Mistletoes have been reported in several host species extending from sea level to about 3500m in the Himalayan hills (Pundir, 1997).

Dendrophthoe falcata belongs to family Loranthaceae comprises about 31 species spread across tropical Africa, Asia and Australia, among which 7 species are found in India. Two of its varieties are widespread in India viz., var. *falcata* (Honey Suckled Mistletoe) and var. *coccinea* (Red Honey Suckled Mistletoe) distinguished by white and red flowerings, respectively. Mistletoes are reported to have hermaphroditic self -compatible flowers and are usually dispersed through a variety of native and migratory birds (Ramirez and Ornelas, 2012). The partial

stem-parasite is reported to grow on around 401 host plants (Moore and Inamdar, 1976). The parasite appears like another branch of the host but are functionally different in terms of its photosynthetic contributions (Glatzel and Geils, 2009).

Helicanthus elastica is an aerial parasitic plant seen on a wide range of hosts. The chemical composition of a hemi-parasitic plant species depends upon the genetic identity which gets modified as part of modifications in the physiology of the plant based on environmental conditions in which the plant grows (Shinde *et al*. 2007). Leaves of this plant are used for checking miscarriage of foetus and for removing stones in the kidney and urinary bladder (Shanavaskhan *et al*., 2012). It is a less known underutilized medicinally important species belonging to family Loranthaceae commonly called mango mistletoe (Sunilkumar *et al*., 2016).

Macrosolen parasiticus is a hemi parasitic shrub growing on aerial parts of trees. They generally have narrow host range. Plant extracts in various solvents are reported to possess antioxidant and anti-cancerous properties (Lobo *et al*., 2011; Sodde *et al*., 2011). Phytochemical constituents showing antimicrobial and antioxidant properties have been quantified from the methanolic extracts of leaves of *M. parasiticus* (Puneetha *et al*., 2016).

Helixanthera wallichiana, a hemi parasite belonging to the family Loranthaceae grows on woody perennials and fruit trees inhabiting elevated topographies (Devkota *et al*., 2011).

Another hemiparasite, *Taxillus tomentosus*, which is a member of Loranthaceaea was reported to be a potential threat to Amla trees in tropical forest areas (Ticktin *et al*., 2012).

In recent years, there has been a significant increase in the application of molecular genetic methods for assessing the conservation and use of plant genetic resources. Molecular techniques have critical roles in studies of phylogeny and species evolution. Inter Specific Sequence Repeats (ISSR) are semiarbitrary markers amplified by PCR in the presence of one primer complimentary to a

target microsatellite. ISSRs have been successfully used in the molecular characterization of members of related species of fruit crops, vegetable crops etc. (Zeitkiewicz *et al*.; 1994; Fang and Roose, 1997; Pharmawati *et al*., 2005). These techniques help to increase our understanding of the distribution and extent of genetic variation within and between species (Mondini *et al*., 2009). A study on genetic diversity and population structure in *Tristerix corymbosus* (Loranthaceae) using RAPD as molecular marker revealed that the genetic diversity is related to its biome (Amico *et al*., 2014). To describe spatial patterns of genetic structure in *Psittacanthus schiedeanus* (Loranthaceae) 10 polymorphic nuclear microsatellite loci have been successfully isolated and characterized by Gonzalez *et al*. (2015).

2.4 Taxonomy, morphology, and anatomy of Loranthaceae

The bioparts of commonly seen Loranthaceae genera *viz*. *Dendropthoe falcata*, *Helicanthes elastica*, *Macrosolen capitellatus, Helixanthera wallichiana*, and *Taxillus tomentosus* were collected from various locations and studied. Systemic position according to Bentham and Hooker (1890) system of classification and detailed description of morphological characters of the collected samples as per Gamble (1936) and Girija *et al*. (2014) are as follows.

2.4.1Dendrophthoe falcata

Systematic position

Kingdom: Plantae

Class: Dicotyledons

Sub-class: Monochlamydeae

Order: Santalales

Family: Loranthaceae

Genus: *Dendrophthoe*

Species: *falcata*

Dendrophthoe falcata is a large bushy parasite. It is a shrub which is much branched, 1-3 m long. Branches are swollen from the base, with terete stems and the bark is dark grey. Oppositely arranged leaves are 7-15 cm long, sessile, exstipulate, entire, variable in shape; elliptical or oblong to orbicular cordate to linear. Leaf stalks are 1 cm long, and the midriff is red. Flowers occur in stout racemes in leaf axils. Fruits ovoid. They have the widest host range including trees like mango, cocoa, neem, *Syzygium sp*., and *Murraya sp*.

2.4.2Helicanthus elastica

Systematic position

Kingdom: Plantae

Class: Dicotyledons

Sub-class: Monochlamydeae

Order: Santalales

Family: Loranthaceae

Genus: *Helicanthus*

Species: *elastica*

Helicanthus elastica is a semi-parasitic dichotomously branched subshrub; branchlets woody, swollen at nodes, hairless. Leaves are opposite, 4-8 x 1.5-4 cm, ovate or elliptic-oblong, base blunt or flat, tip pointed or blunt, thickly leathery, glaucous beneath, basally 3-nerved; stalkless or nearly stalkless. Flowers are aggregated in short in leaf-axils fascicles. Calyx is minute, flask-shaped, margin flat. Flower are white with green stripes, sessile, 2.5-3.5 cm long, split lengthwise into 5 linear, twisted petals. Stamens are 5, protruding, filaments crimson. Ovary about 1.5 mm long; style 3-3.5 cm long; stigma ovoid. Berry 6-8 x 3-3.5 mm, obovoid, red. Berries are globose, pink. The most common hosts of the parasite

are Mango. They are also seen in other perennial tree crops like Guava, Cocoa, Casuarina, Rubber tree, and Citrus.

2.4.3Macrosolen capitellatus

Systematic position

Kingdom: Plantae

Class: Dicotyledons

Sub-class: Monochlamydeae

Order: Santalales

Family: Loranthaceae

Genus: *Macrosolen*

Species: *capitellatus*

Macrosolen capitellatus is a hairless, parasitic shrub, usually seen on trees with single point of attachment. Oppositely arranged leaves are coriaceous, entire, ovate-lanceolate, blunt tipped, base acute. Flowers are racemes, hexamerous corolla hardly 2 cm long, greenish-red, few, in condensed spikes in leaf axils. Peduncle carrying the spike is stout, 5-8 mm long. Bracts are to 5 mm long, ovate, acute or apiculate. Sepal tube is 5 mm long. Flowers variegated in bud, tube 7 mm long, reddish, broad upwards, more or less straight. Six petals, to 1.3 cm long, spoon-shaped, turned back, greenish. Six stamens protrude out of the flower. Flowering seen during April – June. Jack fruit (*Artocarpus heterophyllus*) is the most commonly parasitized crop.

2.4.4Helixanthera wallichiana

Systematic position

Kingdom: Plantae

Class: Dicotyledons

Sub-class: Monochlamydeae

Order: Santalales

Family: Loranthaceae

Genus: *Helixanthera*

Species: *wallichiana*

Helixanthera wallichiana is a parasitic shrub, with stout round branchlets, which are warty. Flower-stalks are 3 mm long, bract adenate to the flower-stalk, spoon-shaped, ciliate. Calyx is globose, 2 mm long, limb annular, obscure. Flowers are reddish, cylindrical in bud, lobes 4, ovate, acute, hairless. Leaves are simple, alternate and opposite, 5-10 x 3-6 cm, ovate- elliptical, blunt, base narrow or rounded hairless. Lateral nerves are 2 or 3 pairs, leafstalks are 0.8-2 cm long. Flowers are borne in slender hairless racemes up to 3-6 cm long, pale red, terete, slender raceme. Berry is 6 mm long, ovoid, rugose. Seen flowering during March – April. They are commonly seen as parasites on highlands fruit crops like Orange, Litchi and also in certain forest tree species.

2.4.5Taxillus tomentosus

Systematic position

Kingdom: Plantae

Class: Dicotyledons

Sub-class: Monochlamydeae

Order: Santalales

Family: Loranthaceae

Genus: *Taxillus*

Species: *tomentosus*

Taxillus tomentosus is a parasitic shrub with many dark brown branches and epicortical roots. Leaf stalks are 5-7 cm long, rusty-hairy. Leaves are hairless, alternate, ovate-oblong to elliptical. Flowers are green, with 1 cm long green flower-tube, and narrow green petals, 4 mm long. Bracts are prominently longer than the sepal tube, which is the identifying feature of the plant. Flowering seen during December to February. They are a common parasite of *Phyllanthus emblica* in highlands.

Four basic haustorial system types are observed in aerial grown hemiparasites namely, epicortical roots (ERs), wood roses, clasping unions and bark strands. Majority of Loranthaceae genera has ER type of haustorial system. Three types of ERs have been described for Loranthaceae *viz*., basal, cauline and adventitious (Calvin and Wilson, 2006).

Efficient abstraction of solutes from host xylem is possible by hemiparasites by direct luminal continuity between the host and parasite vessels (Cameron *et al*., 2006). Studies showed that solute transfer across haustoria proceeds across cell walls or through contact parenchyma (Hibberd and Jeschke, 2001; Tennakoon and Pate, 1997). A study on the functional status of haustoria of field grown sandal wood tree, another member of the order Santalales, reported that direct lumen to lumen xylem connections between xylem of parasite and host were absent, proposing the movement of xylem sap via pits of its xylem elements (Rocha *et al*., 2015).

2.5 Nutrient dynamics between host and parasite

In hemiparasites, accumulation of many mineral elements are observed due to the active uptake by the parenchymatous cells at the host- parasite interface (Lamont and Southhall, 1982). According to Glatzel, (1983) the haustorium lacks an endodermis to regulate the solute flux in it and thus the mineral content in mistletoes were proposed to be a function of xylem sap. Chemical analyses have

proven enriched concentrations of elements like potassium and phosphorus in mistletoe leaves. A hypothesis for these mineral element accumulations in mistletoes as proposed by Glatzel (1983) is that these elements are integral components as they accompany sugars in the phloem sap. Kujit (1991), reported that predominant nutrient translocation in mistletoes takes place from host to parasite. Sucrose transporters were identified and cloned from a variety of plants (Reismier *et al*., 1992, Hirose *et al*., 1997, Burkle *et al*., 1998). According to some scientists, the nutrient uptake by hemiparasites from the host was suggested to be partly active, where the nutrient and water acquisition might not be tightly coupled, instead some mechanism exists in the haustorium to facilitate active nutrient loading, by the indirect contribution from host phloem (Panvini, and Eickmeier, 1993; Cocoletzi *et al*., 2016). Apart from water, mineral nutrients and metabolites, the host parasitic interactions also results in the uptake of secondary metabolites from the host (Marko and Stermitz, 1997). As observed by Bowie and Ward (2004), some species in Loranthaceae have been found to be capable of tapping carbon and inorganic nutrients from the host phloem. A symplastic continuity between the phloem of tobacco and the parasitic plant *Cuscuta reflexa* has been demonstrated by marking the host phloem with GFP, apart from the apoplastic movement (Haupt *et al*., 2001). Studies by Girija *et. al*. (2006) with labelled ^{14}C in between the parasite *Helicanthus elastica* and host Cocoa has shown that there is two-way movement of nutrients between the host and the parasite. A recent study on the effect of removal of mistletoe, a hemiparasite, resulted in increased nitrogen availability and carbon gain leading to increased growth rates of the hosts, which reveals the exigency of an effective management system for hemiparasites (Yan *et al*., 2016). The results from a study on the bidirectional transport of inorganic nutrients between *Dendrophtoe falcata* and the host Ficus using radioactive ^{32}P ions support the idea that the host is not merely a nutritional substrate for the parasite, but there is a close physiological contact through the infection sites which influences its metabolic processes (Bhattacharya *et al*., 2015).

2.6 Functional dynamics between host and parasite

Studies about the relationship between the hemi-parasite *Loranthus europaeus* and host *Quercus sp.* revealed that with similar rates of assimilation, the rate of transpiration and leaf conductance in the parasite was three times greater than the host (Schulze and Ehleringer, 1984). Bannister *et al*. (2002) reported that there was more storage of elements in mistletoes tissues owing to differential accumulation of minerals due to a higher rate of transpiration in them as compared to their hosts. He also observed that concentrations of elements like K, Na and P was found to be higher in leaves of mistletoes than their hosts. Solute transfer between the host and parasite is driven by water potential gradient between them and is known to occur as passive mass flow (Press, 1989; Press *et al*., 1988). Hemiparasites were generally observed to have higher transpiration rates and abnormal stomatal behaviour since they maintain a higher negative water potential than their hosts (Jiang *et al*., 2003). Luxury use of water and mineral resources due to high transpiration rates in hemiparasites prevents them from effective biomass production thus reducing their overall productivity (Cameroon *et al*., 2005; Phoenix and Press, 2005; Press and Phoenix,2005)

Loranthus are generally known to have little control over water loss (Vareschi and Pannier, 1953; Hellmuth 1971). Opening and closing of stomata are essentially a process homeostatically regulated by the leaf- xylem water potential (Brodribb and Holbrook, 2003; Brodribb *et al*., 2003). As per certain contemporary studies on translocation across haustoria, solute transfer in hemiparasites is hypothesised as based on an active transmembrane transport as water potential difference was not found to be required (Tennakoon and Cameroon, 2006). Since the host is unable to control the water lost by the mistletoe, this default control mechanism is disrupted (Glatzel and Geils, 2009).

Hemiparasites (mistletoes) have succulent leaves enabling them to store more water per unit area than host leaves (Glatzel,1983). Besides having fleshy leaves, high concentrations of osmotically active organic solutes enhances mistletoe

water storage (Popp, 1987; Popp *et al*., 1995). Meagre difference in water use efficiency between hosts and hemiparasites was observed by Bannister and Strong (2001). Some mistletoes are reported to perform well once their host is under environmental stress like water deficit (Glatzel and Geils, 2009). Specific leaf weight has a linear relationship with rate of apparent photosynthesis (Dornhoff and Shibles, 1970). Stage of development and physiological factors significantly influence specific leaf weight (Lugg and Sinclair, 1980).

Photosynthetically Active Radiation (PAR) is the part of electromagnetic radiation that can be used as the source of energy for photosynthesis by green plants (McCree, 1972). Biochemical properties of host xylem and phloem have direct influence on the physiological performance as well as on the further survival of the parasite (Snyder *et al*., 1996; Linhart *et al*., 2003). As reported by Strong and co-workers (2000), the hemiparasites mistletoes were shown to exhibit photosynthetic features more related to shade plants viz., low mean CO_2 assimilation rates, low electron transport rate, low light saturation point and low chlorophyll a/b ratio. Some species of hemiparasites have low photosynthetic efficiency than their hosts (Schulze and Ehleringer, 1984; Strong *et al*., 2000). Under a water deficit condition, the hemiparasites tend to continuously extract water and minerals from the host, to maintain its high rate of photosynthesis, imposing a major stress to the host (Garkoti *et al*., 2002). PAR is measured to quantitatively describe the driving force for photosynthesis by measuring the action spectrum of photosynthetic radiation which is defined as photosynthetic productivity (Mottus *et al*., 2011).

The instantaneous carboxylation efficiency (A/Ci) calculated as the ratio between photosynthesis and the CO_2 concentration inside the mesophyll cells is closely related to the intracellular CO_2 concentration and CO_2 assimilation rate (Machado *et al*., 2005). The hemiparasite maintains a more negative water potential than the host which is governed by elevated transpiration rates, abnormal stomatal closure and accumulation of osmotically active compounds (Glatzel, 1983., Ulmann *et al*., 1985; Ehlinger *et al*., 1986). Photosynthetic activity in the

host leaves was reported to be reduced (Rigling *et al*., 2010) which might be due to the stomatal closure induced because of water abstraction from host by parasite (Zweifel *et al*., 2012). The hemiparasite resulted in developing resistance against herbivores or pathogens at the cost of the host as a result of uptake of secondary metabolite from the host (Strauss *et al*., 2002). Research on the metabolite changes with the induction of Cuscuta haustorium and translocation showed variation in host metabolites (Furuhashi *et al*., 2012).

2.7 Improving the - management strategies for controlling Loranthus

Globally mistletoes have been identified as one of the most troublesome parasitic weeds in perennial fruit crops like mango, jack, citrus, sapota etc. (Mathew and Duraimurugan, 2002). They compete for water and minerals taken up by the host plant through vascular connections termed haustoria and reduce the productivity of the crop bringing ultimately death to the host plant. Out of the total crop loss due to mistletoes in Kerala, highest loss is reported to be due to *Dendrophthoe falcata* (Girija *et al*., 2009).

Dendrophthoe falcata and *Helicanthus elastica* are the two different genera of Loranthaceae family most commonly found in the plains of Kerala. The earlier recommended management practices include manual cutting of the infested branches of tress or complete removal of the affected trees (Perry 1995; Torngren *et al*., 1980) and foliar spray of herbicides like glyphosate (Baillon *et al.,* 1988) or an absolute eradication and prevention of further growth of *Dendrophthoe falcata,* diesel or powerine oil (30-50 ppm) is sprayed on the affected host plants (Ragupathy and Mahadevan, 1991). Knoche and co-workers, (1991) reported an increased efficacy of ethrel when the growth regulator was applied in combination with organosilicone surfactant. Vidhyasekaran, (2004) suggested eradication of the parasite using 2,4-D.

All these practices failed to provide a complete management of the parasite, since re-emergence of the parasite was observed within a period of 6-12 months. A methodology of banding the stem of the parasite with 1 % 2,4-D was reported to

give a complete eradication of the parasite from perennial fruit trees (Mathew and Habeeburrahman, 2013). Application of ethephon @ 25 ml/L was reported to be effective in controlling the parasite without harming the host plant (Girija, 2015).

Materials and Methods

3. MATERIALS AND METHODS

3.1 Morphological and anatomical characterization of five major Loranthaceae genera *viz*., *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana* and *Taxillus tomentosus.*

3.1.1 Morphological characterization

3.1.1.1Collection of specimen

The plant specimens of commonly seen Loranthaceae genera *Dendropthoe falcata*, *Helicanthus elastica* and *Macrosolen capitellatus* were collected from various host tress from the plains and the high range dwelling genera *viz*. *Helixanthera wallichiana*. and *Taxillus tomentosus* were collected from various locations of Nelliyampathy forest (Table 1.) Samples collected were correctly identified and authenticated according to Fischer's collection of Herbarium (FRCH). The following morphological characters of the collected samples of different genera were recorded for the study.

Stem characteristics like branching pattern, type of arrangement of the collected specimens were studied.

Leaf characteristics like leaf length, breadth, thickness, venation and leaf area leaf length and breadth were recorded using a metre scale and leaf thickness was recorded using Vernier calipers. Leaf venation was identified on keen observation of the sample leaves and leaf area was calculated using Leaf Area Meter.

3.1.1.2 Anatomical characterization

Plant samples of common genera viz. *Dendrophthoe falcata* and *Helicanthes elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana*., and *Taxillus tomentosus* were collected from different locations. The collected stem portions along with the haustorial point of attachment was used for permanent slide

preparation. The required plant parts were fixed in FAA (5ml Formalin + 5ml Acetic acid + 90ml 70% Ethyl alcohol).

Table 1. Places of specimen collection

S. No.	Genera collected	Location	GPS cordinates
1.	*Dendrophthoe falcata*	Instructional Farm, KAU.	10º 32’ 54.49” N 76º 16’ 04.36” E
2.	*Helicanthus elastica*	Instructional Farm, KAU	10º 32’ 54.49” N 76º 16’ 04.36” E
3.	*Macrosolen capittelatus*	Near Pambur ROB, Thrissur	10° 33' 11.68" N 76° 12' 29.84" E
4.	*Helixanthera wallichiana*	Orange and Vegetable Farm, Nelliyampathy	10° 32' 5.39" N 76° 12' 29.84" E
5.	*Taxillus tomentosus*	Orange and Vegetable Farm, Nelliyampathy	10° 32' 5.39" N 76° 41' 34" E

3.1.1.2.1Microtomy

Wood specimens of size 1 cm^3 were chiselled out from each sample and used for anatomical studies. The haustorial stem region was neatly trimmed with a sharp end of a blade were then softened by keeping in water bath (Rotex water bath) at 100ºC depending on the nature of specimen. After softening, the specimens were placed on the holder of sledge microtome. Transverse section (TS)longitudinal section (TLS) and radial (RLS) sections of 10-15 µm thickness were prepared using a Leica sliding wood microtome (Leica SM 2000R). The sections were stained using saffranin and toluidine blue and later washed through a series of alcohol solutions at different concentrations (70 %, 90 % and 95 %) to ensure complete dehydration. They were subsequently dipped in acetone followed

by xylene and finally mounted in DPX mountant to prepare permanent slides (Johansen, 1940).

3.1.1.2.2 Image analysis

Permanent slides were examined using an image analyser (CatCam 500E series) which is provided with a microscope (Motic BA 210), Zeiss Stemi 305 Compact stereo microscope, digital camera and a personal computer. Images of the sections were captured and then proportions of fibre, vessel and rays were made using the software Catymage. The haustorial attachment and point of union of each of the Loranthaceae genera were examined and compared for the similarities and differences in presence and arrangement of the nature of tissues.

3.2 Genetic diversity analysis of five major Loranthaceae genera viz. *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus.*, *Helixanthera wallechiana* and *Taxillus tomentosus.*

3.2.1 Molecular characterization

Dendrophthoe falcata and *Helicanthes elastic*, the two species widely seen in the plains of Kerala were collected from the campus trees of College of Agriculture, Vellanikkara. *Helixanthera wallichiana* and *Taxillus tomentosus*, the common species infecting fruit and timber crops of high ranges were collected from the fruit trees of Govt. Orange and Vegetable Farm, Nelliyampathy. *Macrosolen capitellatus* which has a limited host range was collected locally from Jack trees. Leaf samples from the collected plants was used for DNA using methodology suggested by Doyle and Doyle (1990). In order to study the genetic variability within and across different genera an initial screening screening with 40 PCR based Inter Simple Sequence Repeats (ISSR) marker system was done.

3.2.1.2 Genomic DNA extraction

Genomic DNA extraction of five major genera of Loranthaceae viz., *Dendrophthoe falcata* and *Helicanthes elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana*, and *Taxillus tomentosus* was done. The leaves of plant

samples (5g) collected and ground using a pestle and mortar in liquid nitrogen. The powdered plant tissue was then transferred to an extraction buffer that contained detergent to disrupt the membranes. Cetyltrimethyl ammonium bromide (CTAB) which is commonly used for this purpose. The extraction buffer also contained a reducing agent (β- mercaptoethanol) and a chelating agent (ethylenediaminetetraacetic acid, EDTA). This helped to inactivate nucleases that are released from the plant cell. However, to avoid serious degradation of the genomic DNA, reaction was carried out in cold conditions. Phenolic compounds may also be released on disruption of plant tissues and these may interfere with subsequent uses of the DNA. Polyvinyl pyrolidone (PVP) was added to the extraction buffer to remove phenolic compounds.

Phenol extraction helped to remove any traces of proteins and the genomic DNA and this was precipitated using either isopropanol. Precipitated DNA was then collected by centrifugation.

3.2.12.a Equipments required

1. High speed centrifuge
2. Microfuge
3. Auto-pippets 2-20µl, 20-200µl, 200-1000µl
4. Waterbath
5. -20^{o} C Deep freezer
6. Refrigerator.

3.2.1.2.b Reagents

1. CTAB Buffer:

1.4 M NaCl
100 mM Tris-Cl
20 mM EDTA
2% β-Mercaptoethanol
1.5% CTAB

Adjust pH to 8.0 with HCl and autoclave before use.

2. Isopropanol

3. Saturated phenol

4. Chloroform: isoamyl alcohol (24:1) mixture

5. 10:1 TE:

10mM Tris

1mM EDTA

The pH was adjusted to 8.0 with HCl and autoclaved before use.

6. RNase A (10mg / ml):

Dissolved RNaseA in 10mM Tris-Cl, pH 7.5, 15 mM Na Cl. It was then heated at 100 ºC, for 15 min and then cooled to room temperature. Aliquot was stored at -20 ºC.

7. 70% ethanol.

3.2.1.3 Procedure for DNA isolation

Clean young leaf tissue was weighed (5 g) and ground to fine powder with a pestle and mortar after freezing in liquid nitrogen. This was transferred to 50 ml centrifuge tube with 20 ml CTAB buffer maintained at 60º C in a water bath with intermittent vigorous mixing (vortex). This was incubated at 60º C for one hour and mixed intermittently. Tubes were filled with chloroform: isoamyl alcohol by gently mixing and inverting for 5 min. Spin at 17,000 rpm for 10 min. with SS34 rotor in Sorval RC-5C centrifuge at 25º C were given. Aqueous phase was transferred to to a fresh centrifuge tube and equal amount of isopropanol was added. DNA was allowed to settle down for 20 min. and spooled out. (If necessary, we can centrifuge in the microfuge for 2 minutes. Drain out the excess chemicals with a pipette. Add 0.5 ml of 70% ethanol. Mix gently and incubate for 30 min. Decant and repeat the 70% ethanol treatment. Decant off and dry the pellet under vacuum). DNA was dissolved in volume of 10: 1 TE. RNase (0.2 ml) was added and incubated at 37º C for one hour. Equal volume of phenol: chloroform (1:1), was then added and mixed properly and a spin was given for 5 min. DNA supernatant was taken out and after this two chloroform:isoamylalcohol extractions was done. After

each extraction spinning was done. DNA was precipitated by adding 1/10 volume of 3M NaOAc and 2.5 times the total volume of chilled ethanol. Mixed and DNA was spooled out. Extra salts were removed by two washings with 70% ethanol. After that, it was dried under vacuum and a volume of TE (10:1) was added. It was dissolved at room temperature and stored frozen at -20^{o} C.

3.2.1.4 Assessing the quantity and quality of DNA

3.2.1.4.a Visualization of DNA- Agarose gel electrophoresis

Visualization of the quality of the DNA isolated from different genera of the Loranthaceae samples collected were done. When an electric field is applied to an agarose gel in the presence of a buffer solution it will conduct electricity, DNA fragments moves through the gel towards the positive electrode (as DNA is negatively charged) at a rate which is dependent on the size and shape. Small linear fragments move more quickly than large ones, which are retarded by entanglement with network of agarose fibres forming the gel. Different concentrations of gel will allow the optimal resolution of fragments in different size ranges. Ethidium bromide is used as a stain for DNA in agarose gels. Ethidium bromide molecules inserts between stacked bases of double stranded DNA. The intercalated Ethidium bromide molecules emit fluorescent light when irradiated with UV light. 300 nm is the optimal wavelength for exciting fluorescence from the ethidium bromide DNA complex.

3.2.1.4.b Reagents and Equipments

1. Tris base (Molecular biology grade)
2. Disodium EDTA (Molecular biology grade)
3. Glacial acetic acid (Molecular biology grade)
4. Agarose (Molecular biology grade)
5. Ethidium bromide (DNA intercalating dye)

TAE Electrophoresis Buffer 50X stock

Tris base	242 g
Disodium EDTA	18.61 g
Glacial Acetic Acid	57.1 ml
Distilled H_2O to	1 L

Tris base and EDTA were added to approximately 700 ml DDI H_2O and stirred until the Tris and EDTA are completely dissolved. Then acetic acid was added, and volume was adjusted to 1 litre.

TAE Electrophoresis Buffer (Working stock 1X - 500 ml)

The 1x TAE solution is 40mM Tris, 20mM Acetate and 1mM EDTA and typically has a pH around 8.6 (do not adjust). Take 10 ml 50X TAE buffer and add 490 ml of distilled water.

Composition of 6X DNA loading dye

0.25% (W/V) bromophenol blue

0.25% (W/V) xylene cyanol FF

15% (W/V) Ficoll 400

Agarose Gel preparation (1%)

1g agarose was weighed and dissolved in 100 ml 1X TAE buffer by heating and constant stirring in a water bath at 95°C. After cooling (35 °C) 2 µl of (10 mg/ml) Ethidium bromide solution was added into it and gel was casted. After solidifying the gel, the comb was removed carefully, and gel was transferred into the electrophoretic apparatus containing 1X TAE buffer.

Instruments required

1. Electrophoretic apparatus
2. Power pack
3. UV trans illuminator

Procedure

DNA sample (5 µl) was mixed with DNA loading dye and loaded in the wells along with molecular weight DNA marker. It was allowed to run at 50 V for half an hour. After the run, the gel was analyzed under UV transilluminator. DNA bands were observed and photographed.

3.2.1.4.c Quantification and purity assessment of DNA

The quality and quantity of DNA isolated from different genera of Loranthaceae collected were determined using UV-spectrophptpmeter. Isolated DNA extracts 10 µl DNA + 990 µl of distilled water and 1ml distilled water as blank were taken in cuvettes. The OD reading at at 260 nm and 280 nm were recorded and purity of DNA isolated were determined as follows.

Ratio of A260/A280 = 1.8 pure DNA)

Total DNA concentration (ng/µl) = A260 × 50 ng/µl ×100

If the A260/A280 goes below 1.6 and above 2.0 the DNA is not suitable for further molecular biology experiments.

3.2.1.5 ISSR (Inter Simple Sequence Repeat) assay

3.2.1.5.a Screening and analysis of ISSR primers

Fourty ISSR primers (Eurofins Genomics) were screened for ISSR analysis as listed in Table 2(a) and (b) primers were selected based on their polymorphism for ISSR analysis. The amplified products were run on 2% agarose gel using 1x TAE

buffer stained with ethidium bromide along with the marker (5 µl, 50 bp DNA ladder). The DNA amplification mixture of 25 µL contained 25 ng template DNA, 1x PCR buffer, 1.5 mM MgCl2, 200 mM dNTPs, 1 µM ISSR primers, and 0.6 U Taq DNA polymerase and double distilled sterile water. The PCR components were prepared as master mix for each primer to minimize the pipetting error. The amplification reaction was performed in a thermal cycler (SimpliAmp, Thermo Fisher) with amplification cycle condition of initial 4 minutes' strands separation at 94°C followed by 40 cycles of 94°C for 45 secs, 53°C for 1 min, and 72°C for 2 minutes and final extension at 72°C for 10 minutes. The products obtained after PCR amplification were electrophoresed in 2.5% agarose gel in 0.5x TAE buffer at 100 V for around 3 hours and gel was stained with ethidium bromide (0.5 µg/mL). The fragments after staining were visualized in a UV transilluminator and photographed. A 50 bp DNA ladder (Hi-media) was used as a size marker for every gel run. The profile is visualised under UV trans illuminator and carefully examined for amplification of DNA and recorded for further analysis.

3.2.1.5.b Data Analysis

Distinct, reproducible, well resolved fragments were scored as present (1) or absent (0) for each ISSR reaction and were displayed as part of a binary matrix. The data matrices obtained were converted to A (0) and T (1) and were analyzed using MEGA version 10 (Kumar *et. al*., 2018). Genetic divergence between the samples was analyzed by construction of a dendrogram using UPGMA (Unweighted Pair Group Arithmetic Mean Method) in MEGA10 (Kumar *et al*., 2018).

Table 2 (a). List of primers used for screening

S. No.	Sequence Name	Sequence
1	ISSR 841	5'GAGAGAGAGAGAGAGAYC3'
2	ISSR 844	5'CTCTCTCTCTCTCTCTRC 3'
3	ISSR 811	5'CACAGAGAGAGAGAGAC3'
4	ISSR 835	5'AGAGAGAGAGAGAGAGYC3'
5	UBC 866	5'CTCCTCCTCCTCCTCCTC3'
6	ISSR 820	5'GTGTGTGTGTGTGTGTC3'
7	ISSR 847	5'CACACACACACACACARC3'
8	ISSR 848	5'CACACACACACACACARG3'
9	ISSR 827	5'ACACACACACACACAGC3'
10	ISSR 829	5'TGTGTGTGTGTGTGTGC3'
11	ISSR 809	5'AGAAGAGAGAGAGAGAC3'
12	ISSR 815	5'CTCTCTCTCTCTCTCTG3'
13	ISSR 816	5'CACACACACACACACAA3'
14	ISSR 817	5'CACACACACACACACAA3'
15	ISSR 818	5'CACACACACACACACAG3'
16	ISSR 823	5'TCTCTCTCTCTCTCTCC3'
17	ISSR 849	5'CTCTCTCTCTCTCTCTRA3'
18	ISSR 834	5'AGAGAGAGAGAGAGAGYT3'
19	ISSR 836	5'AGAGAGAGAGAGAGAGYA3'
20	ISSR 845	5'CTCTCTCTCTCTCTCTRG3'

Table 2 (b). List of primers used for screening

S. No.	Sequence Name	Sequence
21	ISSR 826	5'ACACACACACACACACC3'
22	ISSR 850	5'CTCTCTCTCTCTCTCTRT3'
23	ISSR 851	5'CACACACACACACACARA3'
24	ISSR 852	5'CACACACACACACACART3'
25	ISSR 853	5'AGAGAGAGAGAGAGAGYG3'
26	ISSR 06	5'GAGAGAGAGAGAGAGAC3'
27	ISSR 07	5'CTCTCTCTCTCTCTTG3'
28	ISSR 08	5'GAGAGAGAGAGAGAGAT3'
29	ISSR 15	5'TCCTCCTCCTCCTCC3'
30	ISSR 5	5'GACACGACACGACACGACAC3'
31	SIGMA	5'GAAGTGGGGAAGTGGG3'
32	UBC 857	5'ACACACACACACACACYG3'
33	UBC 865	5'CCGCCGCCGCCGCCGCCG3'
34	ISSR 808	5'AGAGAGAGAGAGAGAGC3'
35	UBC 873	5'GACAGACAGACAGACA3'
36	UBC 880	5'GGAGAGGAGAGGAGA3'
37	UBC 890	5'VHVGTGTGTGTGTGTGT3'
38	UBC 892	5'TAGATCTGATATCTGAATTCCC3'
39	UBC 895	5'AGAGTTGGTAGCTCTTGATC3'
40	UBC 900	5'ACTTCCCCACAGGTTAACACA3'

3.3 Host parasitic interaction studies

To understand the host parasite interaction two methods were adopted

3.3.1 Translocation and partitioning of assimilates using ^{32}P

3.3.2 Comparison of physiological processes in the host and the parasite

3.3.1 Translocation and partitioning studies

For studying the phosphorous partitioning between the host and parasites, labelled ^{32}P in carrier solution (orthophosphoric acid) of concentration 1000 mg L^{-1} was used. Cocoa plantation maintained by Cocoa Research Station at the Instructional farm of Kerala Agricultural University, Vellanikkara, Thrissur, Kerala was identified to be infested with *Dendrophthoe falcata* and *Helicanthus elastica* were selected for the study. The plantation had unform plants planted in 1996.

The carrier solution of ^{32}P was artificially fed to the host (cocoa) through root feeding. To study the host parasite interaction between *Dendrophthoe falcata* and cocoa five cocoa plants were selected and tagged, similarly, to study the interaction between *Helicanthus elastica* and cocoa, two cocoa trees were identified and tagged. Holes were prepared on the ground near the surface running roots of Cocoa in three concentric circles at 30cm, 45 cm and 60 cm from the central trunk with 4 holes in each circle. PVC pipes (1-inch diameter) of varying length *viz*. 15cm, 30cm, 45 cm and 60 cm were inserted into each hole in respective concentric circles around each cocoa tree (Plate 1 and 2). Root application was done by feeding the carrier solution through the PVC pipes inserted in each hole around the Cocoa plant @ 7ml/ hole so that each host tree received an initial radio activity of 2mCi/plant and covered the maximum root activity (Plate 3). Leaf samples from the host as well as from the parasite was collected 7 and 14 days after treatment.

Plate 1. (a). Selection and numbering of cocoa tree infested with *D falcata* and /or *H. elastica*; (b) & (c) Land preparation for root application of ^{32}P using PVC pipes around the surface running roots of cocoa

Plate 2. Land prepared for ^{32}P application to study the host parasitic interaction

Plate 3. Root application of ^{32}P through surface running roots of host cocoa

Plate 4. Leaf application of ^{32}P on the leaves of Loranthus

Similarly, in order to study the interaction of the parasite with its host ^{32}P in carrier solution was fed to the parasite through foliar application. Two cocoa trees, each infected with *Dendrophthoe falcata* and *Helicanthus elastica* were selected for the study. Foliar application of ^{32}P was done by brushing the leaf lamina with ^{32}P in carrier solution (Plate 4). Leaf samples from the parasite and host were collected 2, 7 and 14 days after treatment. The leaf samples of root treated, and foliar treated host and parasite were oven dried and 1g of each sample with three replications for each treatment were taken and acid digested (Piper, 1996) and prepared for assessing recovery of isotopic activity. Each of the samples was kept for pre-digestion in di-acid mixture; nitric acid: perchloric acid @ 9:4. A quantity of 15 ml per gram of the samples was used for digesting the dried leaf samples. Then, the isotopic activity was assessed using liquid scintillation counter (Hidex Triathler Multi Label Tester, Turku, Finland). The counts of leaf tissue sample of each treatment with three replications were recorded and corrected for background radiation.

3.2.1.1 Design of experiment and statistical analysis

For root feeding, three trees each infected with either of the parasites *D. falcata* and *H. elastica* were selected. For foliar application, three cocoa trees each (which was not selected for root feeding) with parasite infestation were selected. Experimental design was Completely Randomised Design (CRD). Samples were taken 7 and 14 days after root feeding and 2,7 and 14 days after foliar application. Statistical analysis (ANOVA) was carried out based on the scintillation counts obtained. The counts were finally expressed as counts per minute per gram ($cpmg^{-1}$) of dry tissue.

3.3.2 Physiological studies

Four cocoa trees each infected with *Dendrophthoe falcata* and *Helicanthus elastica* maintained at the Instructional farm of Kerala Agricultural University, Vellanikkara, Thrissur, Kerala were selected and tagged for the study. The following physiological observations were taken for a period of six months.

3.3.2.1 Photosynthetic rate

Rate of photosynthesis was measured directly, by measuring either the production of oxygen or the uptake of carbon dioxide. Infra-Red Gas Analyser (IRGA, Li-COR, LI-6400) was used for the measurement of photosynthetic rate of *Dendrophthoe falcata* and *Helicanthus elastic* for a period of six months. IRGA is a CO_2 assimilation system designed for non-destructive measurement of photosynthetic CO_2 assimilation rates in the field. The IRGA works on the principle that heteroatomic gas molecules (such as CO_2, H_2O, NH_3, N_2O etc.) absorb infra-red radiation in specific infra-red wavebands and this absorption follows the Beer-Lambert's law (Pandey *et al*., 2017) The LI-6400XT Portable Photosynthesis System provides a stable platform for a variety of applications. The decrease in the CO_2 concentration in the sample cell is monitored relative to the reference cell. The rate of photosynthesis is directly read from the display of the console and expressed as μmol CO_2 /m^2/sec.

3.3.2.2 Transpiration rate

Infra-Red Gas Analyser (IRGA, Li-COR, LI-6400) was used for the measurement of transpiration rate of *Dendrophthoe falcata* and *Helicanthus elastic* and expressed as mmolH_2O/m^2/sec. Understanding the variations in plant transpiration has significance in understanding water related dynamics in plants (Wang *et al*., 2013). Transpiration rate of *D. falcata* and *H. elastica* infected in Cocoa plants were recorded for a period of six months.

3.3.2.3 Stomatal conductance

To study the stomatal conductance responses of *Dendrophthoe falcata* and *Helicanthus elastica* infected on cocoa plants, Infra-Red Gas Analyser (IRGA, Li-COR, LI-6400) reading were recorded for a period of six months and expressed as molH_2O/m^2/sec.

3.3.2.4 Relative water content

Several physiological variables such as leaf turgor, growth, transpiration and photosynthesis is dependent on the leaf water status in plants (Kramer and Boyer, 1995). Relative water content of *Dendrophthoe falcata* and *Helicanthus elastica* found on Cocoa plants were calculated using weight method. Relative water content was calculated by measuring the fresh weight, dry weight, and turgid weight of known number of leaf discs from the treatment plants. After measuring the fresh weight of the sample, it was submerged in distilled water for 3 hours and then the turgid weight was taken. The dry weight of the sample was measured after keeping the samples in oven at 80º C for 3 consecutive days. The RWC of the treatments was calculated using the following formula.

RWC % = [Fresh weight – dry weight) / (turgid weight –dry weight)] x 100

3.3.2.5 Total phenol content

Quantification of phenols was done by Folin-Ciocalteau method (Mayr *et al*., 1995). Phenol was estimated from 0.5 g sample and reflexed in 10ml of 80 per cent methanol for 20 min. The tissue was ground thoroughly in a mortar with pestle and filtered through a double layered cheese cloth. The filtrate was subjected to centrifugation at 1000 rpm for 10 min. The supernatant was collected and made to a known volume using 80 per cent methanol. 0.1ml aliquot was drawn to a test tube and made up to 3ml using 80 per cent methanol. To this, 0.5ml of Folin-Ciocalteau reagent and 2ml of 20 per cent Na_2CO3 were added. It was kept in a boiling water bath for 5 min till a white precipitate was formed and was then again centrifuged at 5000 rpm for 5 min. The absorbance of the clear supernatant was read at 650nm against the blank and expressed as µg/ml of the extract.

3.3.2.6 Water use efficiency

Water use efficiency (WUE) was calculated as the ratio between photosynthesis and transpiration at a time (de Santana *et al*., 2015).

WUE = Rate of photosynthesis / Rate of transpiration.

3.3.2.7 Specific leaf weight

From each plant, fully expanded third leaf (from main stem apex) was collected. Leaves were dried at 80ºC for 2 days and the dry weight was taken. Specific Leaf Weight was calculated using the formula.

SLW (g/cm^2) = Leaf dry weight/ leaf area

3.3.2.8 Photosynthetically Active Radiation (PAR)

Photosynthetically active radiation (PAR) is defined as electromagnetic radiation in the waveband between 400 and 700 nm, or 0.400–0.700 mm (CIE, 1993). In order to study the efficiency of phototsynthesis in *Dendrophthoe falcata* and *Helicanthus elastica,* PAR was recorded using Infra-Red Gas Analyzer (IRGA, Li-COR, LI-6400).

3.3.2.9 Carboxylation efficiency

Response of photosynthetic rate to increasing ambient concentration of CO_2 in leaves of *Dendrophthoe falcata* and *Helicanthus elastica* was studied by recording the carbon use efficiency. Instantaneous carboxylation efficiency (A/Ci) defined as the ratio between photosynthesis and the CO_2 internal concentration (Dias *et al*., 2017) where in photosynthetic rate and CO_2 internal concentration were available from Infra-Red Gas Analyser (IRGA, Li-COR, LI-6400).

3.3.2.10 Light Use efficiency

The light use efficiency of a plant canopy is defined as the ratio of net primary productivity to absorbed photosynthetically active radiation. In order to estimate the light use efficiency of *Dendrophthoe falcata* and *Helicanthus elastica*, canopy

photosynthetic light use efficiency of the host (cocoa) was taken into consideration. The canopy photosynthetic light use efficiency (Ɛc) is calculated as photosynthesis (A) per unit absorber photosynthetically active radiation (PAR) (Medlyn, 1998).

$$Ɛc = A/ PAR$$

3.2.2.2 Design of experiment and statistical analysis

For studying the physiological parameters, design of experiment followed was Completely Randomised Design (CRD). Four cocoa trees each infected with *Dendrophthoe falcata* and *Helicanthus elastica* was selected for the study and continuous observations were taken for a period of six months. For data analysis, ANOVA of values of host and parasite samples for six months were done in ICAR Goa WASP 2.0. For comparison between parasite and host, students t-test for values of host and parasite samples for six months were done in ICAR Goa WASP 2.0.

3.4 Improving the management strategies for controlling Loranthus

An experiment was laid out to improve the management strategy of Loranthus, at the Kerala Agricultural University in the cocoa orchard maintained by Cocoa Research Station (CRS) in the Instructional Farm, Vellanikkara. Cocoa trees infected with *Dendrophthoe falcata* and *Helicanthus elastica* were identified and labelled.

Two non-ionic surfactants were mixed with the recommended dose of 25ml/l of ethephon and the efficacy of the combination was tested on Loranthus. Non-ionic surfactants are those which contains no charge and they do not react with the active ingredient. The two-chemical type of non-ionic adjuvants used were

(a) Nonyl phenol ethoxylate (NPE) (Product name: Surfact-V, Victus Laboratories Private Limited) and

(a) Organosilicone (OS) (Product name: Ultrawet, Laboratories Private Limited).

Plate 5. Ethrel for spraying and 2,4-D for base banding

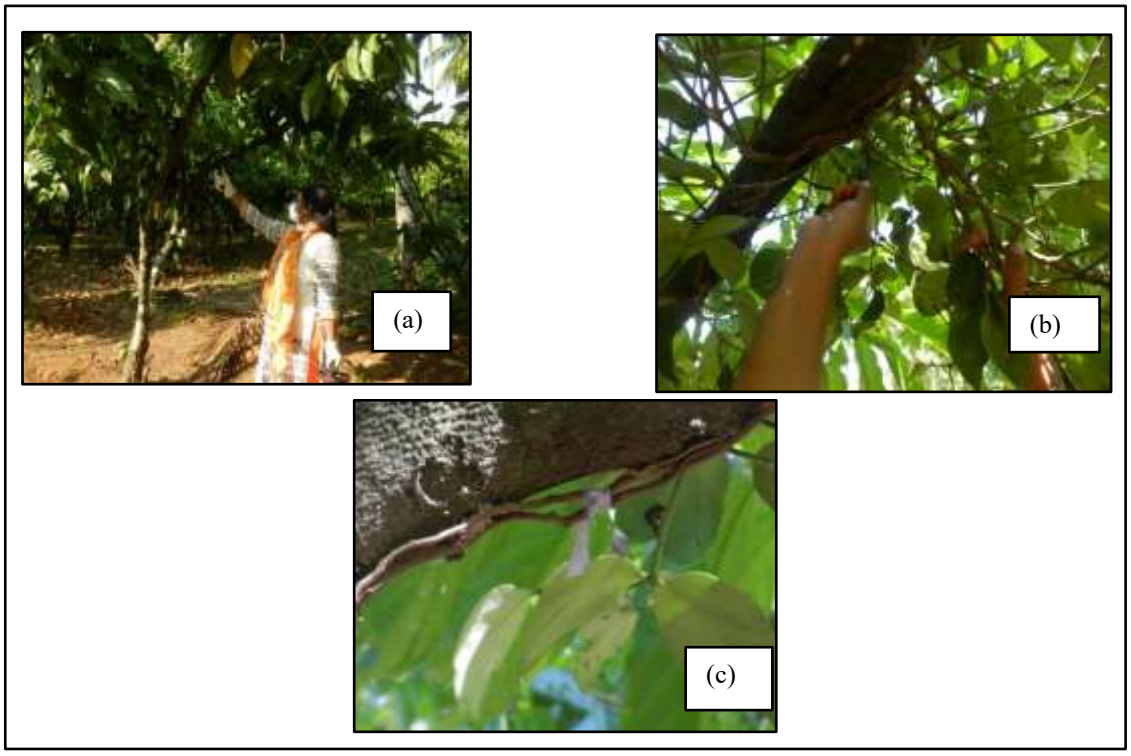

Plate 6. (a) Spraying of ethrel; (b) making wound in the active runner root of parasite prior to base banding (c) base banding with cloth strips immersed in 2,4-D

Three different concentrations of these two surfactants were sprayed to both the parasites observed on cocoa trees of the orchard. Two controls were kept for the experiment,

i) Treatment of ethephon devoid of the surfactants and

ii) Treatment of 1 per cent 2,4-D applied as base banding.

The treatment combination and concentration of each surfactant used is given in table 3. For application of base banding, 1 per cent 2,4-D solution was prepared. (Plate 5). The extent of drying and defoliation were observed, and occurrence of regrowth was noted and expressed as regrowth percentage.

Table 3. Treatment combinations for management of Loranthus.

S. No.	Treatment Name	Treatment combination
1	T1	25ml/L Ethephon + 3ml/L NPE
2	T2	25ml/L Ethephon + 5ml/L NPE
3	T3	25ml/L Ethephon + 8ml/L NPE
4	T4	25ml/L Ethephon + 0.2ml/L OS
5	T5	25ml/L Ethephon + 0.5ml/L OS
6	T6	25ml/L Ethephon + 0.8ml/L OS
7	T7	25ml/L Ethephon (Control)
8	T8	1% 2,4-D base banding (Control)

NPE- Nonyl Phenol Ethoxylate, OS- Organosilicone.

Ethephon treatments were given as foliar application on two different genera of Loranthaceae *viz*., *Dendropthoe falcata* (D) and *Helicanthus elastica* (H) seen in

cocoa plantation. The method of stem banding with 1 per cent 2,4-D (Mathew and Habeeburrahman, 2013) was considered as the second control for the treatments. Base banding with 1 per cent 2,4-D was done by soaking a strip of cotton cloth of 10 cm length and 1cm width in 1 per cent 2,4-D (Plate 6).

The bark of the parasite stem was scrapped to a length of about 0.5 cm at the point of its attachment to the host. The strip of cotton cloth soaked with 1 per cent 2,4-D was tied to the point of infection in the cocoa trees infected with *Dendropthoe falcata* (D) and *Helicanthus elastica* (H).

3.4.1 Observation

The treatments were given on 18.01.2018 during morning hours using a knap-sack sprayers and observation on defoliation and regrowth were taken for a period of twelve months. Number of branches with Loranthus infestation was counted and each constellation was taken as one unit of measurement. Observations included i) time taken for defoliation expressed as per cent, and ii) regrowth pattern expressed as scores. Scoring was given based on visual observation as follows.

Scores-

0- no regrowth
1- sporadic regrowth
2- stunted regrowth
3- normal regrowth
4- profused regrowth

3.4.2 Interpretation of data

Observation on treated trees infected with *Dendrophthoe falcata* and *Helicanthus elastica* up to 12 months after treatment were recorded. Observation on time taken for defoliation was recorded up to 12 months after spraying and

expressed as per cent defoliation. Observation on regrowth pattern was also recorded for a period of 12 months and scoring based on visual observation was given.

Results

4. RESULTS

4.1 Morphological and anatomical characterization of five major Loranthaceae genera *viz.*, *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus.*, *Helixanthera wallichiana* and *Taxillus tomentosus.*

4.1.1 Morphological characterization

Morphological characters of commonly seen Loranthaceae genera *viz*. *Dendropthoe falcata*, *Helicanthes elastica*, *Macrosolen capitellatus, Helixanthera wallichiana*, and *Taxillus tomentosus* collected from plains and highlands of Kerala were studied. Basic morphological features and haustorial characteristics of different genera of Loranthaceae observed are detailed below.

4.1.1.1 *Dendrophthoe falcata*

Dendrophthoe falcata was observed as a bushy shrub, branched. It was seen parasitized on the branches of cocoa. Samples were collected, identified and authenticated according to Fischer's collection No. 5861. Leaves were oppositely arranged, oblong falcate in shape. The observations on different leaf parameters *viz*., leaf length, breadth, thickness and leaf area is given in table 4. The maximum length of leaf observed was 16.8 cm and minimum was 13.1cm with a mean of 15.38cm. The maximum breadth observed for the leaf of *D. falcata* was 4.2 cm and minimum was 3.2cm and the mean observed was 3.64 cm. Maximum thickness recorded for the parasite was 0.7 cm and minimum 0.5 cm with an average of 0.53cm. Leaf area of *D. falcata* was recorded to be 46.93 cm^2 and minimum area was 35.77cm^2 with an average of 42.406 cm^2. Flowers buds were pale green - white tubes. Fruits were ovoid and red in colour (Plate 7,8) Flowering was observed from November to February.

The haustorial system observed in *D. falcata* was epicortical roots (ERs), holdfast on the basal portion of haustorial attachment to host was found to be swollen and basal epicortical roots were also observed (Plate 9).

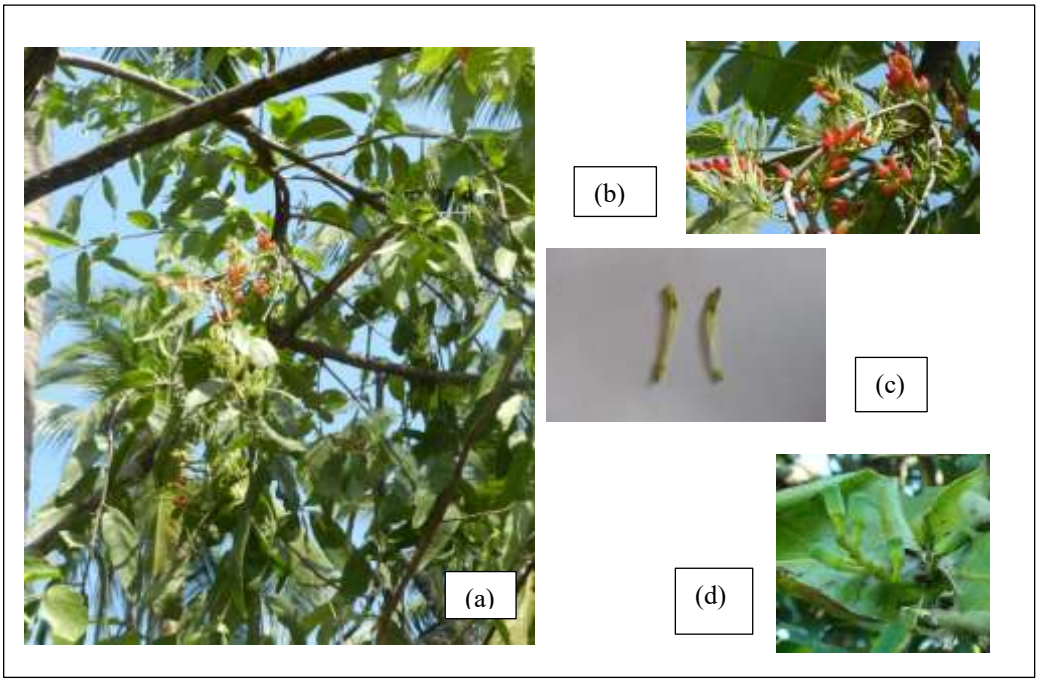

Plate 7. *D. falcata* (a). Parasite on host Cocoa; (b) *D. falcata*- inflorescence in branch; (c) *D. falcata*- flowers; (d) *D. falcata* - fruits

Plate 8. *D.falcata* -different flower colours

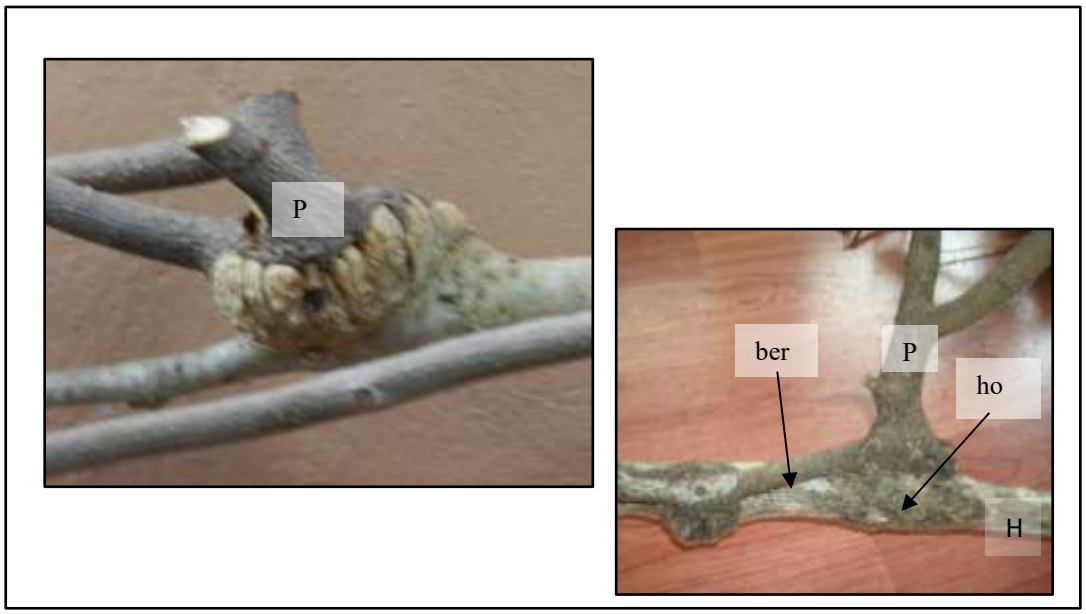

Plate 9. (a) Haustorial attachment of parasite *D. falcata* with the host (Mulberry), (b) *D. falcata* branching pattern; ber- basal epicortical root, ho-holdfast.

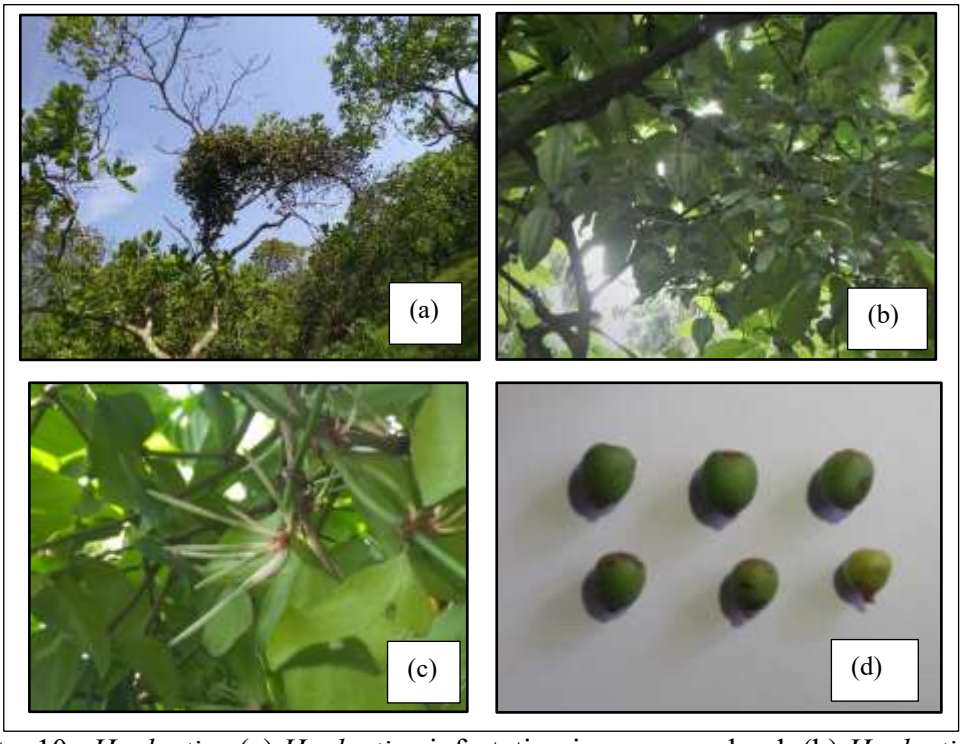

Plate 10. *H. elastica* (a) *H. elastica* infestation in cocoa orchard; (b) *H. elastica* constellation in Cocoa; (c) *H. elastica* inflorescence; (d) *H. elastica* seeds

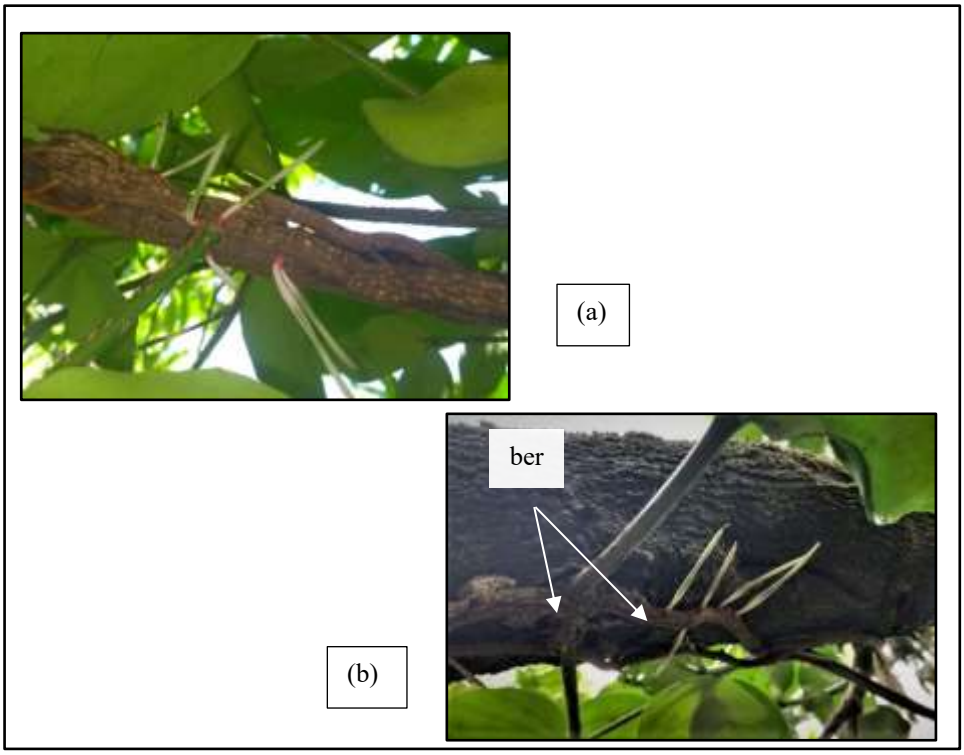

Plate 11. (a) & (b) *H. elastica* – Branching pattern; ber-basal epicortical root

Plate 12. (a) *M. capitellatum* infestation on Jack fruit tree;
(b) *M. capitellatum* inflorescence;(c) *M. capitellatum* fruits

4.1.1.2 *Helicanthus elastica*

Helicanthes elastica was observed as a thickly branched woody shrub on the branches of cocoa. Samples were collected identified and authenticated according to Fischer's collection No. 5851. Leaves were dark green, oblong with blunt base and tip, oppositely arranged with reticulate venation and pale green fascicle like flowers were observed (Plate 10). Leaves showed a maximum length of 12.8 cm and minimum of 10.2 cm with a mean of 11.76 cm. maximum breadth observed for the parasite was 7.4 cm and minimum was 5.9cm with an average breadth of 6.8 cm. The parasite had leaves with maximum thickness of 0.5 cm and minimum of 0.3 cm, where the average thickness was found to be 0.38cm. Maximum leaf area of *H. elastica* was found to be 58.61cm^2 and minimum 53.14 cm^2 with a mean of 56.49cm^2 (Table 4). Flowering was observed in September – December and during April -May. Fruits observed were young dark green globose shaped berries. White coloured sticky gum like exudes were observed when the berries are detached from its stalk.

Haustorial system of *H. elastica* was found to be basal epicortical root (Plate 11), which bears leaf axils, floral nodes and secondary branches, growing parallel to the host branch.

4.1.1.3 *Macrosolen capitellatus*

Macrosolen capitellatus was observed in plains as a parasitic shrub, appeared as another branch of the host, on branches of nutmeg and jackfruit tree. Samples were collected, identified and authenticated according to Fischer's collection No. 5884. They were observed to have limited host range compared to other genera. They have a single point of attachment to the host stem and appear to branch in all directions. Leaves were observed as narrow lanceolate shaped with reticulate venation and oppositely arranged. Leaf characteristics are detailed in Table 4. Maximum leaf length measured was 8.4 cm and minimum was 6.9cm with a mean of 7.48 cm. Leaf was measured to have a maximum breadth of 4.2 cm and minimum of 3.4 cm with a mean of 3.74 cm. Leaf thickness of *M. capitellatus* ranged between 0.4 cm and 0.3 cm with an average thickness of 0.32 cm.

Plate 13. (a). *M. capitellatum* branches and inflorescence; (b) *M. capitellatum* branching pattern

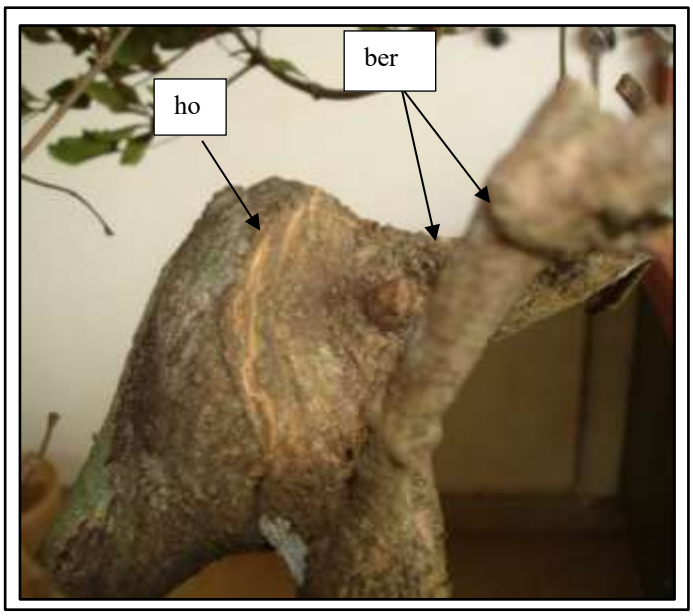

Plate 14. *M. capitellatum* haustorial point of attachment; ber-basal epicortical root, ho-holdfast

maximum leaf areas was found to be 24.19 cm^2 and minimum was 16.71 cm^2 with a mean of 18.73 cm^2. Flowers were racemes, short tube like and pedicellate, buds were greenish in colour and petals were greenish to pale yellow. Flowering was observed during April – June. Fruits were dark green globose berries (Plate 12 & 13). Basic haustorial type was identified to be basal epicortical root (ber), with a single point of attachment to the host stem, slightly swollen holdfast (Plate 14) Axillary branches were observed to emerge from the portion above ber.

4.1.2.1.4 *Helixanthera wallichiana*

Helixanthera wallichiana was observed as a stout shrub on the branches of litchi and orange trees in the high ranges (Plate 15 (a)). Samples were collected, identified and authenticated according to Fischer's collection No. 2773. The leaves were shiny green, narrow ovate in shape, with reticulate venation and oppositely arranged (Plates. 15 (b) & 16). Leaf characteristics are detailed in Table 4. Maximum leaf length of *H. wallichiana* recorded was 6.8 cm and minimum was 5.7 cm with an average length of 6.3 cm. Leaf breadth of the parasite ranged between 2.6 cm and 2.0 cm with an average breadth of 2.34 cm. the average leaf thickness of the parasite recorded was 0.4 cm with a maximum of 0.5 cm and minimum of 0.3cm. Maximum leaf area of *H. wallichiana* was found to be 15.88 cm^2 and minimum was 1.4cm^2 with a mean leaf area of 13.06 cm^2. Flowering was seen during March-April. Basic haustorial system was observed to be basal epical root (Plate 17), with several axillary branches arising from either side of the stem. Elongation of the parasite stem is parallel to the host stem along the axis of basal epicortical root growth (Plate 18).

4.1.2.1.5 *Taxillus tomentosus*

Taxillus tomentosus was observed as brown coloured shrub with branches on the stem of Phyllanthus emblica in highlands. Leaves were yellow green to brownish in appearance with comparatively delicate texture and waxy, narrow ovate shaped (Plate 19). Leaf characteristics are detailed in table 4. *T. tomentosus* leaves had a maximum length of 4.4 cm and a minimum of 3.7 cm, with an

Plate 15. (a) *H. wallichiana* infestation on Litchi tree; (b) *H. wallichiana* branch with infllorescence

Plate 16. (a) *H. wallichiana* leaves; (b) *H. wallichiana* inflorescence

Plate17. *H. wallichiana* branching pattern; ber- basal epicortical root, ho- holdfast.

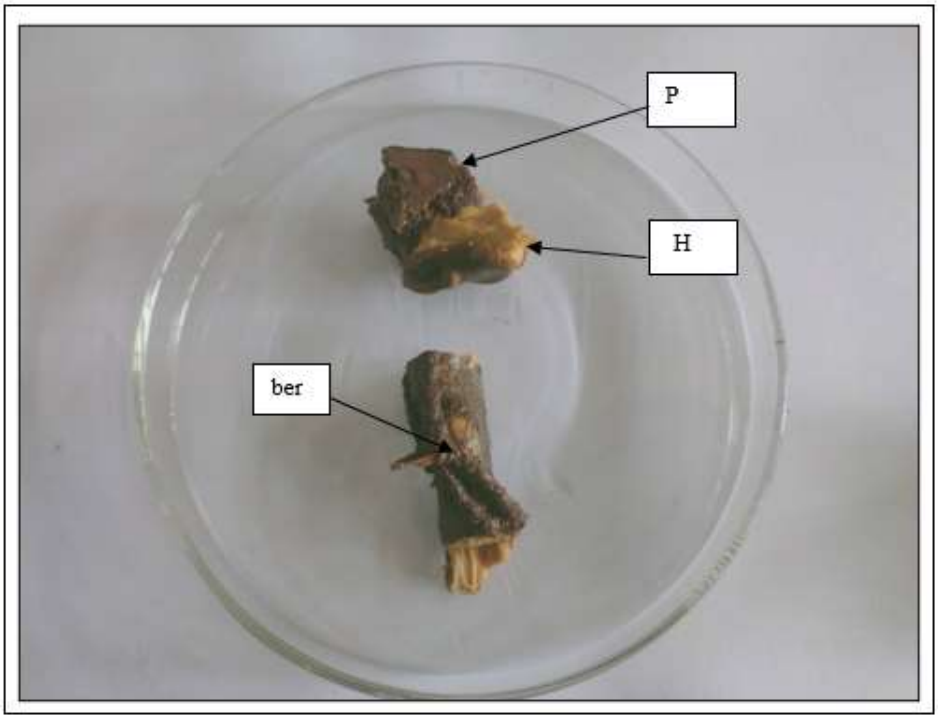

Plate 18. *H.wallichiana* haustoria and branching pattern;ber-basal epicortical root,H-host, P-parasite.

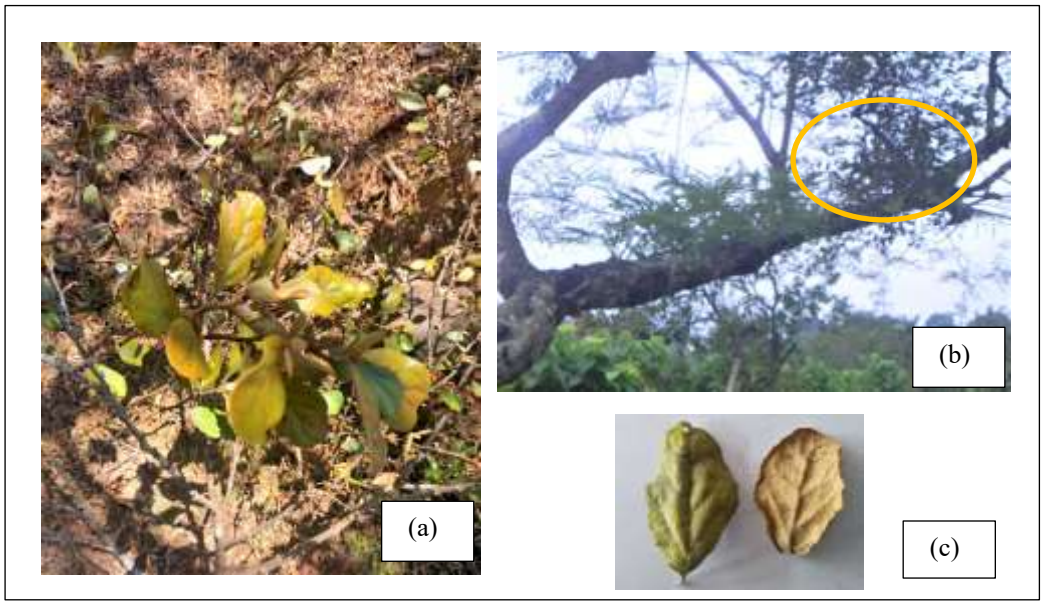

Plate 19. (a) *T. tomentosus* branch; (b) *T. tomentosus* infected on *Emblica officinalis;* (c) *T. tomentosus* leaves.

Plate 20. (a) & (b) *T. tomentosus* inflorescence; (c) *T. tomentosus* fruits

Table 4. Morphological observation of leaves - different genera of Loranthus

S.No.	Genera	Parameter			
		Length (cm)	Breadth (cm)	Thickness (cm)	Leaf area (cm^2)
1	*D. falcata*	15.38±1.43	3.64±0.48	0.53±0.07	42.406±4.46
2	*H. elastica*	11.76±0.99	6.8±0.76	0.38±0.08	56.498±4.44
3	*M. capitellatus*	7.48±0.59	3.74±0.38	0.32±0.04	18.73±3.02
4	*H. wallichiana*	6.3±0.52	2.34±0.24	0.4±0.1	13.066±2.36
5	*T. tomentosus*	3.9±0.29	2.42±0.36	0.3±0.00	7.316±1.58

average of 3.9cm. Leaves were observed to have a maximum breadth of 2.9 cm and a minimum breadth of 2.1 cm with a mean value of 2.42 cm. Leaf thickness of the parasite was observed to be 0.3 cm and maximum leaf area recorded was 9.99cm^2 and minimum was 6.14 cm^2 with a mean value of 7.316cm^2. Flowering season was from December to February. Flowers were short stalked, light brown in colour. Fruits were elongated berries, stout towards the basal end (Plate 20). The basic haustorial system was observed to be basal epicortical root and holdfast was also identified (Plate 21).

4.1.2.2 Anatomical characterization

The anatomical characters of the haustoria of major genera of Loranthaceae in Kerala *viz*. *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana*., and *Taxillus tomentosus* have been studied. The plant samples with the wooden portion containing the haustoria was collected and permanent slides were preapared. These slides were observed using an image analyser (CatCam 500E series) which was provided with a microscope (Motic BA 210) and Zeiss Stemi 305 Compact stereo microscope.

The anatomical characters of *D. falcata* is given in Plate 22. The transverse section (T.S.) shows structure of a typical dicot stem after secondary thickening.

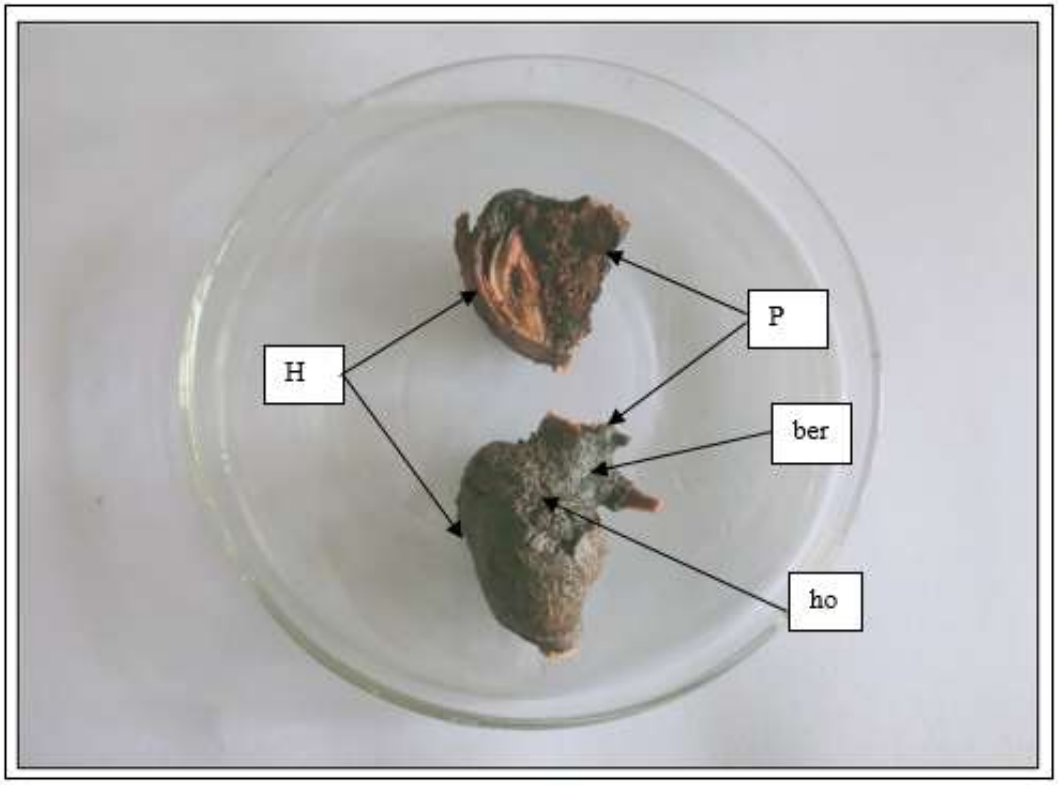

Plate 21. *T. tomentosus* haustoria; H- host; P-parasite, ber- basal epicortical root, ho-holdfast.

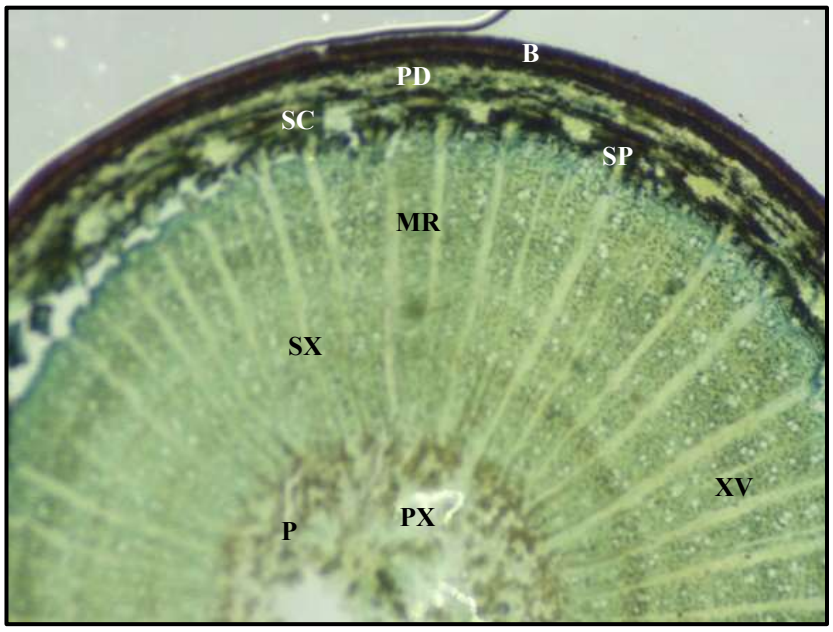

Plate 22. CS of *D. falcata* in biocular light microscope. (Magnification X 40. PX- Primary Xylem Crushing Pith, P- Pith, SX- Secondary Xylem, XV- Xylem Vessels; MR- Medullary rays, SP- Secondary Phloem, PD- Periderm, SC-Secondary Cortex, B-Bark).

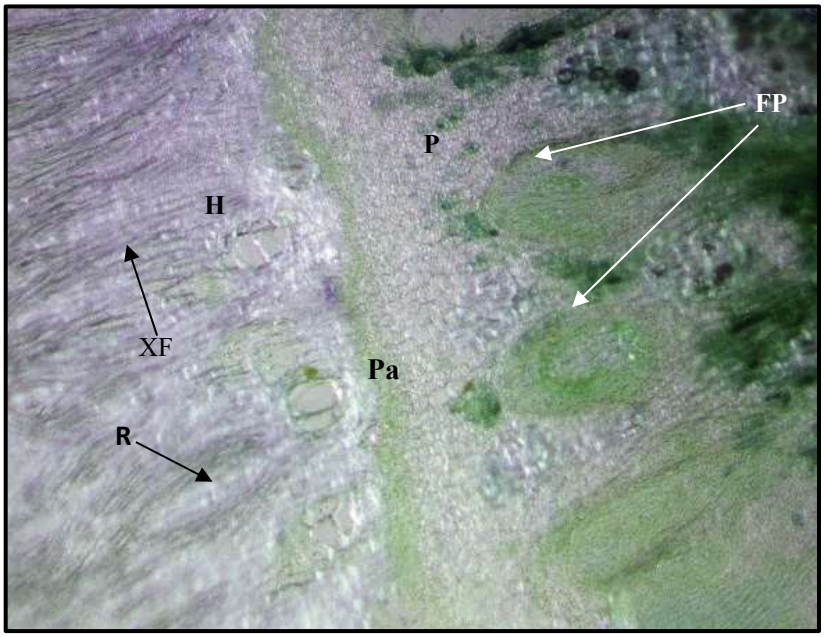

Plate 23. Haustoria of *D. falcata* in phase contrast microscope. (Magnification 10X. H- Host region, P-Parasite Region, Pa- Parenchymatous region. R- Rays, XF- Xylem Fibres, FP-finger like projections (could be elongated parenchyma cells formed by parasite)).

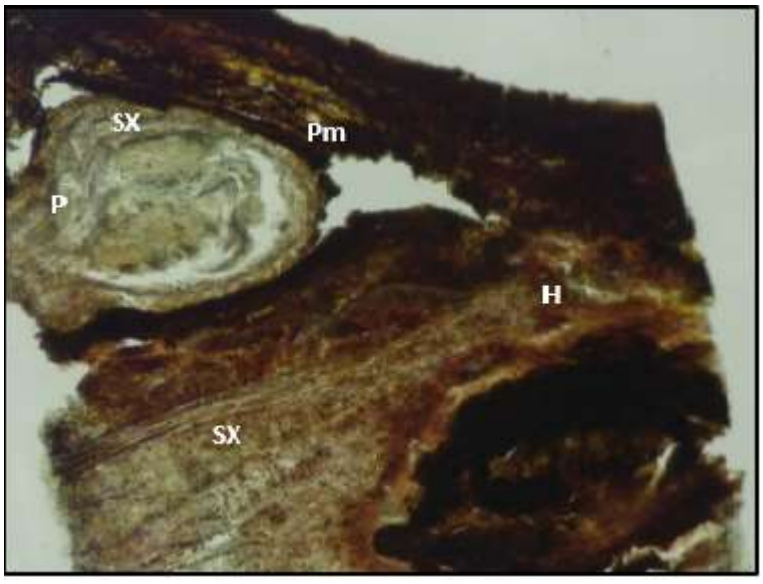

Plate 24. Haustorial attachment of *H. elastica* in biocular light microscope. (Magnification X 2.0.H- Host region, P-Parasite region, Pm- Periderm, SX- Secondary Xylem).

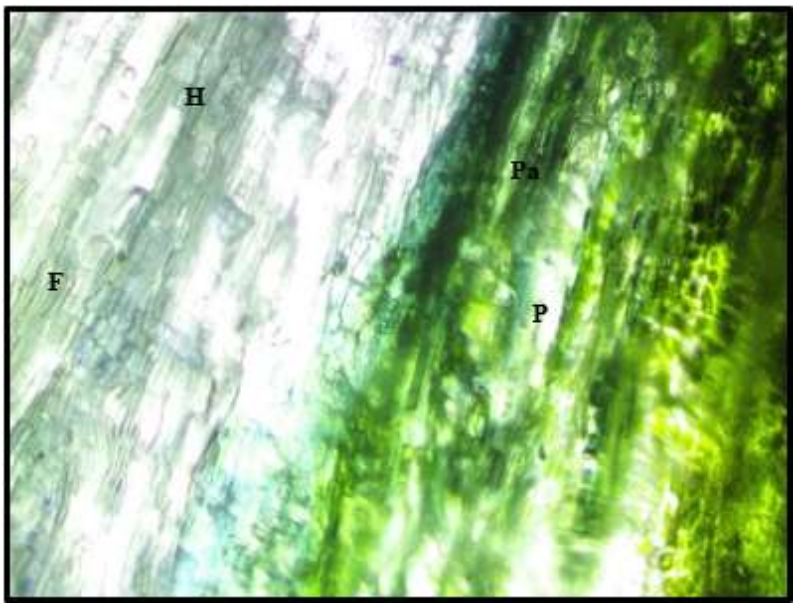

Plate 25. Haustorial portion of *H. elastica* under phase contrast microscope. (Magnification X 10. H- Host region, P-Parasite Region, Pa- Parenchymatous region, F- Fibre).

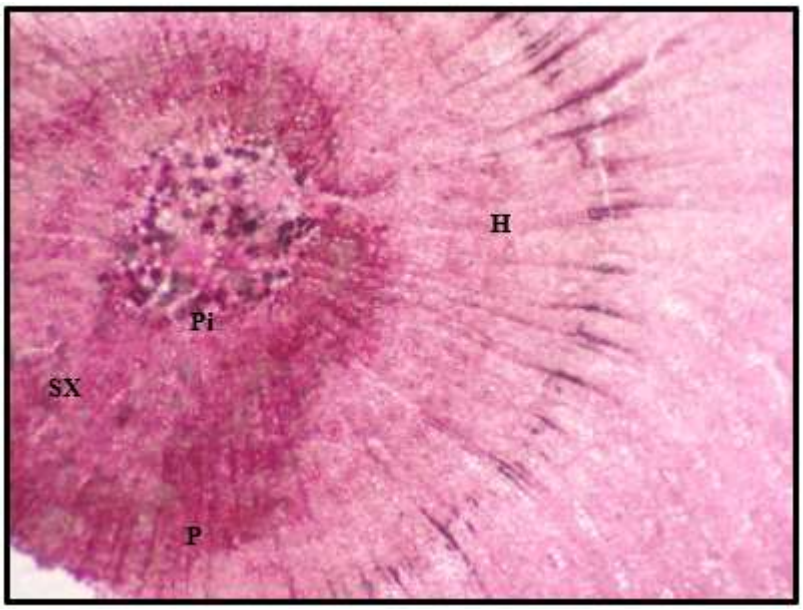

Plate 26. Haustorial portion of *M. capittelatus* in biocular light microscope. (Magnification X 4.0. H- Host region, P-Parasite Region, SX- Secondary Xylem, Pi- Crushed Pith with lignin deposition).

The outer layer is the periderm followed by secondary phloem. The secondary phloem crushes the cortical cells and the primary phloem due to secondary thickening. The secondary xylem cells alternating with the medullary rays are seen after the secondary phloem. Primary xylem is seen crushing the pith at the innermost position. Plate 23 shows the intersection of the parasite entering the host tissues (Mulberry). Junction at which the parasite invades the host tissues has parenchyma cells (Pa) with slight deposition of chlorophyll pigments. Secondary tissues like ray cells are prominent at the host portion. An arrangement of xylem vessels at the junction of haustorial cells is also visible.

Haustorial attachment of *H. elastica* with the host, mango is given in plate 24. Intrution of parasite tissues breaking the periderm of the host is visible in the image. Periderm and secondary tissues of both the host and parasite are prominently seen where the parasite joins the host. The point of invasion by parasite into the host is represented in plate 25. Parenchyma cells with chloroplast is seen in parasite portion. Fibers formed after secondary thickening is seen at the host portion.

M. capittelatus is seen to be joining with the host (Nutmeg) cells in plate 26. The parasite cells have so completely merged with the host cells that it is difficult to see a demarcation between the two. Both the host and the parasite shows cells after secondary thickening. The pith region is seen with some depositions (lignin). Parasite (*M. capittelatus)* and host (Nutmeg) interacting region is visible in the figure given in plate 27. Parenchyma cells with chloroplast are seen at the haustorial portion. Secondary xylem elements are seen at the host part.

Haustorial portion of *H. wallechiana* with its host orange is shown in plate 28. The figure represents the junction at which the parasite cells invade and intervene the host tissues. Secondary xylem elements like fibres and rays of the host are present. The figure 29 shows the point where the parasite haustoria invades the host tissues as seen under stereo microscope of a magnification of 4.0.

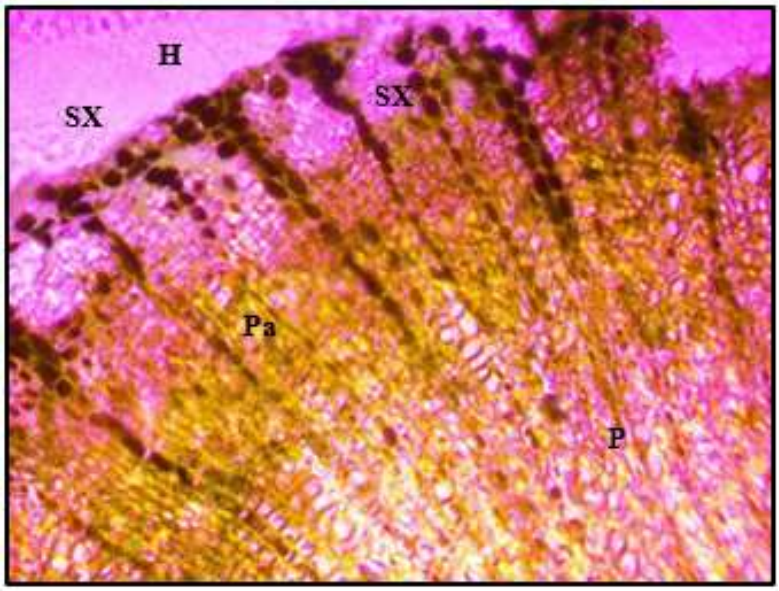

Plate 27. Haustorial portion of *M. capittelatus* in phase contrast microscope. (Magnification X10. H- Host region, P-Parasite region, SX- Secondary Xylem Pa- Parenchyma cells with chloroplast).

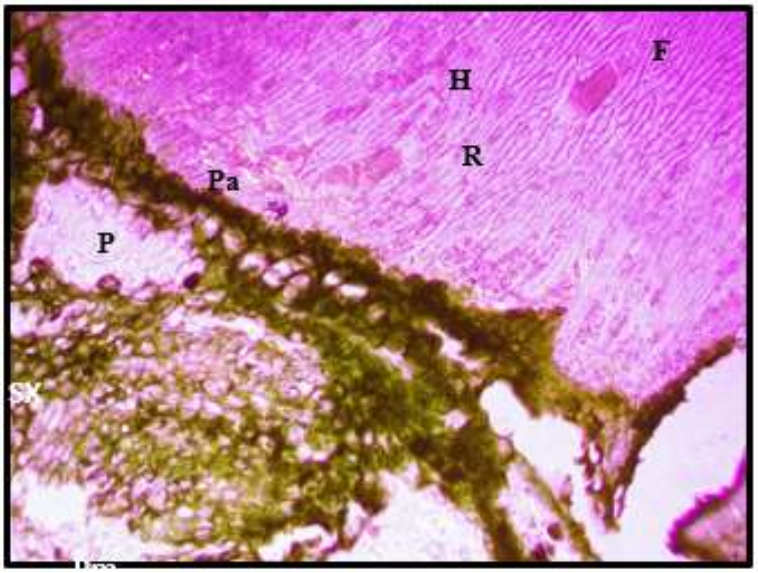

Plate 28. Haustorial portion of *H. wallechiana* in phase contrast microscope. (Magnification X 10. H- Host region, P-Parasite region, Pa- Parenchymatous region, R- Rays, F- Fibres).

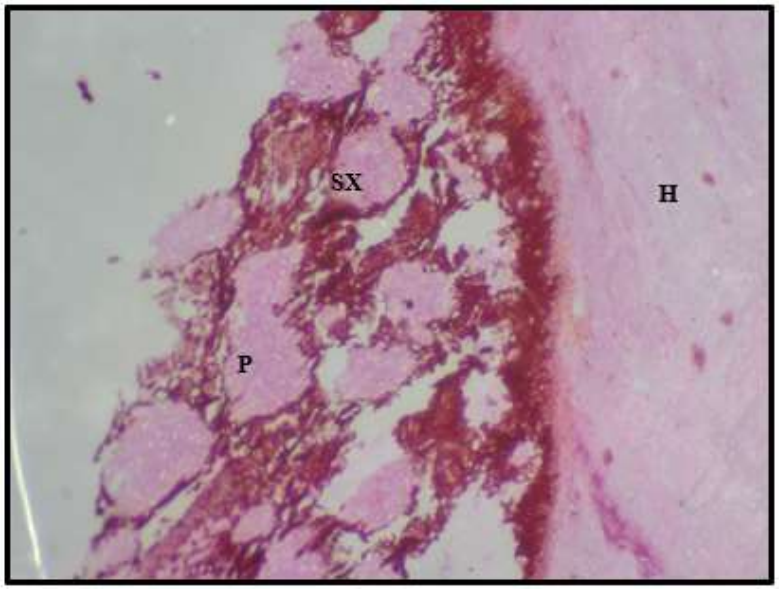

Plate 29. Haustorial portion of *H. wallechiana* in biocular light microscope. (Magnification X 4.0. H-Host region, P-Parasite region, SX- Secondary Xylem elements).

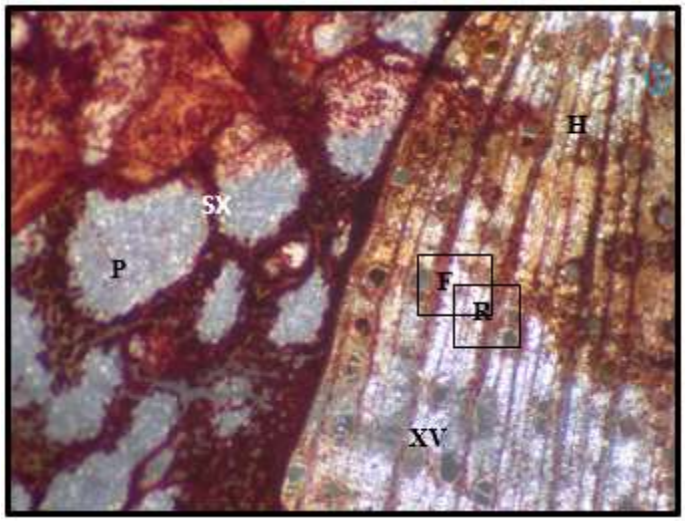

Plate 30. Haustorial portion of *T. tomentosus* in biocular light microscope. (Magnification 4.0. H-Host region, P-Parasite region, R- Rays, F- Fibres, SX Secondary Xylem, XV Xylem Vessels).

Haustorial portion of *T. tomentosus* in stereo microscope (Plate 30) shows a clear demarcation between the host region (*Phyllanthus emblica*) and the parasitic region. The host region shows typical secondary thickening with prominent secondary xylem elements like xylem vessels, fibers and medullary rays. Haustorial portion of *T. tomentosus* in phase contrast microscope of magnification 10x is given in plate 31. The xylem vessels are prominent in the host region with medullary rays.

4.2 Genetic Diversity analysis of five major Loranthaceae genera *viz.* *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana,* and *Taxillus tomentosus.*

4.2.1 Molecular characterization

To explore the genetic diversity of a plant species, molecular markers independent of environment have been successfully used. Samples of five different major genera of Loranthaceae were collected from different locations. Their DNA isolation method were standardised.

4.2.1.1 DNA isolation

The genomic DNA was extracted from the collected leaf samples using the CTAB method with slight modifications (Doyle and Doyle, 1987). The leaves after being finely ground to fine powder in liquid nitrogen were mixed with freshly prepared CTAB extraction buffer and incubated at 50°C for 15–20 minutes in hot water bath before being subjected to centrifugation at 12000 rpm for 5 minutes. The resultant supernatant was treated with chloroform: isoamyl alcohol (24 : 1) followed by another centrifugation at 13000 rpm for 1-2 minutes. The pellet obtained after 7.5 M ammonium acetate treatment was washed several times with 70% ice-cold ethanol and dried before being resuspended in sterile DNase-free double distilled water. The DNA sample obtained was further incubated at 65°C for 20 minutes to destroy any DNase if present and stored at 4°C for subsequent analysis. DNA quality and quantity were determined through spectrophotometry at 260 and 280 nm, respectively. The purity and integrity were

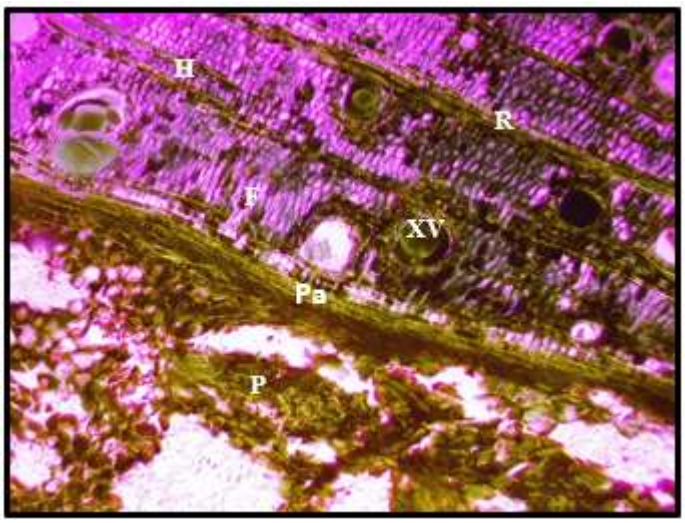

Plate 31. Haustorial portion of *T. tomentosus* in phase contrast microscope. (Magnification X10. H- Host region, P Parasite region, Pa Parenchymatous region, R- Rays, F- Fibres, XV- Xylem Vessels).

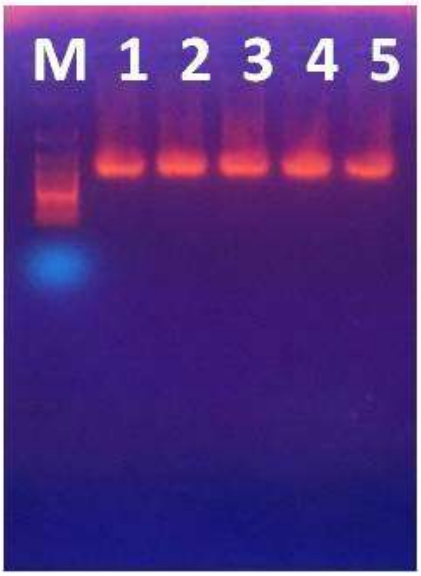

Plate 32. Visualization of genomic DNA in Agarose gel; (Lane M- DNA ladder. Lane 1-5 Genomic DNA isolated from samples 1-5 (1- *D. falcata*, 2- *H. elastica*, 3- *H. wallichiana*, 4. *T. tomentosus* and 5- *M. capitellatus)*).

later checked by performing 1.0% agarose gel electrophoresis and comparing the intensity of the resultant bands with 50bp DNA ladder. The DNA samples were finally diluted to 50 ng/µL and stored at −20°C for further use.

4.2.1.2 Quantification of DNA

The quantification of DNA isolated was done using UV visible spectrophotometry. For the assessment of purity, the reading of absorbance ratio at A260/A280 were recorded (Table 5).

Table 5. Quality and quantity of DNA of plant samples by UV visible spectrophotometry

S.No.	*Genera*	A260nm	A280nm	A260/A280	Concentration ng/µl
1	*D. falcata*	0.215	0.112	1.91	1075
2	*H. elastica*	0.241	0.126	1.91	1205
3	*M. capitellatus*	0.239	0.129	1.85	1195
4	*H. wallichiana*	0.264	0.135	1.95	1320
5	*T. tomentosus*	0.255	0.129	1.97	1275

4.2.1.3 Agarose gel electrophoresis

One g agarose was weighed and dissolved in 100 ml 1X TAE buffer by heating and constant stirring in a water bath at 95ºC. After cooling (35 ºC) add 2 µl of (10 mg/ml) Ethidium bromide solution into it and caste the gel. After solidifying and transferring the gel into the electrophoretic apparatus containing 1X TAE buffer DNA of samples (5 µl)was mixed with 1X gel loading buffer and loaded on to agarose gel (1%) containing ethidium bromide (5µg/mL) along with 50 bp molecular weight marker (Himedia) for electrophoresis at 50 V for half an hour. After the run, the gel was analyzed under UV transilluminator. DNA bands were observed and photographed (Plate 32).

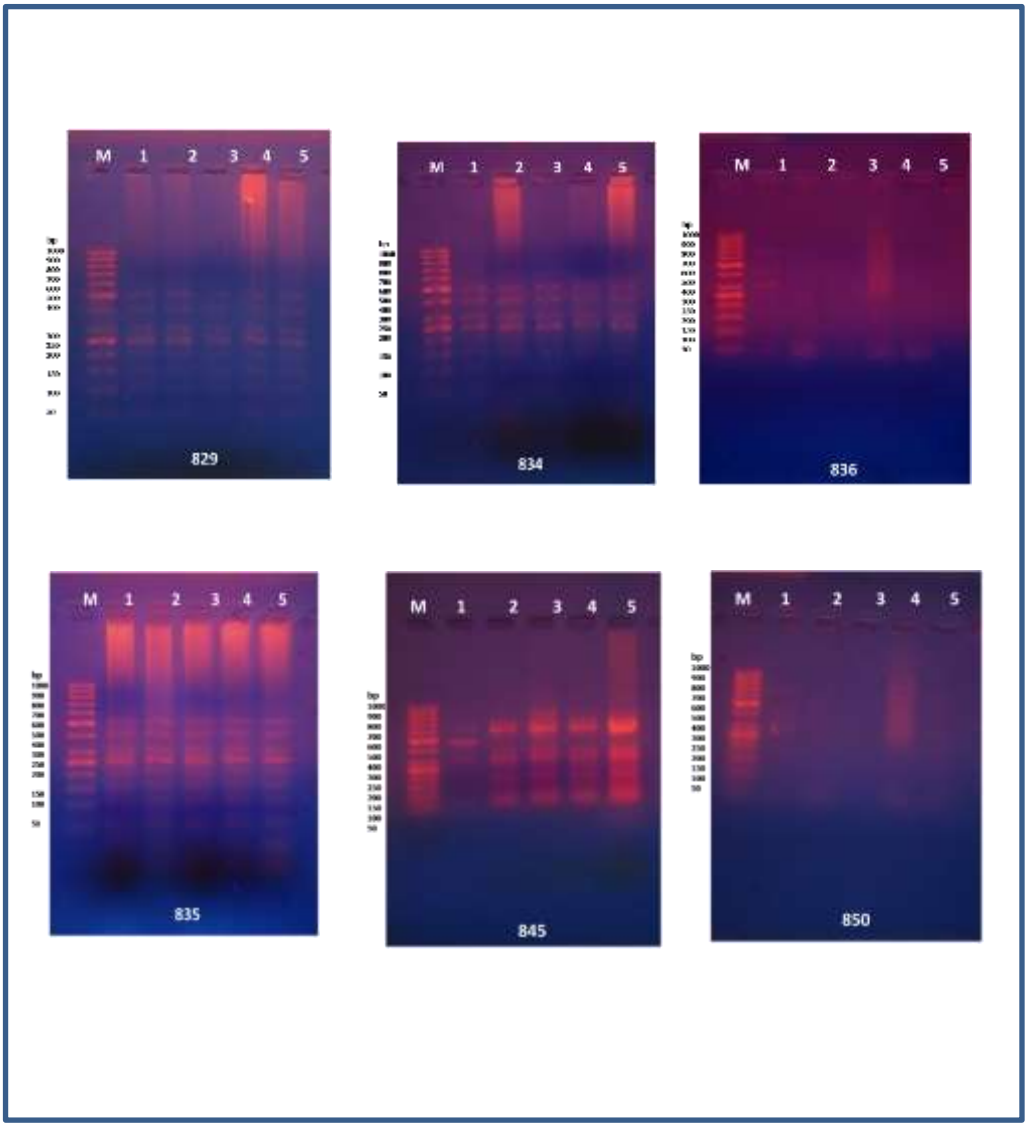

Plate 33. (a) Amplification profiles generated by ISSR primers

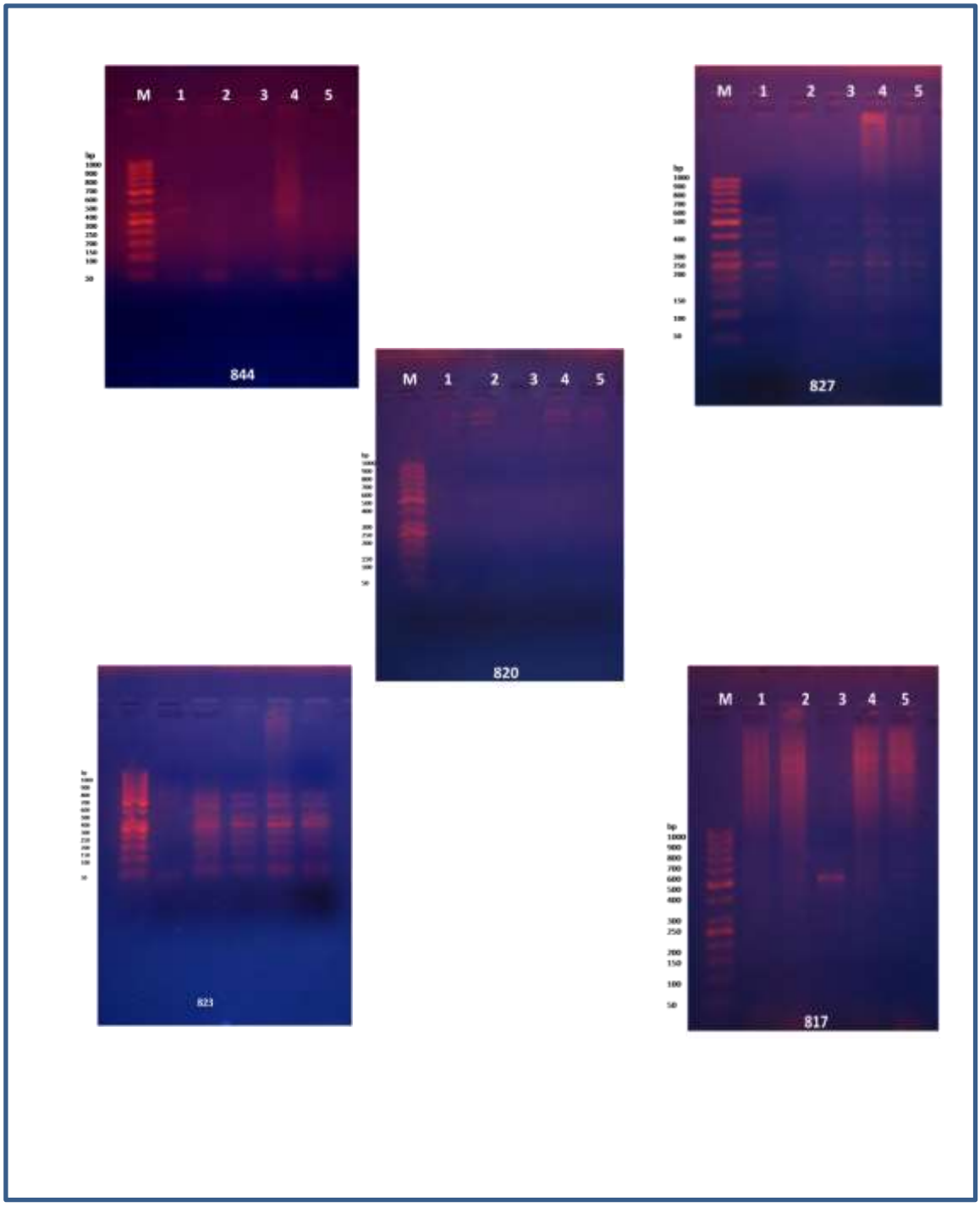

Plate 33 (b). Amplification profiles generated by ISSR primers

4.2.1.4 *Inter Simple Sequence Repeat (ISSR) assay*

The genetic profile study of the given plant samples was done using 25 ISSR markers, selected after screening from forty ISSR primers. The selected primers showed good, reliable, repetitive, and distinct bands which enabled effective scoring for genetic diversity study within and among the populations. Details of markers used for ISSR assay are given in table 6.

DNA amplification mixture of 25 µL contained 25 ng template DNA, 1x PCR buffer, 1.5 mM MgCl2, 200 mM dNTPs, 1 µM ISSR primers, and 0.6 U Taq DNA polymerase and double distilled sterile water. PCR components were prepared as master mix for each primer to minimize the pipetting error. Amplification reaction was performed in a thermal cycler (SimpliAmp, Thermo Fisher) with amplification cycle condition of initial 4 minutes' strands separation at 94°C followed by 40 cycles of 94°C for 45 secs, 53°C for 1 min, and 72°C for 2 minutes and final extension at 72°C for 10 minutes. The products obtained after PCR amplification were electrophoresed in 2.5% agarose gel in 0.5x TAE buffer at 100 V for around 3 hours and gel was stained with ethidium bromide (0.5 µg/mL). Fragments after staining were visualized in a UV transilluminator and photographed. A 50 bp DNA ladder (Hi-media) was used as a size marker for every gel run.

4.2.1.5 Phylogenetic analysis

The genetic variation analyzed among five plant samples using 25 ISSR primers. All the plants showed a high percentage of monomorphic bands (bands common) in all the five samples. A few polymorphic bands were also identified (Unique to one population). Among the ISSR primers, primer 816, 817, 818, 820, 823, 827, 829, 834,835, 836, 844, 845 showed good amplification (Plate 33 (a) and (b)).

Table 6. List of markers selected after screening

S.No.	Name	Sequence
1	ISSR 808	5’AGAGAGAGAGAGAGC3’
2	ISSR 809	5’AGAAGAGAGAGAGAC3’
3	ISSR 811	5’CACAGAGAGAGAGAC3’
4	ISSR 815	5’CTCTCTCTCTCTCTG3’
5	ISSR 816	5’CACACACACACACAA3’
6	ISSR 817	5’CACACACACACACAA3’
7	ISSR 818	5’CACACACACACACAG3’
8	ISSR 820	5’GTGTGTGTGTGTGTC3’
9	ISSR 823	5’TCTCTCTCTCTCTCC3’
10	ISSR 826	5’ACACACACACACACC3’
11	ISSR 827	5’ACACACACACACAGC3’
12	ISSR 829	5’TGTGTGTGTGTGTGC3’
13	ISSR 834	5’AGAGAGAGAGAGAGAGYT3’
14	ISSR 835	5’AGAGAGAGAGAGAGAGYC3’
15	ISSR 836	5’AGAGAGAGAGAGAGAGYA3’
16	ISSR 841	5’GAGAGAGAGAGAGAGAYC3’
17	ISSR 844	5’CTCTCTCTCTCTCTCTRC 3’
18	ISSR 845	5’CTCTCTCTCTCTCTCTRG3’
19	ISSR 847	5’CACACACACACACACARC5’
20	ISSR 848	5’CACACACACACACACARG5’
21	ISSR 849	5’CTCTCTCTCTCTCTCTRA5’
22	ISSR 850	5’CTCTCTCTCTCTCTCTRT5’
23	ISSR 851	5’CACACACACACACACARA5’
24	ISSR 852	5’CACACACACACACACART5’
25	ISSR 853	5’AGAGAGAGAGAGAGAGYG5’

4.2.1.6 ISSR Data analysis

From the results of ISSR assay, well resolved fragments were scored for presence (1) and absence (0) of bands of various molecular weight sizes. The data obtained were converted into binary data matrices containing arrays of 0 and 1s. the data was analysed using MEGA X.

Based on the dendrogram, as per ISSR band clustering five groups were obtained. The genera present in the same cluster are more phylogenetically similar

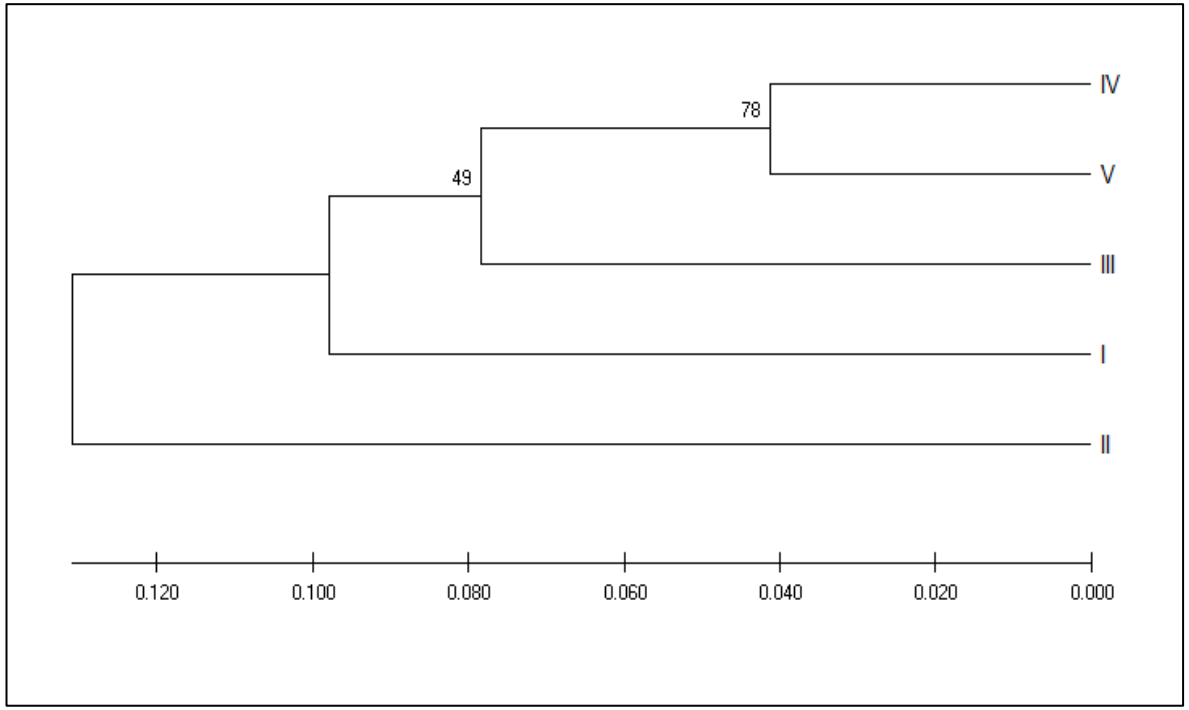

Plate 34. Dendrogram showing similarity relationship of Loranthaceae, (I- *D. falcata*, II- *H. elastica*, III- *H. wallichiana*, IV- *T. tomentosus* and V- *M. capitellatus*).

compared to those present in distant cluster. In the dendrogram, roman letters I, II, III. IV and V indicate the five genera of Loranthaceae, *viz.*, *Dendrophthoe falcata*, *Helicanthus elastica*, *Helixanthera wallichiana*, *Taxillus tomentosus* and *Macrosolen capitellatum* respectively. Cluster analysis was generated by the Unweighted Pair Group Method (UPGMA). Two main clusters are formed with genera I, III, IV and V together and genera II as a lone cluster. Genera IV and V were observed to be more similar compared to other genera (Plate 34).

4.3. Host parasitic interaction studies

4.3.1 Translocation and partitioning studies

The interaction between host and parasite was studied using radioactive ^{32}P application on host as well as on parasite and the leaf samples were collected to measure radioactivity using scintillation counters. ^{32}P was applied to the surface running roots of cocoa plant and leaf samples of *Dendrophthoe falcata* and *Helicanthus elastica* collected and used for the study.

Table 7. Radioacivity (counts per minute per gram) of host and parasite after root feeding cocoa with labelled ^{32} P.

S. No.	Treatments	Sampling frequency	
		7DAT	14DAT
1	C leaves for D	144.66 (12.03)	0.1^{d} (0.707)
2	D leaves	139.33(11.80)	372.67^{ab} (19.30)
3	C leaves for H	248.33 (15.17)	153^{c} (12.33)
4	H leaves	117.33 (10.83)	220.33^{bc} (14.79)
5	C young bud leaves	125.33 (11.19)	509.67^{a} (22.12)
CD (5%)		**NS**	**4.759**

*transformed values after square root transformation are given in paranthesis. (C-Cocoa, D- *D. falcata*, H-*H. elastica*, C- Cocoa, DAT- Days After Treatment).

The results of radioactivity from cocoa leaves on 7^{th} and 14^{th} day revealed that, there was movement of labelled ^{32}P into the parasite from the host. Radioactivity counts of samples taken for observation is given in table 7. Observation on the 7^{th}

day revealed that, the amount of ^{32}P translocated from host to parasite and the developing bud of cocoa (the host) was uniform and statistically there was no difference. However, sampling on the 14th day revealed, young bud of cocoa (509.67 c/m/g) accumulated maximum ^{32}P followed by *D. falcata* (372.67 c/m/g) and *H. elastica* (220.33 c/m/g). Least accumulation was seen in cocoa leaves bearing the parasites, *H. elastica* (220.33c/m/g) and *D. falcata* (0.1 c/m/g).

Labelled ^{32}P was fed to the parasites, *D. falcata* and *H. elastica* by foliar application. Leaf samples of host collected from nearest branches of application and from branches distant from the point of application. The first sampling was taken on the 2nd day after foliar application of ^{32}P followed by the 7th and 14th days after foliar application. The radioactivity detected by scintillation counter is given in Table 8. After two days of foliar application, radioactivity as detected in host, revealing that, labelled ^{32}P applied on the parasite was translocated to the host (cocoa), indicating a clear two way communication between the host and parasite. Even though there is no statistical difference in the radioactive counts from the host leaves two days after ^{32}P application, highest accumulation was observed in new developing bud of the host (750.33 c/m/g) followed by the leaves of the host far away from the leaves of the parasite where ^{32}P was applied rather than the nearer host leaves. Radioactive counts of cocoa leaves farther from the point of application of parasite *D. falcata* observed on 2nd day after ^{32}P foliar application was comparatively higher (496.67 c/m/g) than that of host leaves (206.67c/m/g) farther from the point of application of ^{32}P on *H. elastica.*

The results from seven days after foliar application of ^{32}P to the parasite, *H. elastica* also reveal that there is translocation of ^{32}P from the parasite to the host. Maximum translocation of ^{32}P was observed in developing bud of cocoa (3131.66 c/m/g) followed by the cocoa leaves (154.67) nearer to the point of application in *H. elastica* and was found statistically significant. The radioactive counts of cocoa leaves 7 days after foliar application of ^{32}P in *D. falcata* was highest in distant leaves (91.67 c/m/g).

Table 8. Radioactivity (counts per minute per gram) of host after foliar application on *D. falcata* and *H. elastica*.

S. No.	Treatments	Sampling frequency		
		2 DAT	7 DAT	14 DAT
1	C near leaves to H	143 (11.97)	154.67^{b} (11.12)	361.33^{b} (18.21)
2	C distant leaves to H	206.67 (14.14)	70.33^{bc} (7.79)	186^{b} (12.72)
3	C new leaves to H	750.33 (27.19)	3131.66^{a} (55.29)	2037.66^{a} (45.03)
4	C near leaves to D	96.67 (9.77)	0.0001^{c} (0.707)	246.33^{b} (15.67)
5	C distant leaves to D	496.67 (19.4)	91.67^{bc} (9.57)	0.0001^{c} (0.707)
CD (0.05)		**NS**	**10.684**	**7.974**

*transformed values after square root transformation are given in paranthesis. (C-Cocoa, D- *D. falcata*, H-*H. elastica*. DAT- Days After Treatment)

The observations on 14 days after foliar application of ^{32}P to the parasite, *H. elastica* reveal that the translocation and accumulation of ^{32}P is higher than that in two and seven days after foliar application of ^{32}P. The developing young bud of cocoa accumulated maximum ^{32}P from *H. elastica* (2037.66 c/m/g). Radioactive counts of cocoa leaves nearer to point of foliar application of ^{32}P after 14 days of application in both the parasites, *D. falcata* (246.33 c/m/g) and H. *elastica* (361.33 c/m/g) were on par with that of cocoa leaves (186 c/m/g) distant from *H elastica*. However, the results clearly establish movement of ^{32}P from parasite to host. Thus, it can be concluded that there exists a two way communication between host and parasite.

Labelled ^{32}P was applied on the leaves of parasites residing on the same host (cocoa) as foliar application and the scintillation counts from the leaves of same parasite as well the other parasite on the same host was taken on 2^{nd}, 7^{th} and 14^{th} days after treatment. The observation from these experiments show that there is

movement of ^{32}P from one parasite to the other infected on the same host (Table 9).

Table 9. Radioactivity (counts per minute per gram) after foliar application of labelled ^{32}P on *D. falcata* and *H. elastica*.

S. No.	Treatments	2DAT	7DAT	14DAT
1	H near leaves to H	73.333^{c} (8.4)	158.67 ^{b} (12.53)	625.33^{b} (24.61)
2	H distant leaves to H	208.67^{a} (14.44)	116.33 ^{c} (10.69)	1546.67^{a} (39.24)
3	D distant leaves to H	0.0001^{d}(0.017)	0.0001^{d} (0.017)	183.67^{c} (13.35)
4	D distant leaves to D	140.333^{b} (11.84)	1092.67^{a} (32.86)	0.0001^{d} (0.017)
5	H leaves to D	667^{bc} (9.77)	290.67^{b} (16.87)	437 ^{b} (20.08)
	CD (5%)	**2.16**	**4.734**	**8.088**

*transformed values after square root transformation are given in paranthesis. (D- *D. falcata*, H-*H. elastica*. DAT- Days After Treatment).

The radioactive counts from the parasite leaves two days after treatment on *H. elastica* showed that maximum translocation was to distant leaves leaves of same plant itself (208.66 c/m/g). Similar result was observed for *D. falcata* also (140.33 c/g/m). Translocation of ^{32}P from *H. elastica* to its nearer leaves was found to be lesser (73.33 c/m/g) as compared to the leaves of *D. falcata* (667 c/m/g). It was also found that there was movement of ^{32}P from *D. falcata* to *H. elastica* leaves two days after treatment.

From the results of 7th day after ^{32}P application on parsasite *D. falcata*, the highest accumulation was recorded on the distant leaves of *D. falcata* (1092.67 c/m/g). The next higher accumulation of ^{32}P was found on *H. elastica* leaves (158.66 c/m/g) nearer to the point of application and *D. falcata* leaves (290.67

c/m/g) when foliar application was given to. *H. elastica* followed by *H. elastica* leaves (116.33 c/m/g).

After fourteen days of ^{32}P treatment as foliar application in *H. elastica*, the maximum count was observed in *H. elastica* leaves (1546.67 c/m/g) located at a distant branch from the point of application compared to the leaves of nearer branches (625.33 c/m/g). Radioactive counts of *H. elastica* leaves (437 c/m/g) farther from the point of application on *D. falcata* leaves was statistically on par with that of *H. elastica* leaves located nearer to foliar applied branch of *H. elastica*. ^{32}P when applied on *H. elastica* was also found to be translocated to *D. falcata* leaves (183.67 c/m/g). This result clearly shows, ^{32}P applied on one parasite was translocated to the other parasite inhabiting on the same host indicating two -way trafficking of the mineral nutrient between parasites.

4.2.2 Physiological studies

The observation on physiological parameters like photosynthetic rate, transpiration rate and stomatal conductivity along with photosynthetically active radiation recorded at monthly intervals for the period from January 2019 to June for both D. falcata and its host cocoa is given in table 10.

It was observed that, the stomatal conductance of *D. falcata* over a period of six months was found to be statistically not significant, but that of cocoa was observed to be statistically significant. The stomatal conductance of *D. falcata* for the period of observation between the host and parasite were statistically significant. Highest stomatal conductance for cocoa was during the month of May, 2019 (0.555 molH_2O/m^2/sec), followed by March (0.248 molH_2Om^2/sec) and April (0.199 molH_2O/m^2/sec) which were statistically on par, followed by February (0.181 molH_2O/m^2/sec) and June (0.182 molH_2O/m^2/sec), which were also statistically on par. and the least during the month of January 2019 (0.087 molH_2O/m^2/sec). Compared to the host, cocoa (0.242 molH_2O/m^2/sec) *D. falcata* had a higher rate of stomatal conductance (2.82 molH_2O/m^2/sec).

The rate of transpiration of the host and *D. falcata* was also observed for a period of six months from January – June 2019 and was found to be statistically significant. The mean of transpiration rate of *D. falcata* for the period of observation was 14.58 mmolH_2O/m^2/sec and the mean transpiration rate of cocoa for the period of observation was 5.2 mmolH_2O/m^2/sec. The parasite *D. falcata* showed a higher rate of transpiration than the host, cocoa for all the months during observation. Transpiration rate of the parasite was also observed to be significantly different across the period of observation with the highest recorded during April (21.7 mmolH_2O/m^2/sec) and March (17.08 mmolH_2O/m^2/sec) followed by January (11.06 mmolH_2O/m^2/sec) and May (11.06 mmolH_2O/m^2/sec) and lowest during the month of June (10.4 mmolH_2O/m^2/sec). Rate of transpiration of host was also found to be statistically significant across the period of observation. Highest rate was observed during the months of May, April and March *viz*., 8.02 mmolH_2Om^2/sec, 7.61 mmolH_2O/m^2/sec and 6.99 mmolH_2O/m^2/sec respectively followed by February (5.27 mmolH_2O/m^2/sec) and lowest during June (1.1 mmolH_2O/m^2/sec).

Photosynthetically active radiation (PAR) received by the host and parasite were also recorded for a period of six months from January to June, 2019 and was statistically insignificant indicating that the host and the parasite received light energy with not much variation. The average PAR received by *D. falcata* was recorded to be 440.16 mol/m^2/s and that received by cocoa was 431.6 mol/m^2/s.

The rate of photosynthesis of *D. falcata* and cocoa for a period of six months from January- June 2019 was recorded. Photosynthetic rate between host and parasite was found to be statistically significant for the period of observation. *D. falcata* showed a higher rate of photosynthesis throughout the period of observation with a mean photosynthetic rate of 10.38 µmolCO_2/m^2/sec than cocoa with a mean photosynthetic rate of 3.91 µmolCO_2/m^2/sec throughout the period of observation. Highest rate of photosynthesis was recorded by *D. falcata* during the month of February (15.5 µmolCO_2/m^2/sec) followed by January (13.56 µmolCO_2/m^2/sec), and June (10.04 µmolCO_2/m^2/sec) and lower rates were

recorded during the months of April, May and March which were statistically on par *viz*. 9.08 μmolCO_2/m^2/s, 7.27 μmolCO_2m^2/sec and 6.81 μmolCO_2m^2/sec respectively. Photosynthetic rate of cocoa was observed to be highest in the month of May (8.15 μmolCO_2/m^2/sec) and the rates of all the other months *viz*., January (2.87 μmolCO_2/m^2/sec), February (2.47 μmolCO_2/m^2/sec), March (2.25 μmolCO_2/m^2/sec), April (4.23μmolCO_2/m^2/sec) and June (3.47 μmolCO_2/m^2/sec) were statistically on par.

Carboxylation efficiency across the period of observation between *D. falcata* and cocoa was found to be significantly different. However, carboxylation efficiency was higher for *D. falcata* compared to cocoa throughout the period of observation. *D. falcata* recorded higher carboxylation efficiency during the month of February (0.065 mol/m^2/sec) and January (0.06 mol/m^2/sec) which was found to be statistically on par, whereas all other months viz., March (0.026 mol/m^2/sec), April (0.035 mol/m^2/sec), May (0.026 mol/m^2/sec) and June (0.035 mol/m^2/sec) showed similar results which were statistically on par. Cocoa recorded maximum carboxylation efficiency during the month of May (0.032 mol/m^2/sec), followed by April (0.015 mol/m^2/sec) followed by January, February and June which were statistically on par (0.014 mol/m^2/sec, 0.011 mol/m^2/sec and 0.013 mol/m^2/sec respectively), and least during March (0.008 mol/m^2/sec). Table 11 shows the carboxylation efficiency of host and parasite across the period of observation.

Light use efficiency of parasite *D. falcata* and host cocoa was found to be statistically significant on comparison across the period of observation, with the parasite having a higher light use efficiency with a mean value of 0.03 μmolCO_2/mol than the host with a mean value of 0.013 μmolCO_2/mol. However, the variation in light use efficiency of parasite when compared within the period of observation was found to be statistically insignificant, whereas that of cocoa showed significant difference. Highest light use efficiency of cocoa was recorded during the month of May (0.034 μmolCO_2/mol) while rest of the months the plant showed light use efficiency with meagre variation which was statistically on par

viz., 0.005 μmolCO_2/mol, 0.007 μmolCO_2/mol, 0.006 μmolCO_2/mol, 0.014 μmolCO_2/mol and 0.012 μmolCO_2/mol for January, February, March, April and May respectively (Table 11).

Table 10. Comparison of stomatal conductance, photosynthetic rate and transpiration rate between host (cocoa) and parasite (*D. falcata*).

Mean of	Stomatal conductance ($molH_2O/m^2/sec$)		Transpiration rate ($mmolH_2O/m^2/sec$)		Photosynthetically Active Radiation ($mol/m^2/s$)		Photosynthetic rate ($\mu molCO_2/m^2/sec$)	
Month	*D. falcata*	Cocoa	*D. falcata*	Cocoa	*D. falcata*	Cocoa	*D. falcata*	Cocoa
Jan	2.64	0.087^{c}	11.06^{bc}	2.19^{c}	551.25	538.5	13.56^{ab}	2.87^{b}
Feb	1.54	0.181^{bc}	16.44^{ab}	5.27^{b}	554.5	529.75	15.5^{a}	2.47^{b}
Mar	1.43	0.248^{b}	17.08^{a}	6.99^{a}	539.25	497	6.81^{c}	2.25^{b}
Apr	2.76	0.199^{b}	21.7^{a}	7.61^{a}	483.75	406.5	9.07^{c}	4.23^{b}
May	1.83	0.555^{a}	10.82^{bc}	8.02^{a}	274.25	323.75	7.26^{c}	8.12^{a}
Jun	3.52	0.182^{bc}	10.4^{c}	1.1^{c}	238	294	10.04^{bc}	3.47^{b}
Mean	**2.28**	**0.242**	**14.58**	**5.2**	**440.16**	**431.6**	**10.38**	**3.91**
CD (5%) (Within the parasite)	**NS**	**0.1**	**5.956**	**1.475**	**NS**	**NS**	**4.306**	**2.018**
T-value (Between parasite and host)	**5.979****		**4.236****		**NS**		**3.893****	

* represents significance level at 0.05%, ** represents significance level at 0.01% and 0.05%.

Table11. Comparison of carboxylation efficiency, light use efficiency and water use efficiency between host (cocoa) and parasite (*D. falcata)*.

Mean of	Carboxylation Efficiency ($mol/m^2/sec$)		Light Use Efficiency ($\mu molCO_2/mol$)		Water Use Efficiency ($\mu molCO_2/mmolH_2O$)	
Month	*D. falcata*	Cocoa	*D. falcata*	Cocoa	*D. falcata*	Cocoa
Jan	0.06^{a}	0.014^{bc}	0.025	0.005^{b}	1.34^{a}	1.36^{b}
Feb	0.065^{a}	0.011^{bc}	0.037	0.007^{b}	0.994^{ab}	0.529^{c}
Mar	0.026^{b}	0.008^{c}	0.018	0.006^{b}	0.404^{c}	0.313^{c}
Apr	0.035^{b}	0.015^{b}	0.03	0.014^{b}	0.504^{c}	0.59^{c}
May	0.026^{b}	0.032^{a}	0.03	0.034^{a}	0.677^{bc}	1.03^{b}
Jun	0.035^{b}	0.013^{bc}	0.042	0.012^{b}	0.965^{ab}	3.15^{a}
Mean	**0.041**	**0.016**	**0.03**	**0.013**	**0.814**	**1.16**
CD (5%) (Within the parasite)	**0.018**	**0.007**	**NS**	**0.013**	**0.455**	**0.415**
t-value (Between parasite and host)	**NS**		**3.050***		**NS**	

* represents significance level at 0.05%, ** represents significance level at 0.01% and 0.05%.

Water use efficiency of cocoa and *D. falcata* was also calculated and compared for a period of six months as shown in table 8. It was observed that there was significant difference in water use efficiency between cocoa and *D. falcata* with a far higher mean for the host (1.163 μmolCO_2/mmolH_2O) than the parasite (0.814 μmolCO_2/mmolH_2O). However *D. falcata* showed a significantly different water use efficiency across the period of observation with highest recorded during the month of January (1.34 μmolCO_2/mmolH_2O), followed by February (0.994 μmolCO_2/mmolH_2O) and June (0.965 μmolCO_2/mmolH_2O), which was on par. Lower values were observed during May (0.677 μmolCO_2/mmolH_2O) followed by April and March, which were statistically on par (0.504 μmolCO_2/mmolH_2O and 0.404 μmolCO_2/mmolH_2O respectively). Water use efficiency of cocoa was maximum during the month of June (3.15 μmolCO_2/mmolH_2O) followed by January (1.36 μmolCO_2/mmolH_2O) and May (1.03 μmolCO_2/mmolH_2O) and lowest during the months of March, February and April which were statistically on par (0.313 μmolCO_2/mmolH_2O, 0.529 μmolCO_2/mmolH_2O, 0.59 μmolCO_2/mmolH_2O respectively).

Physiological parameters of *H. elastica* and its host cocoa also observed for a period of six months using Infra-Red Gas Analyser (IRGA). Table 12 shows comparison of stomatal conductance, photosynthetically active radiation, photosynthetic rate, and transpiration rate of *H. elastica* and cocoa. In general, the parasite *H. elastica* had a higher stomatal conductance with a mean of 1.84 mol$H_2O$$m^2$/sec than cocoa, the host having a mean value of 0.261 mol$H_2O$$m^2$/sec throughout the period of observation which was statistically significant. The stomatal conductance of parasite varied significantly, where in a maximum of 2.74 mol$H_2O$$m^2$/sec was recorded during the month of May, followed by March (2.07 mol$H_2O$$m^2$/sec) and April (2.35 mol$H_2O$$m^2$/sec), which were on par, followed by June (1.64 mol$H_2O$$m^2$/sec) and February (1.38 mol$H_2O$$m^2$/sec). Minimum value of 0.866 mol$H_2O$$m^2$/sec was recorded during the month of January.

H. elastica had a higher transpiration rate than cocoa for all the months of observation with a mean value of 12.76 mmolH_2O/m^2/sec compared to cocoa 4.98 mmolH_2O/m^2/sec. *H. elastica* observed highest rate of transpiration rate during the months of April (15.36 mmolH_2Om^2/sec), February (15.1 mmolH_2Om^2/sec) and May (13.38 mmolH_2Om^2/sec) which were statistically on par, followed by March (12.68 mmolH_2O/m^2/sec) and least value was obtained during the month of June (9.9 mmolH_2Om^2/sec) and January (10.13 mmolH_2Om^2/sec) which were on par. Transpiration rate of cocoa was observed to be statistically significant with maximum during the month of May (8.89 mmolH_2Om^2/sec), followed by that recorded during the month of February (6.31 mmolH_2Om^2/sec) and April (6.04 mmolH_2Om^2/sec), followed by January (2.99 mmolH_2Om^2/sec) and March (4.54 mmolH_2Om^2/sec) which were statistically on par and least during the month of June (1.1 mmolH_2Om^2/sec).

Photosynthetically active radiation (PAR) received by *H. elastica* as well as cocoa was found to be statistically insignificant throughout the period of observation. On comparing the PAR received by the parasite and host across the months during the observation period was statistically insignificant. This indicates that host and the parasite were receiving light energy without much variation.

On comparing the photosynthetic rate of *H. elastica* and cocoa, it was found to be statistically significant with a mean value of 11.68 µmolCO_2/m^2/sec for former and a mean value of 4.38 µmolCO_2/m^2/sec for latter. Photosynthetic rate of *H. elastica* and cocoa for the period of observation across the months were found statistically insignificant.

Table 12. Comparison of stomatal conductance, photosynthetic rate and transpiration rate between host (cocoa) and parasite, *H. elastica*..

Mean of	Stomatal conductance ($molH_2O/m^2/sec$)		Transpiration rate ($mmolH_2O/m^2/sec$)		Photosynthetically Active Radiation ($mol/m^2/s$)		Photosynthetic rate ($\mu molCO_2/m^2/sec$)	
Month	*H. elastica*	Cocoa	*H. elastica*	Cocoa	*H. elastica*	Cocoa	*H. elastica*	Cocoa
Jan	0.866^{c}	0.106	10.13^{b}	2.99^{bc}	542.5	542	14.51	3.86
Feb	1.38^{bc}	0.397	15.1^{a}	6.31^{ab}	456.75	442.5	13.57	6.11
Mar	2.07^{ab}	0.198	12.68^{ab}	4.54^{bc}	320	431.25	9.49	1.96
Apr	2.35^{ab}	0.196	15.36^{a}	6.04^{ab}	420	295.5	9.71	2.85
May	2.74^{a}	0.486	13.38^{a}	8.89^{a}	377.75	357.5	11.38	8.01
Jun	1.64^{abc}	0.182	9.9^{b}	1.1^{c}	222	294	11.4	3.47
Mean	**1.84**	**0.261**	**12.76**	**4.98**	**389.8**	**393.79**	**11.68**	**4.38**
CD (5%) (Within the parasite)	**1.138**	**NS**	**2.829**	**4.202**	**NS**	**NS**	**NS**	**NS**
t-value (Between parasite and host)	**4.775****		**5.280****		**NS**		**5.903****	

* represents significance level at 0.05%, ** represents significance level at 0.01% and 0.05%

Carboxylation efficiency, Light Use efficiency and Water Use efficiency of *H. elastica* and cocoa during the period from January to June, 2019 was recorded (Table 13). Carboxylation efficiency of *H. elastica* was observed to be higher with a mean of 0.047 mol/m^2/sec than cocoa (0.018 mol/m^2/sec) throughout the months from January to June, 2019 and this was found to be statistically significant. The carboxylation efficiency of *H. elastica* across the months were found to be statistically significant with highest value recorded during the month of January (0.068 mol/m^2/sec), followed by that during the month of February (0.057 mol/m^2/sec), followed by May (0.042 mol/m^2/sec) and June(0.041 mol/m^2/sec) and lowest during March (0.036) and April (0.037) which were statistically on par whereas that of cocoa was found to be statistically insignificant.

Light use efficiency of host and parasite *H. elastica* was compared and observed to be statistically significant throughout the period of observation. It was noted that *H. elastica* had a higher rate of light use efficiency with a mean value of 0.393 µmolCO_2/mol than cocoa which had a mean value of 0.133 µmolCO_2/mol. Light use efficiency of *H. elastica* was observed to be statistically insignificant across the period of observation whereas that of cocoa was observed to be significantly different. The highest light use efficiency of host was observed during the month of May (0.029 µmolCO_2/mol) and the rest of the months showed statistically similar light use efficiency *viz*., 0.007 µmolCO_2/mol, 0.013 µmolCO_2/mol, 0.008 µmolCO_2/mol, 0.011 µmolCO_2/mol and 0.012 µmolCO_2/mol for the months of January, February, March, April and June respectively.

Water use efficiency was another physiological parameter observed to establish the relationship between the host and the parasite. Water use efficiency was found to be slightly higher for cocoa than host with a mean value of 1.195 µmolCO_2/mmolH_2O than the parasite, *H. elastica* had an average value of 0.956 µmolCO_2/mmolH_2O. During the period of observation, both cocoa and *H. elastica* showed significantly different value for water use efficiency. Water use efficiency

of *H elastica* was higher during the month of January (1.46 µmolCO_2/mmolH_2O), followed by June (1.15 µmolCO_2/mmolH_2O). The values obtained for February (0.917 µmolCO_2/mmolH_2O), March and May were statistically on par (0.729 µmolCO_2/mmolH_2O and 0.839 µmolCO_2/mmolH_2O respectively). Lowest value for water use effieicncy was during April (0.623 µmolCO_2/mmolH_2O). Cocoa had the highest water use efficiency during the month of June (3.15 µmolCO_2/mmolH_2O) followed by January (1.29 µmolCO_2/mmolH_2O. Water use efficiency during February and May were on par (0.935 µmolCO_2/mmolH_2O and 0.913 µmolCO_2/mmolH_2O). Lowest was during March (0.404) µmolCO_2/mmolH_2O and April (0.475 µmolCO_2/mmolH_2O).

Relative water content, specific leaf weight and phenol content of *D. falcata* and *H. elastica* were analysed for a period of six months. It was found that the difference in relative water content of *D. falcata* and *H. elastica* for the period of observation were not statistically significant (Table 14). The relative water content of *D. falcata* was found to have significant difference across the period of observation and highest relative water content was observed during the months of May (94.42%) and June (94.23%) which were statistically on par, followed by relative water content of March (87.58 %) and April (88.36%)which were also on par and the least for January (78.17%) and February (81.86%), which were also on par. Relative water content of *H. elastica* over the period of six months were also found to be relatively significant with highest value recorded during May (95.56%), April (95.33%) and June (94.67%) which were statistically on par. The relative water content during January, February and March were 79.08 %, 88.75% and 79.06 % respectively.

Specific leaf weight of the two parasites, was observed to be statistically insignificant. On the other hand, specific leaf weight of *D. falcata* and *H. elastica* when compared independently for a period of six months were found to be statistically significant. *D. falcata* had specific leaf weight which were statistically on par on all months *viz*., January (0.019 g/cm^2), February (0.017 g/cm^2), March

(0.021 g/cm^2), April (0.017 g/cm^2) and June (0.017 g/cm^2) except on May (0.012 g/cm2). Specific leaf weight of *H. elastica* were also found to have significantly different for the months from January to June, 2019. Highest specific leaf weight was recorded during the month of June (0.021 g/cm^2) followed by January, March and April, recorded the same specific leaf weight of 0.017 g/cm^2 which was followed by that during May (0.016 g/cm^2) and lowest value was observed during the month of February (0.012 g/cm^2).

Table 13. Comparison of carboxylation efficiency, light use efficiency and water use efficiency between host (cocoa) and parasite- *H. elastica*.

Mean of	Carboxylation Efficiency ($mol/m^2/sec$)		Light Use Efficiency ($\mu molCO_2/mol$)		Water Use Efficiency ($\mu molCO_2/mmolH_2O$)	
Month	*H. elastica*	Cocoa	*H. elastica*	Cocoa	*H. elastica*	Cocoa
Jan	0.068^{a}	0.02	0.027	0.007^{b}	1.466^{a}	1.29^{b}
Feb	0.057^{ab}	0.027	0.045	0.013^{b}	0.917^{bc}	0.935^{c}
Mar	0.036^{c}	0.007	0.04	0.008^{b}	0.729^{cd}	0.404^{d}
Apr	0.037^{c}	0.012	0.038	0.011^{b}	0.632^{d}	0.475^{d}
May	0.042^{bc}	0.03	0.035	0.029^{a}	0.839^{cd}	0.913^{c}
Jun	0.041^{bc}	0.013	0.051	0.012^{b}	1.15b	3.15^{a}
Mean	**0.047**	**0.0182**	**0.03933**	**0.0133**	**0.956**	**1.19**
CD (5%) (Within the parasite)	**0.019**	**NS**	**NS**	**0.014**	**0.275**	**0.312**
t-value (Between parasite and host)	**4.433****		**5.639****		**NS**	

Table 14. Variation in relative water content, specific leaf weight and phenol content of *D. falcata* and *H. elastica*. attached to the same host (cocoa) for the period from January- June 2019.

	RWC (%)		SLW(g/cm^2)		Phenol (µg/ml)	
Month	Genera					
	D	H	D	H	D	H
Jan-2019	78.17^{c}	79.08^{b}	0.019^{a}	0.017 ^{ab}	4.17^{a}	5.31^{a}
Feb- 2019	81.86^{c}	88.75^{b}	0.017^{a}	0.012 ^{c}	1.88^{b}	3.88^{b}
Mar-2019	87.58^{b}	79.06^{b}	0.021^{a}	0.017 ^{ab}	0.507^{e}	4.65^{b}
Apr-2019	88.36^{b}	95.33^{a}	0.017^{a}	0.017 ^{ab}	1.33^{c}	1.48^{d}
May-2019	94.42^{a}	95.56^{a}	0.012^{b}	0.016 ^{bc}	0.93^{d}	4.61^{b}
Jun-2019	94.23^{a}	94.67^{a}	0.017^{a}	0.021 ^{a}	0.43^{e}	0.62^{e}
Mean	87.44	88.74	0.017	0.017	1.491	3.288
CD (5%) (Within genera)	4.358	9.016	0.004	0.004	0.134	0.228
t-value (Between genera)	NS		NS		NS	

The phenol content of *D. falcata* and *H. elastica* were also analyzed and compared for a period of six months from January to June, 2019. It was inferred that the phenol content of *D .falcata* and *H. elastica* was significantly different when compared independently across the months and was found statistically insignificant when compared between the genera. The maximum phenol content in *D. falcata* was observed during the month of January (4.17 µg/ml), followed by that during February (1.88 µg/ml), which was followed by phenol content during April (1.33 µg/ml) and May (0.93 µg/ml) and least during the months of June

(0.43 µg/ml) and March (0.507 µg/ml) which were statistically on par. The phenol content in *H. elastica* was observed to be maximum during the month of January (5.31 µg/ml), followed by the phenol content observed during February (3.88 µg/ml), March (4.65 µg/ml), and May (4.61 µg/ml) which were statistically on par and followed by that recorded during the month of April (1.48 µg/ml)and lowest during the month of June (0.62 µg/ml).

4.3 Effect of treatment formulations in controlling Loranthus

With an aim to standardize an improved and efficient management strategy for managing Loranthus, the existing practices were modified and compared. The prevailing recommendation practice for Loranthus management is foliar application of 25ml/L of Ethephon. This was modified using surfactants with an idea to improve its efficiency. Seven different treatment combinations with two different non-ionic surfactants in three different concentrations along with 25ml/L Ethephon were formulated. Another practice followed for controlling Loranthus namely base banding with 1 per cent 2,4-D was also compared. The detailed treatment formulations is illustrated in table 1. Treatments T1, T2, and T3 were combinations of 25ml/L Ethephon and nonyl phenoxy ethoxylate (NPE) at three different concentrations @ 3, 5 and 8 ml/L and treatments; T5, T6 and T7 were combinations of 25ml/L Ethephon and with organosilicone (OS) surfactant @ 02.,0.5 and 0.8 ml/L respectively. T7 was 25ml Ethephon without addition of any surfactant and T8 was 1 percent 2,4-D for base banding and both were considered as control.

Ethephon formulations were applied to *D. falcata* in vegetative stage of growth, infected on cashew as foliar spray using a knap-sack sprayer and observation on leaf defoliation and drying were taken for a period of twelve months. Table 15 shows the extent of defoliation after application of different treatments. The extent of defoliation is expressed as per cent defoliation in number of days after application.

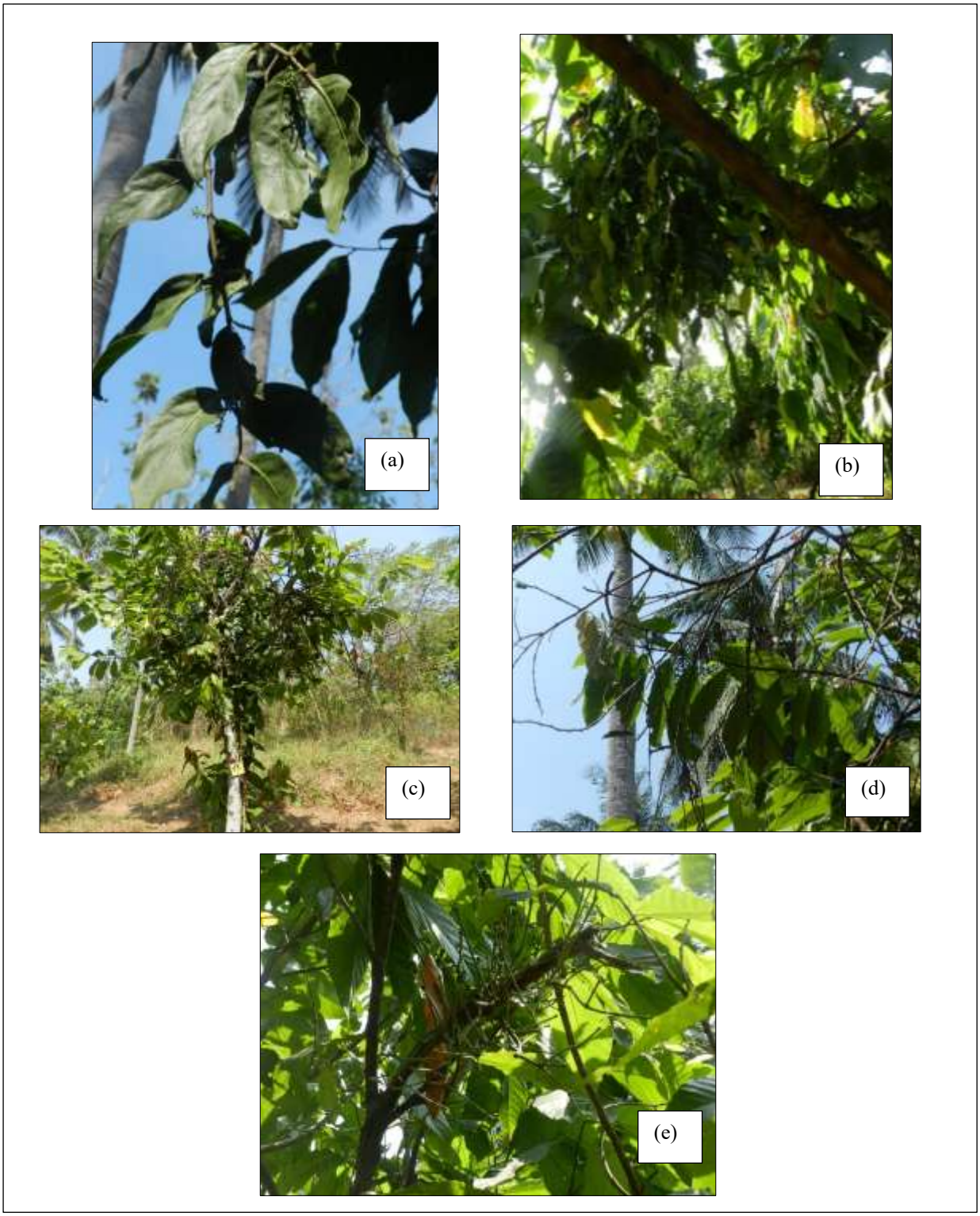

Plate 35. (a) *D. falcata* before treatment (T5) (b) *H. ealstica* before treatment (T5); (c) & (d) *D. falcata* after treatment (T5); (e) *H. ealstica* after treatment (T5)

Table 15. Effect of treatments on the control of *D. falcata* expressed as percentage defoliation.

Treatment	Percentage defoliation (days after spraying)				
	25%	50%	75%	90%	100%
T1- E+ 3 NPE	0 DAS	1 DAS	2 DAS	3 DAS	7 DAS
T2- E+ 5 NPE	2 DAS	3 DAS	5 DAS	10 DAS	25 DAS
T3- E+ 8 NPE	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS
T4-E+ 0.2 OS	0 DAS	1 DAS	3 DAS	5 DAS	5 DAS
T5-E+ 0.5 OS	0 DAS	0 DAS	1 DAS	2 DAS	3 DAS
T6- E+ 0.8 OS	0 DAS	1 DAS	2 DAS	4 DAS	7 DAS
T7-E(Control)	0 DAS	0 DAS	0 DAS	3 DAS	10 DAS
T8-1% 2,4-D (Control)	0 DAS	0 DAS	0 DAS	0 DAS	0 DAS

DAS- Days After Spraying.

The results from the experiment indicate that, hundred per cent defoliation of *D. falcata* were observed after the application of all treatment formulation except T8. After one day of the application of T1, 50 per cent leaves were found to have defoliated and it took seven days for 100 percent defoliation. *D. falcata* treated with T2 took 2 days after treatment for 25 percent defoliation and up to 90 per cent defoliation was achieved within ten days after application and complete defoliation was observed to occur within 25 days after foliar application of the formulation. On the other hand, after application of T3, 25 percent defoliation was observed one day after application and hundred percent defoliation was seen in five days after treatment application. For treatments T4, 50 per cent defoliation was observed one day after treatment and for T5 (Plate 35, 36), 75 per cent defoliation was observed one day after treatment and hundred per cent defoliation was seen for T4 and T5 in 5 and 3 days after application respectively. For treatment T6, fifty per cent defoliation was attained one day after treatment and for complete defoliation it took 7 days. For T7, ninety percent defoliation was observed on 3rd day after treatment application, but for complete defoliation it took ten days where as T8 resulted in no defoliation.

Plate 36. (a) Defoliation after spraying formulation; (b) Drying of leaves after spraying formulation.

Table 16. Regrowth of *D. falcata* on the host (cocoa) after spraying.

		Weeks after spraying						Months after spraying								
		1 m		2 m		3 m										
S. no.	Treatment	2 w	4 w	6 w	8 w	10 w	12 w	4 m	5 m	6 m	7 m	8 m	9 m	10m	11m	12m
1	T1-E+3 NPE	0	1	1	2	2	3	3	3	3	3	3	3	3	3	3
2	T2-E+5 NPE	0	1	1	2	3	3	3	3	3	3	3	3	3	3	3
3	T3-E+8 NPE	0	0	1	2	3	3	3	3	3	3	3	3	3	3	3
4	T4-E+0.2OS	0	0	1	1	2	2	2	2	2	1	1	1	1	0	0
5	T5-E+0.5OS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	T6-E+0.8OS	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
7	T7-E(Control)	0	0	0	1	1	2	3	3	3	3	3	3	3	3	3
8	T8-1% 2,4-D (Control)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Scoring pattern- 0-no regrowth, 1-sporadic regrowth, 2- stunted regrowth, 3-normal regrowth, 4- profuse regrowth.

The time taken for regrowth of *D. falcata* after the application of treatments were also observed (Table 16). Scoring pattern was followed to analyse the data on regrowth of the parasite, where scoring from 0, 1, 2, 3 and 4 were given for no regrowth, sporadic regrowth, stunted regrowth, normal regrowth and profuse regrowth respectively. For treatments T1, T2 and T3 regrowth was observed within four weeks after application whereas for T3, regrowth was observed six weeks after application. For T4, regrowth was observed 6 weeks after spraying which failed to sustain as time progressed and finally reached at no regrowth 11 months after spraying whereas treatment T5 showed no regrowth. *D. falcata* treated with T6 showed regrowth ten weeks after spraying and plants treated with T7 took only 8 weeks after spraying to regrow. T8 had resulted in no defoliation and maintained normal growth. The result showed that, addition of surfactant has a positive effect on defoliation and improving the efficiency of ethephon. Organosilicone surfactant in combination with ethephon (treatments T4, T5 and T6) have shown enhanced efficiency in managing *D. falcata*.

Different treatment formulations applied on *D. falcata* was also applied to *H. elastica* infected on cocoa tree, which were in their vegetative phase of growth and observations on defoliation and drying was taken for a period of 12 months. Defoliation was expressed as percentage as shown in table 17.

For treatments T1, T3 and T6, 25 per cent of defoliation was observed one day after spraying, whereas in *H. elastica* treatments with T2 and T5 gave, fifty percent defoliation was observed one day after spraying. For *H. elastica* treated with T1 it took 25 days after spraying for complete defoliation, and for T2 and T3 it took only 5 days after spraying for complete defoliation. For plants treated with T4, 75 per cent defoliation occurred one day after spraying, whereas for complete defoliation it took 3 days after spraying. Parasites treated with T6 and T7 took seven days for complete defoliation but for the plants treated with T6, 25 per cent defoliation occurred one day after spraying and for T7 treated plants 90 per cent defoliation was observed to occur four days after spraying. *H. elastica* treated with T8 showed no defoliation.

Table 17. Effect of treatments on the control of *H. elastica* expressed as percentage defoliation.

Treatment	Percentage defoliation (days after spraying)				
	25%	50%	75%	90%	100%
T1-E+ 3 NPE	1 DAS	2 DAS	5 DAS	15 DAS	25 DAS
T2- E+ 5 NPE	0 DAS	1 DAS	2 DAS	4 DAS	5 DAS
T3- E+ 8 NPE	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS
T4- E+ 0.2 OS	0 DAS	0 DAS	1 DAS	2 DAS	3 DAS
T5- E+ 0.5 OS	0 DAS	1 DAS	2 DAS	2 DAS	3 DAS
T6- E+ 0.8 OS	1 DAS	2 DAS	3 DAS	4 DAS	7 DAS
T7- E(Control)	0 DAS	0 DAS	0 DAS	4 DAS	7 DAS
T8- 1% 2,4-D (Control)	0 DAS	0 DAS	0 DAS	0 DAS	0 DAS

DAS- Days After Spraying

On the whole, treatment combination of ethephon with NPE surfactants (T1, T2 and T3) took longer time for complete defoliation whereas treatment combination of ethephon with OS surfactants (T4, T5 and T6) showed comparatively shorter period for complete defoliation. Treatment without surfactant (T7) has taken a maximum of 7days for complete defoliation.

The regrowth pattern for *H. elastica* treated with different treatment formulation was also observed for a period of 12 months and scoring was done based on visual observation (Table 18). For treatment T1, regrowth was observed 4 weeks after spraying whereas for treatments T2, T6 and T7, regrowth was seen 12 weeks after spraying. For treatments T3 and T4, regrowth was observed 5 months after spraying, whereas T5 has shown no regrowth. T8 had no effect on defoliation and the plants continued to maintain normal growth.

Table 18. Regrowth of *Helicanthus elastica* on the host (cocoa) after spraying.

		Weeks after spraying						Months after spraying								
S.No.		1 m		2 m		3 m		4 m	5 m	6 m	7 m	8 m	9 m	10 m	11 m	12 m
	Treatment	2 w	4 w	6 w	8 w	10 w	12 w									
1	T1- E+3NPE	0	1	1	1	1	1	1	1	2	2	2	3	3	3	3
2	T2- E+5NPE	0	0	0	0	0	1	1	1	2	2	2	2	2	3	3
3	T3- E+8NPE	0	0	0	0	0	0	1	1	1	1	1	2	2	2	2
4	T4-E+0.2 OS	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
5	T5-E+0.5 OS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	T6-E+0.8 OS	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
7	T7-E(Control)	0	0	0	0	0	1	1	1	1	1	2	2	3	3	3
8	T8-1% 2,4-D (Control)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Scoring pattern- 0-no regrowth, 1-sporadic regrowth, 2- stunted regrowth, 3-normal regrowth, 4- profuse regrowth.

Discussion

5. DISCUSSION

5.1 Genetic diversity of Loranthaceae

To understand the genetic diversity of Loranthaceae, representative species from five major genera of the family namely *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana*., and *Taxillus tomentosus* were selected. *D. falcata*, *H. elastica* and *M. capitellatus* were collected from different locations in the plains and *H. wallichiana* and *T. tomentosus* which are found infecting the fruit crops of the highlands were collected from Nelliyampathy. Genomic isolation from the leaves of the collected samples were done followed by ISSR assay with selected primers. The results from the assay were used to prepare dendrogram using the tool MEGA X.

The dendrogram shows two major clusters (Fig. 34), with four genera namely *T. tomentosus, M. capitellatus, H. wallichiana*, and *D. falcata* together in aa major cluster and *H. elastica* formed a separate lone cluster. This shows that, the former four genera are closely related than the lone one. From the dendrogram, it was inferred that, *T. tomentosus* and *M. capitellatus* were more similar compared to the members of other genera. Morphological similarity in haustorial branching pattern was also observed substantiating this. Both were having basal epicortical root system of haustorial pattern with slight modification than the other genera. The haustorial connection had single point of attachment (holdfast) and axillary branches were observed to develop form the shoot arising from the hold fast. (hyaline body).

In the dendrogram, *H. elastica* formed a separate lone cluster, indicating its genetical variance from other genera. Morphological observations supporting such a uniqueness in this parasite was noted in haustorial branching type, where basal epicortical root with lateral tendril like structures from nodes and internodes were observed. The parasite also showed other phylogenetically advanced characters according to Angiosperm Phylogeny Group (APG system) (Angiosperm Phylogeny Group, 2009) of classification like fused corolla and calyx.

5.2 Morphological and anatomical features of five major genera of Loranthaceae

Morphology and anatomy of five major genera of Loranthaceae were considered for the study. Among the selected genera, *D. falcata*, *H. elastica* and *M. capitellatus* were seen to be parasitising the major fruit and timber crops of the plains, whereas the genera, *H. wallichiana* and *T. tomentosus* were observed to parasitise fruit crops of the highlands.

Basic haustorial system of all the genera was found to be basal epicortical root. These are adaptations from a terrestrial root habitat to an aerial habitat. This is based on the extent and pattern of elongation and frequency of lateral root formation of the parasites. Haustoria helps in the direct contact of the parasite with the host. Basal epicortical root arises from the stem of the host from close proximity to the holdfast (Kujit, 1991). New axillary branches and new haustoria develops on the basal ER. These distinctive patterns of roots help in the extension and spreading of the parasite in the host plant (Calvin and Wilson, 2006). Leaf arrangement of all the genera were opposite (Gamble, 1936). Loranthaceae genera *D. falcata*, *H. elastica* and *M. capitellatus*, infecting tropical fruit and timber crops was observed to have longer leaves reaching upto an average length of 15.38cm in *D. falcata,* 11.76 cm in *H. elastica* and 7.48 cm in *M.capitellatus. H. wallechiana* and *T. tomentosus*, were observed to infest trees seen in highranges with cooler climatic conditions. They were found to have smaller leaves with an average leaf length of 6.3cm and 3.9 cm respectively. This might help them in reducing transpiration to thrive in their habitat. *D. falacta* was found to have brilliant colour variants within the same species. In general the inflorescent type was observed to be tubular in all the five genera studied (Sasidharan, 2012). Fruits were berry ovoid -globate and found to be having gum like substance in all the genera which aids in dispersal and pollination. The parasites are generally ornithophilous (pollinated by birds). The seeds of the plant from the faecal

matter of birds adheres to the branches of tress with the help of a non-digestive gummy mucilage around the seed, and it germinates in the bark (Subhashini *et al*., 2019).

5.3 Haustorial anatomy

In order to study the anatomical characters of common Loranthus genera viz. *Dendrophthoe falcata*, *Helicanthes elastica*, *Macrosolen capitellatus*, *Helixanthera wallichiana* and *Taxillus tomentosus*, the samples of these genera were collected.

In *D. falcata*, finger like projections were observed at the haustorial region. They are elongated parenchyma cells at the host- parasite interface which helps in formation of penetration peg which ensures attachment of haustorial roots to the host cells. The demarcation at the host parasite interphase with elongated or irregular shaped parenchyma cells were evident in the haustorial sections of all the genera studied. Parenchyma cells at the haustorial interphase was distinguishably different from the parenchyma seen inside the host and parasite. These undifferentiated parenchyma cells might be functioning as transfer cells at the interphase of haustorial region. Similar results were reported in the haustorial anatomy of *Santalum album*, a hemiparasite, invading the roots of the host. A dark staining material at the interphase of parasitic plants was observed in all the haustorial sections of different genera of Loranthaceae studied. It can be described as ‘tip lyses’ which are secretions of tubular / elongated parenchyma which helps in the firm adhesion of the parasite on the host (Tennekoon and Cameroon, 2006). Other anatomical evidence like poorly defined boundaries of haustoria, few uniseriate to multiseriate rays, presence of fibres from the investgations in the transverse sections of different genera of Loranthaceae were observed. These observations were found to be in congruent with the results of wood anatomy studies of Loranthaceae indigenous to New Zealand (Rajni, 1991). No phloem cells were observed at the site of contact of haustoria suggesting that the transfer of solutes, molecules and signals takes place through xylem tissues.

5.4 Host parasitic interaction dynamics

In the prevailing study, translocation and partitioning of mineral nutrient phosphorous between the parasites, *Dendrophthoe falcata* and *Helicanthus elastica* and the host cocoa has been investigated using radioactive ^{32}P. ^{32}P in carrier solution was given through the surface running roots of the host (cocoa) and the samples from both host and parasite were collected after 7 and 14 days of treatment, dried, powdered and acid digested to estimate radioactivity present in these samples using liquid scintillation counter. Samples were collected from cocoa leaves, emerging leaves of cocoa, leaves of *D. falcata* and *H. elastica* were analyzed. At 7 days after treatment, movement of phosphorous was maximum to *H. elastica* than to any other parts of host itself or to *D. falcata*, the other parasite inhabiting the host (Fig. 1), indicating that the host considers its parasite as a sink without discriminating between host and parasite. But after 14 days of ^{32} P application to the host, phosphorous was found to get accumulated in young emerging leaves in host plant. Here the emerging leaves acts as the immediate alternate sink for translocation of phosphorous.

^{32}P in carrier solution was given as foliar application by leaf swabbing to the parasites, *D. falcata* and *H. elastica*. Leaf samples of parasites as well as host were collected 2, 7 and 14 days after treatment with radioactive ^{32}P. Counts of foliar applied ^{32} P leaf samples of *H. elastica* showed significant counts at 2 days after treatment in the leaves of emerging cocoa bud near to the tree trunk (Fig 2). This shows the effective transport of foliar applied ^{32}P on parasite to the host. Next significant count was observed on the cocoa leaves in the canopy far from the applied *H. elastica* branch than in the cocoa leaves adjacent to the treated parasite leaves. Similar results were seen in *H. elastica* leaves far from the applied leaf than the nearby leaves of *H. elastica*. This indicates that there is a prioritized partitioning of phosphorous to the sink depending on demand despite the difference whether it is host or parasite. Parasite induced additional sink generation has been reported by Hibberd *et al*. (1999) and Jeschke *et al*. (1994). At 7 days after ^{32}P foliar application in the *H. elastica*, the counts were

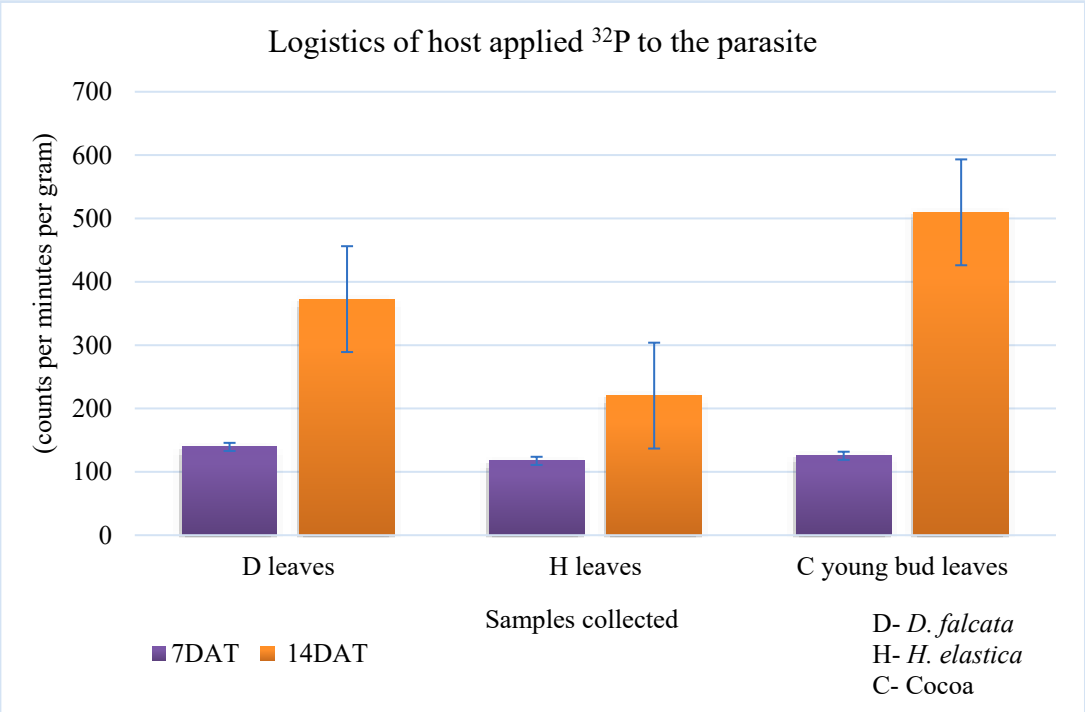

Fig. 1. ^{32}P counts in parasites from root feeding studies on cocoa

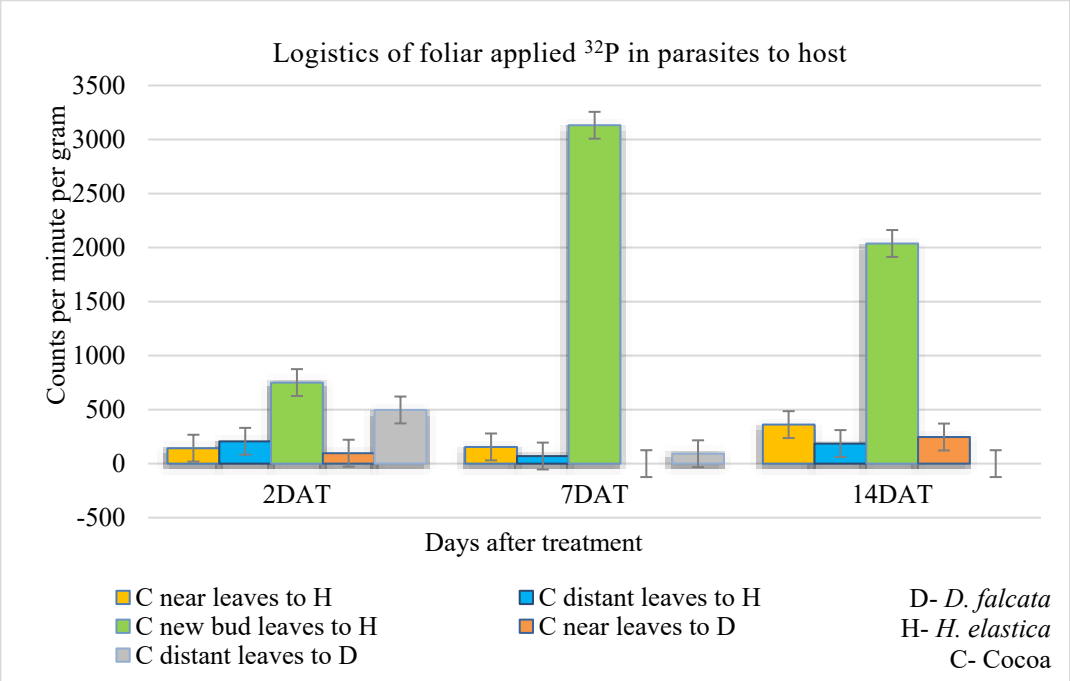

Fig.2. ^{32}P counts in host by foliar application studies on parasite

significantly higher in leaves of new buds of cocoa than other leaves of host or parasites. Radioactivity of leaf samples from the newly emerged bud of cocoa and distant leaves of *H. elastica* from the treated leaf were higher at 14 days after treatment followed by adjacent leaves of *H. elastica*. Overall, the transportation of ^{32}P from the *H. elastica* to the host leaf was observed to be maximum after 7 days of foliar application. The rate of flow was found to be reduced as time progressed, which showed comparatively lower counts in the leaves of emerging buds of cocoa. From the results it is evident that, there is flux of ^{32}P from the parasite to the host tissue. It can be assumed that the flow can either be symplastic or apoplastic. Structural feature like secondary plasmodesmata can be helpful for cell-to-cell symplastic translocation. Secondary plasmodesmata are thought to provide specific pathways for transport of macromolecules and for non- cell autonomous regulation (Kragler *et al*., 1998). This complex symplastic continuum aids the translocation of phloem sap from parasite to the host. Phosphorous being a signaling molecule has profound significance in host-parasite signal transduction. It can be conceived as extracellular ATP (eATP) through apoplast. eATP is known to induce a number of cellular responses (Tanaka *et al*., 2010) and serve as a growth signal (Kim *et al*., 2006). These postulates are supplemented by the presence of transition cells of parenchymatous nature between the host and parasitic tissues in the anatomical investigation of haustoria (Plates 23,25,28,29,30 and 31).

The transportation of ^{32} P from the leaves of *D. falcata* to the host leaves at both 2 and 14 days after treatment were found to be statistically non-significant. From the obtained scintillation counts, maximum radioactivity were observed in the leaves of cocoa far from the treated parasite leaf than in the leaves of parasite adjacent to the treated leaves. Counts were also observed in *H. elastica* leaves, which is the other parasite habiting in the same host indicates transportation of ^{32}P from one parasite species to another in the same host (Fig. 3). Similar results were observed on cocoa leaves and *H. elastica* leaves at 12 days after application of ^{32}P. Leaf samples of *D. falcata* not adjacent to the ^{32}P treated leaf had higher

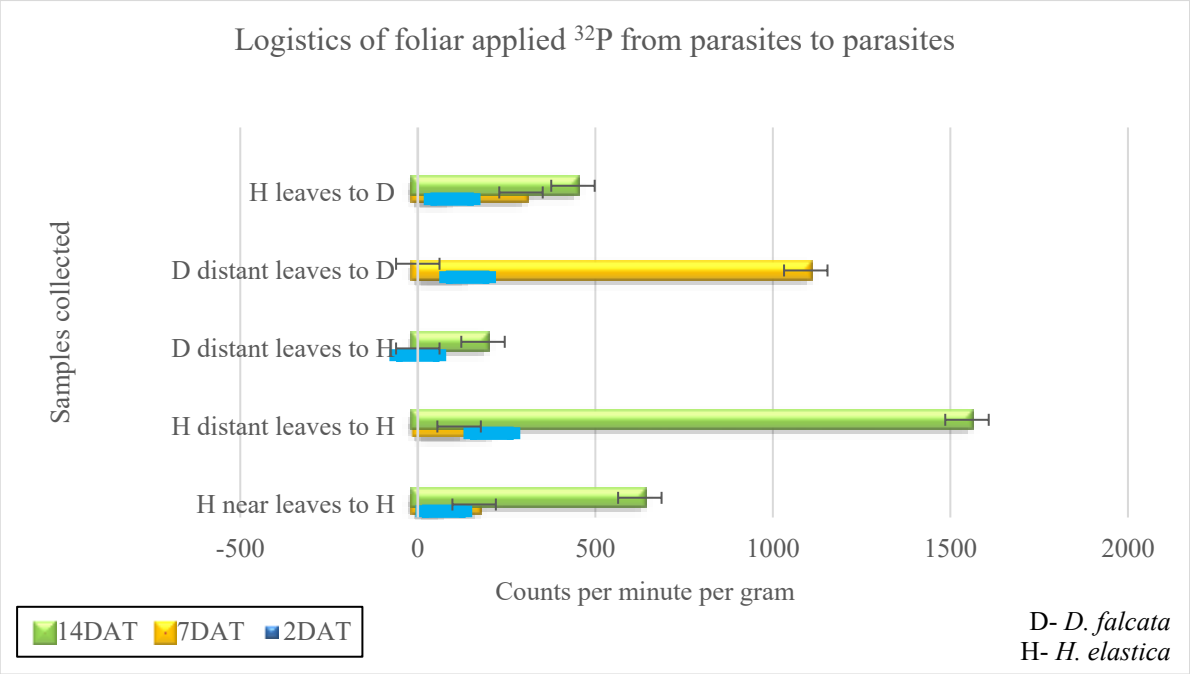

Fig. 3. Parasite to parasite movement of foliar applied ^{32}P

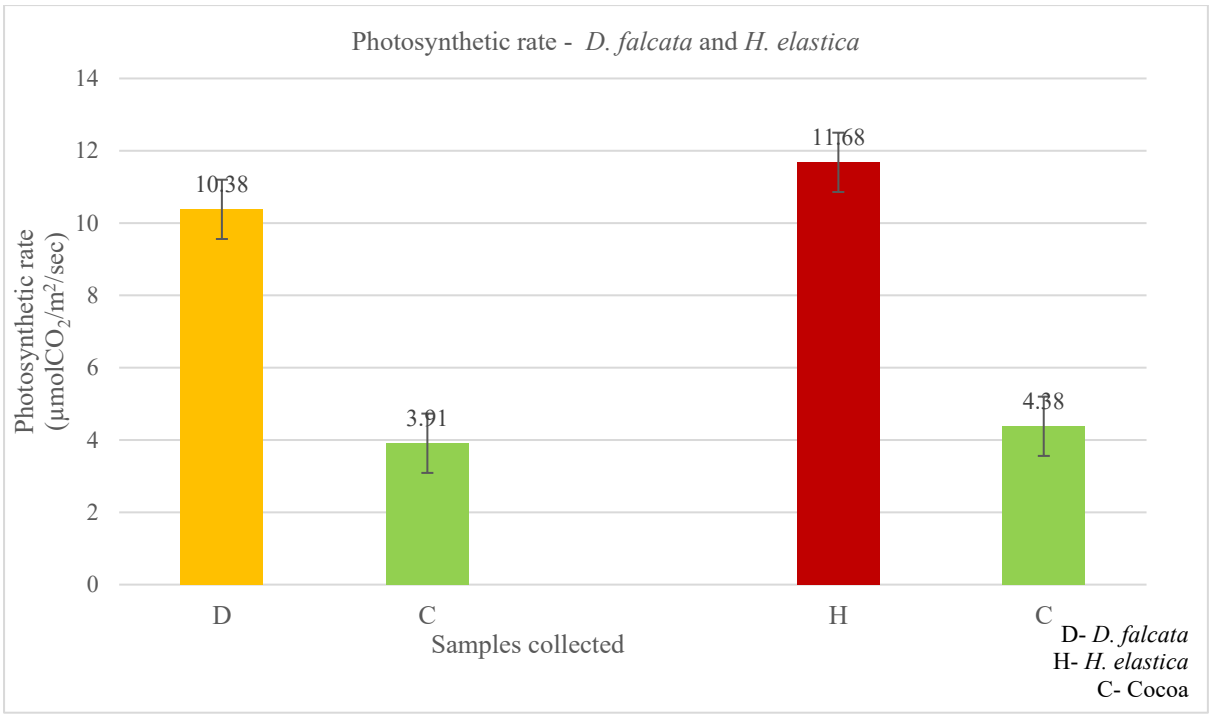

Fig.4. Comparison of photosynthetic rate of *D. falcata* and *H. elastica*

counts than cocoa leaves and *H. elastica* leaves at 7 days after application. Substantially this showed that there is an implicit communication from parasite to the host. The bidirectional transport of inorganic nutrients between *D. falcata* and the host ficus using radioactive ^{32}P ions has also been observed by Bhattacharya *et al*. (2015) and he was of the opinion that such a connection indicates metabolic interaction between host and parasite. A report on reverse translocation of ^{32}P from labelled *Santalum album* to its host tree Casuarina also showed a two-way communication between the host and parasite (Rocha *et al*., 2015). These observations also suggest that the host and the parasites inhabited in the host acts as a single system and follows a pattern in nutrient partitioning as the sink demands. This also indicated that the impact of parasitism can also be mutualistic at community levels (Phoenix and Press, 2005). The overall mineral trafficking between host and parasite in the current study using ^{32}P is illustrated in plate 37 and 38.

5.4.1 Physiological parameters influencing host parasite interaction

Physiological activity of the host is dependent on the parasite and *vice-versa*. In order to apprehend the host parasitic interdependence physiological parameters of infected host and the parasite were observed for a period of six months from January 2019 to June 2019. Concurrent observations on rate of photosynthesis, rate of transpiration, stomatal conductance, and Photosynthetically Active Radiation (PAR) of *D. falcata, H. elastica* and the host cocoa over a time span of six months were recorded to understand the physiological dependence of them.

Photosynthetically active radiation (PAR) is the energy source of photosynthesis in plants. The quality and quantity of incident PAR play an important parameter dependent on climatic conditions of the day. The action spectrum in PAR decides photosynthetic productivity (Mottus *et al*., 2011). However, though equal PAR was received by host and parasite, it was observed that photosynthetic efficiency was higher for the parasites than the host plant.

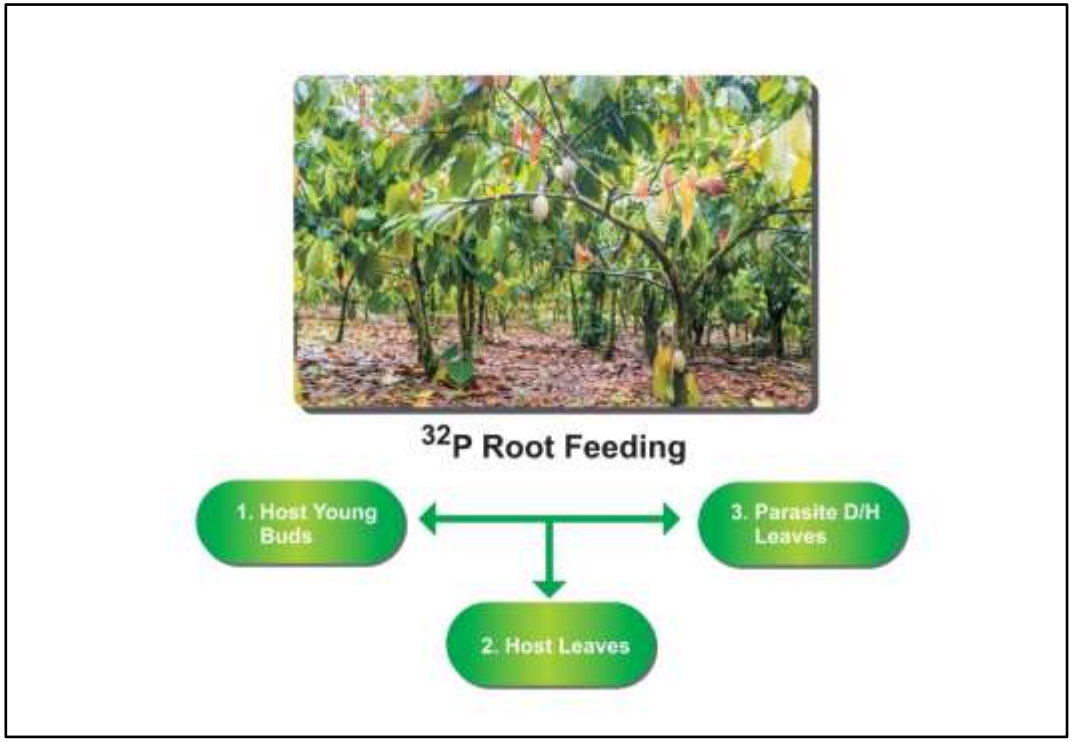

Plate 37. Dynamics of prioritized partitioning of root fed ^{32}P to host

(numerals indicates priority in partitioning)

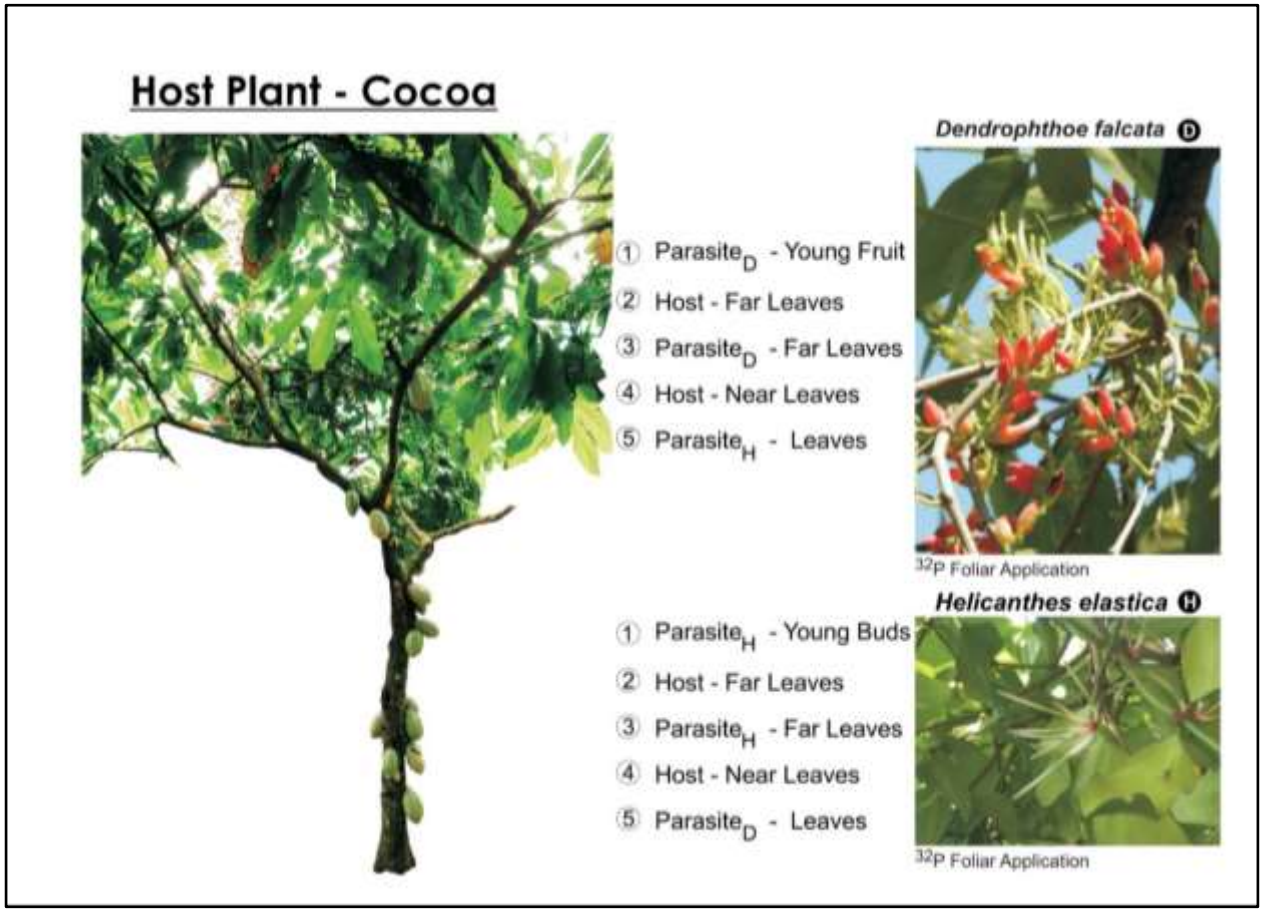

Plate 38. Dynamics of prioritized partitioning of foliar applied ^{32}P to *D. falcata* and *H. elastica* (numerals indicates priority in partitioning)

Photosynthetic rate of *H. elastica* was higher than to *D. falcata* (Fig.4) and both the parasites were having higher rate of photosynthesis than the host. This was found to be in contrast to the earlier reports by Strong and co-workers (2000) who reported lower assimilation rate in hemiparasites while, Schulze and his co-workers (1984) observed equal rates of photosynthesis of host and parasite. The current study indicates increased rate of photosynthesis of the hemi parasites. This might be a survival strategy or it indicates competitiveness of the hemiparasite, which is in accordance with the assumption as suggested by Glatzel and Geils in 2009.Here, any limiting factor in the field emulating a stress might have added to the increased rate of photosynthesis by the parasites than their host plant.

D. falcata and *H. elastica* had higher status of stomatal conductance than compared to their host (Fig 5). It has been reported by Vareschi and Pannier (1953) and Hellmuth (1971) that, Loranthus have little control over the stomatal opening and closure, since the activity is host dependent. In order to maintain a balance within the system the opening and closing of stomata is regulated by the leaf xylem water potential of the host. Results of the current study did not vouch this statement. However, further information on stomatal frequency may be necessary to explain the phenomenon.

Transpiration rate also followed the same trend as that of photosynthetic rate when compared with their host (Fig.6). This result was in accordance with the findings of Schulze *et al*. (1984). Contrary to the rate of photosynthesis, the rate of transpiration was higher for *D. falcata* than *H. elastica.* This might be due to a higher stake on competitiveness of *D. falcata* compared to *H. elastica* when they were infected on same tree. This is in accordance with the observations of (Girija *et al*., 2013). Who has reported similar finding with O_2 discrimination studies of *D. falcata* and *H. elastica.*

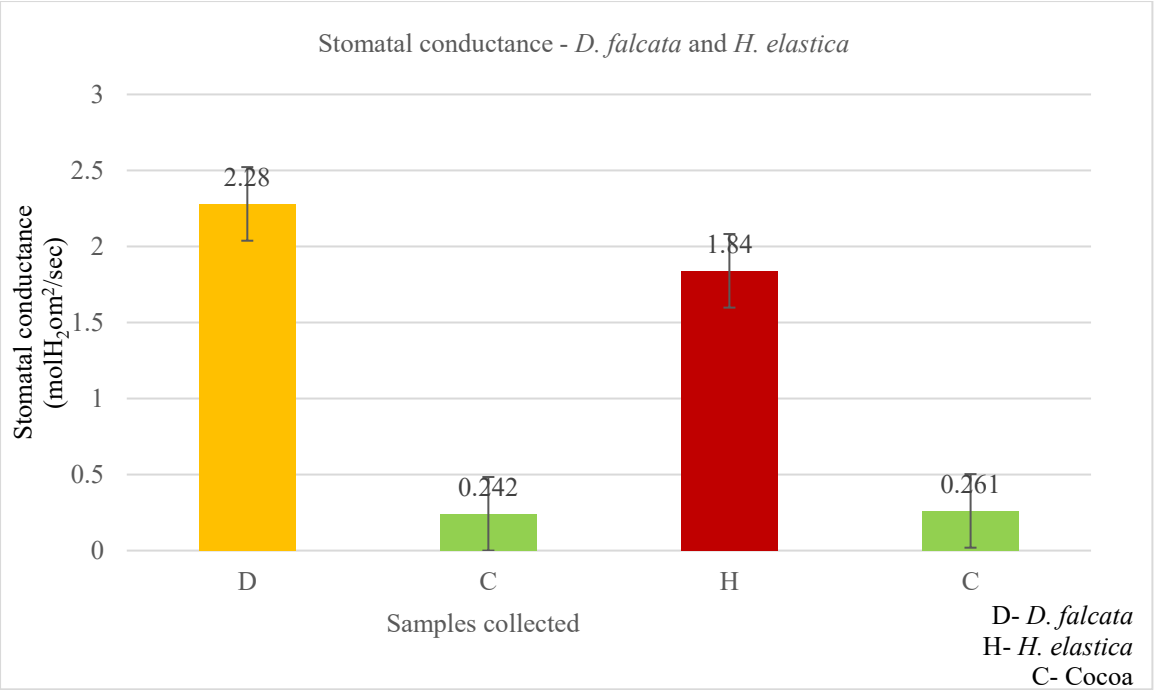

Fig. 5. Comparison of stomatal conductance of *D. falcata* and *H. elastica*

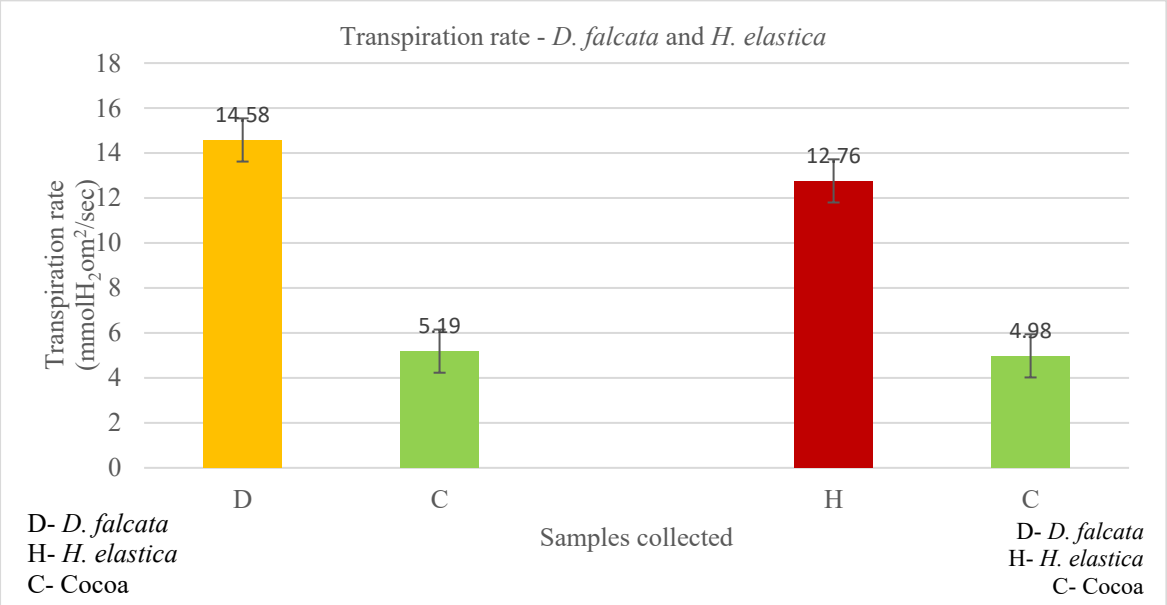

Fig. 6. Comparison of transpiration rate of *D. falcata* and *H. elastica*

From the data of carboxylation efficiency observed for a period of six months (Fig.7), it is evident that the carboxylation efficiency of parasites are far higher than their hosts. For the quantum of PAR received the CO_2 assimilation is efficiently carried out by the parasites when compared to the host plant. These results are in accordance with earlier findings by Schulze and Ehleringer in 1984 and Strong *et al*., in 2000. This may be because, raw materials are a limiting factor for the parasites since they are dependent on the host. Hence an efficient mechanism for utilising the available resources is mandatory for their survival. This can be substantiated from the graph given in Fig 7, where highest carboxylation efficiency is observed during the months of January and February but CE is found to decrease over the months of March and April where water deficit in the field situations might have contributed to the reduction during summer months. During the months of May and June, CE was found to increase at a slow pace indicating water availability with the onset of summer showers and S-W monsoon. Meterological data during the period of observation is given in Appendix I.

Light use efficiency (LUE) of the parasites also followed the same trend as that of photosynthetic rate and carboxylation efficiency. *D. falcata* and *H. elastica* showed a higher LUE than the host (Fig. 8). There was not much difference in water use efficiency between the host and parasite (Fig. 9). While comparing the month wise data recorded (Table 8) there existed only a meagre discrepancy in the water use efficiency of host and parasite. Similar results were also reported by Bannister and Strong (2001).

A comparison of the two parasites residing in the same host showed that relative water content, specific leaf weight and phenol content had no statistical difference for these among the parasites.

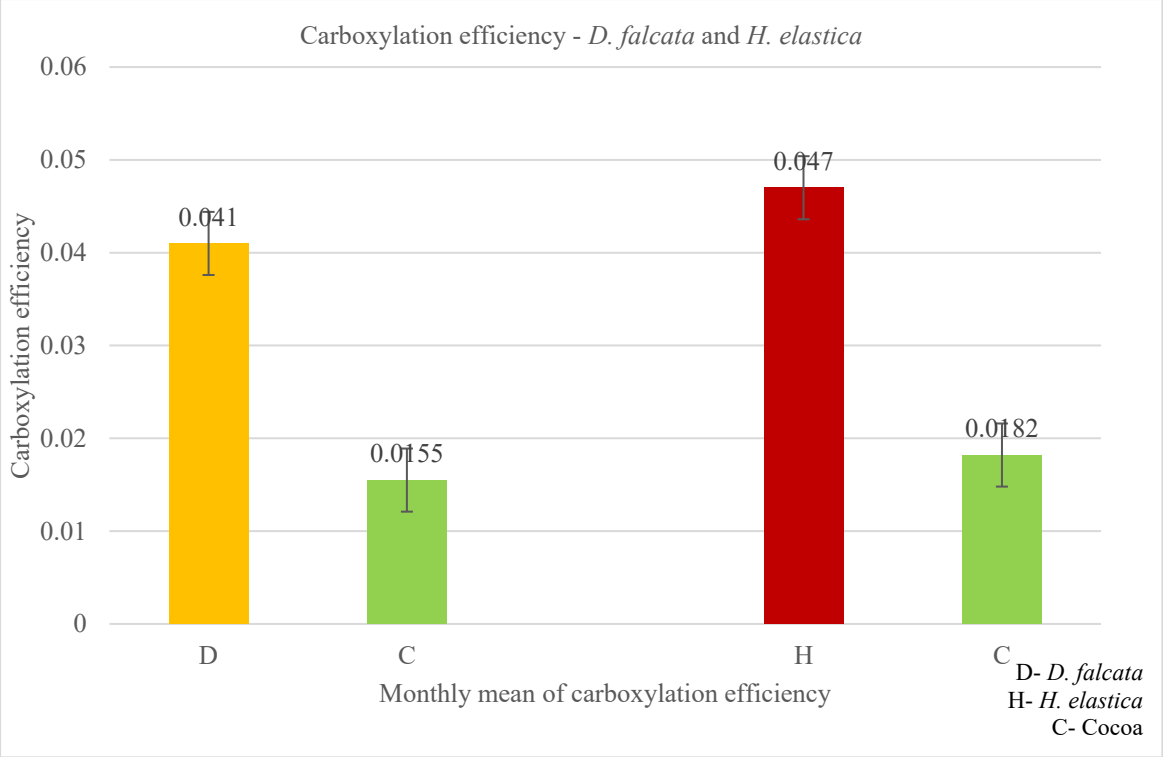

Fig.7. Comparison of carboxylation efficiency of *D. falcata* and *H. elastica*

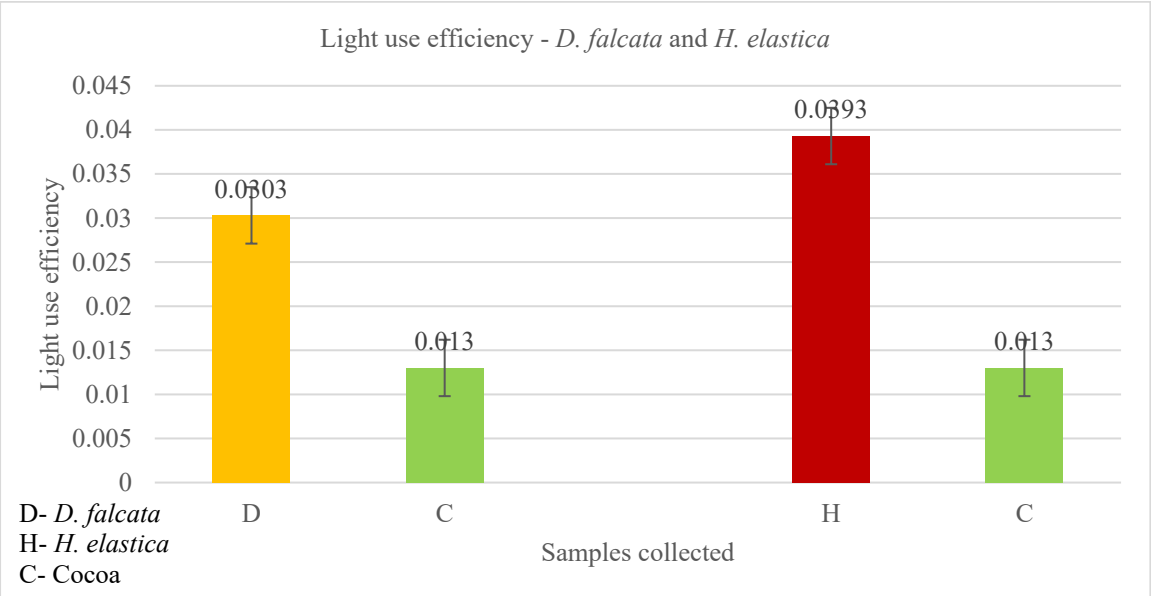

Fig. 8. Comparison of light use efficiency of *D. falcata* and *H. elastica*

5.5 Improved strategies for management of Loranthaceae

Loranthus a parasitic weed of parasite of perennial cash crops and timber crops need a complete management strategy to control its spread and regrowth on the host. Even though a number of practices are followed to manage this parasite, the occurrence of regrowth has been a major concern. Application of plant hormone Ethephon at the rate of 25 ml/L has been found successful in managing this parasitic weed (KAU, 2016).

With an aim to improve the efficiency of ethephon, two non-ionic surfactants; Nonyl phenol ethoxylate (NPE) and Organosilicone (OS) were used along with ethephon. The results of the study indicated that both the surfactants improved the efficiency of ethephon, and complete defoliation (Fig. 10 & 11) was observed within 3 to 7 days which was faster than the control with no surfactant which required 10 days for defoliation. This was true in the case of both the parasites. Among the two surfactants tested OS @ 0.5 ml/L gave the best result since it took only 3 days for complete defoliation.

Observation taken on regrowth of parasites in the treatment applied indicated that regrowth was faster in treatment 1 and 2, where NPE was added to ethephon @ 3 and 5 ml per litre as compared to treatments where OS was used as surfactant. Treatment T5 (E+ OS) showed no regrowth (Fig. 11 & 12) even after one year of application indicating that this is the best combination which can be taken forward for further confirmatory studies. Concurrent results were observed for both the parasites.

Loranthus species is a major tree parasite. Different species of Loranthaceae attach to the host through direct haustorial connection to the xylem and can influence the metabolism of the host plant. Partitioning of nutrient to the parasite can contribute to productivity decline as the host and parasite shows a mutualistic association sharing resources. Organosilicone surfactant added with ethephon can be recommended as a suitable control measure for managing Loranthus in tree crops. An occurrence of zero regrowth of the parasite is a significant one. The

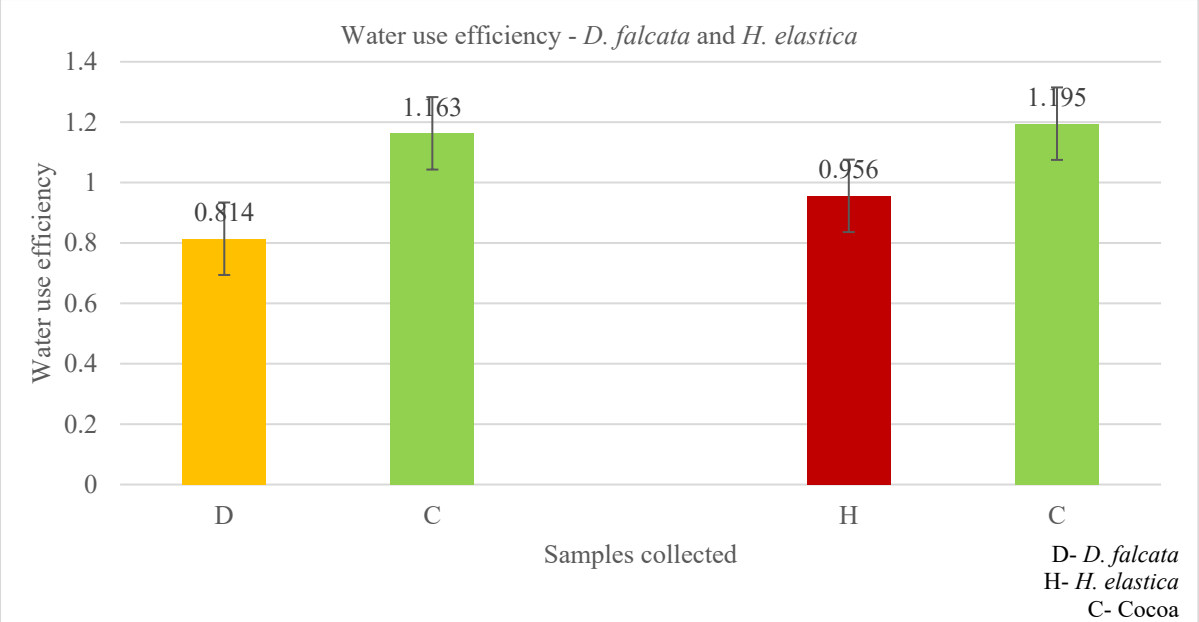

Fig. 9. Comparison of water use efficiency of *D. falcata* and *H. elastica*

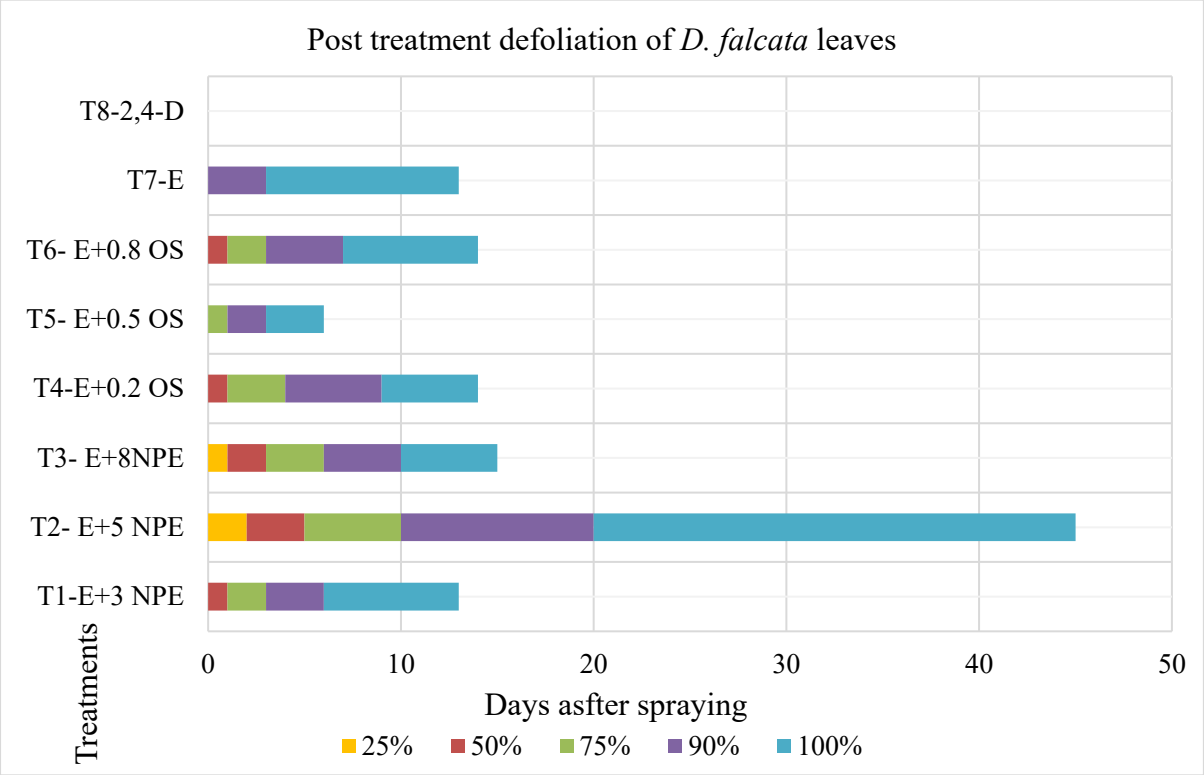

Fig. 10. Defoliation in *D. falcata* after spraying of treatment formulations expressed in %

treatment combination T5 was found to be the best performing among the applied formulations. Hence the formulation can be suggested for further field trials in farmer's field.

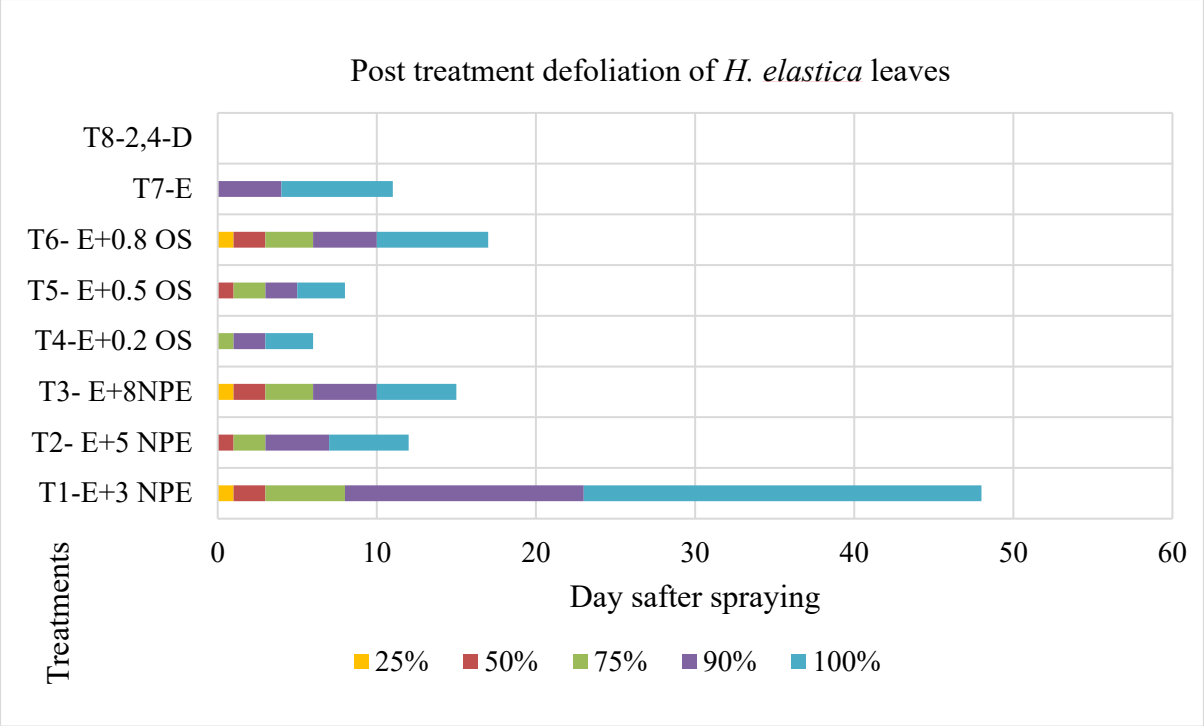

Fig. 11. Defoliation in *H. elastica* after spraying of treatment formulations expressed in %

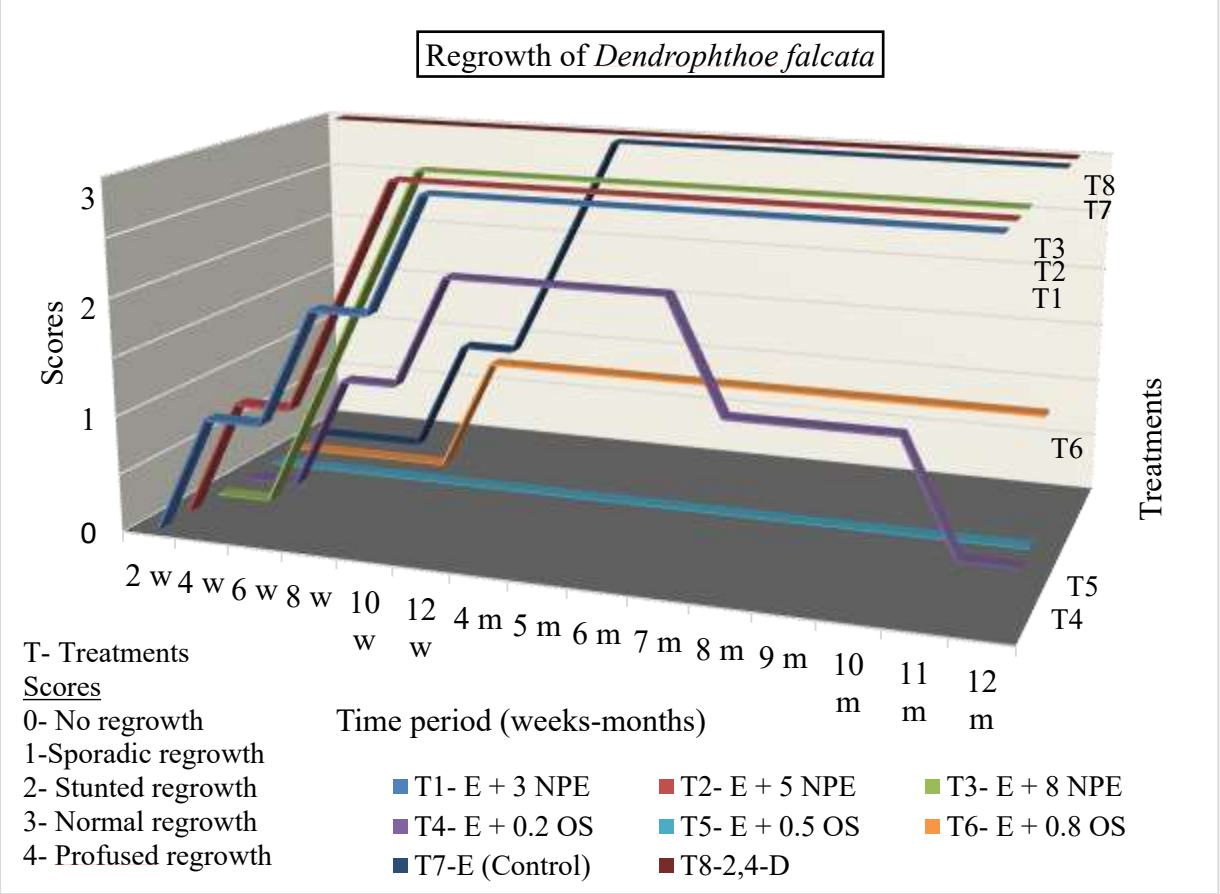

Fig. 12. Regrowth of *D. falcata* after spraying treatment formulations

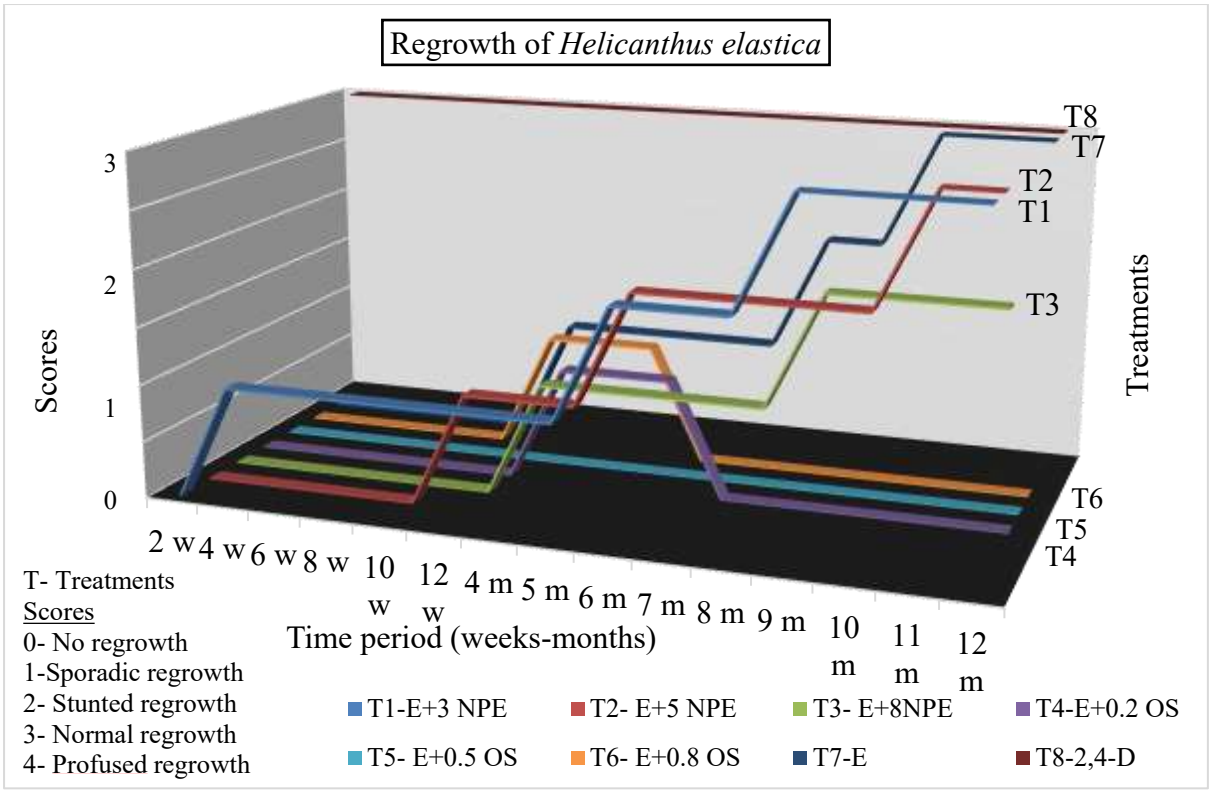

Fig. 13. Regrowth of *H. elastica* after spraying treatment formulations

Summary

6. SUMMARY

Physiological, molecular and management studies on different genera of Loranthaceae were done in the Department of Plant Physiology with the following objectives; (i) to identify the genetic diversity of Loranthus genera in Kerala,(ii) toubderstand the dynamics of host parasite interaction and (iii) to modify and improve he prevailing management strategies. Morphological characterization and haustorial anatomy of the five major species of Loranthaceae *viz Dendropthoe falcata*, *Helicantues elastica*, *Macrosolen capitellatus, Helixanthera wallichiana*, and *Taxillus tomentosus,* were also done. Plant samples were collected from various locations in the plains and highlands of Thrissur. Morphological characterization revealed that each genera varies in characters like leaf shape, texture, leaf length and leaf area, the arrangement of leaves were opposite in all the genera studied. Maximum leaf length was observed for D. falcata whereas maximum area was found highest for *H. elastica*. *H. wallichiana* and *T. tomentosus* had narrow ovate shape with least leaf area.

The haustorial anatomy of the representative members of these five genera showed presence of undifferentiated parenchyma cells at the interphase region between host and parasite tissues. They function as transfer cells and aid in translocation of materials between host and parasite. It was also observed that only xylem elements were observed at the haustorial union suggesting that bidirectional movement between host and parasite takes place through xylem.

The haustorial branching pattern of the five different members from different genera showed that, the basic haustorial pattern in them is basal epicortical root with slight modifications.

Genetic diversity analysis of *D. falcata*, *H. elastica*, *M. capitellatus, H. wallichiana*, and *T. tomentosus* by ISSR assay and the resultant dendrogram showed that *M. capitellatus* and *T. tomentosus* were phylogenetically more similar compared to the other genera. In the dendrogram four genera namely

T. tomentosus, M. capitellatus, H. wallichiana and *D. falcata* together formed a major cluster and *H. elastica* formed a separate lone cluster.

Host parasitic dynamics between the parasites *D. falcata* and *H. elastica* and the host cocoa was studied using labelled ^{32}P. Radioactive ^{32}P was given as root application to the host and as foliar application to the parasites. Leaf samples were collected from host and the parasites and oven dried di-acid digested leaf extracts were subjected to radioactive assay. The results from the study revealed that there existed a two-way communication between the host and parasite. A prioritized partitioning of ^{32}P between host and parasite based on the sink demand was observed. Radioactive ^{32}P when fed to cocoa as root application, the initial translocation was to the juvenile developing branches of the host, second to the mature leaves of the host and finally to the parasites. When labelled ^{32}P was given as foliar application to the parasites, *D. falcata* and *H. elastica*, the initial movement was to the juvenile developing shoots of the host, then to host leaves, followed to their own leaves and finally to the leaves of the other parasite, seen on the same host.

Studies on physiological parameters of the host and parasite showed that, photosynthetic rate, transpiration rate, and stomatal conductivity was higher for both the parasites *D. falcata* and *H. elastica* compared to its host. Carboxylation efficiency and light use efficiency was also found to be higher for parasites than cocoa. However, water use efficiency was observed to be higher for cocoa than its parasites.

Organosilicone surfactant improved the efficiency of ethephon in controlling Loranthus. The surfactant @ 0.5ml/L along with 25ml/L ethephon contributed in complete eradication of the parasite with defoliation of parasite in minimum number of days and it was also efficient in checking the regrowth of the parasite on the host.

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Appendix

APPENDIX I

Meteorological data of Vellanikkara, Thrissur from January to July 2019

S. No.	Parameter	Months						
		January	February	March	April	May	June	July
1	Mean Max ºC	32.9	35.3	36.8	36.1	34.6	32.2	30.4
2	Mean Min ºC	20.4	23.4	24.8	25.5	24.9	23.5	22.8
3	Highest Max ºC	34.8	38.7	40.4	37.9	36.3	35.4	32.6
4	Lowest Min ºC	16.5	18.8	21.8	21.6	21.0	21.8	21.5
5	Mean RH (%) (morning)	71	71	85	86	89	93	95
6	Mean RH (%) (evening)	38	41	45	54	59	73	76
7	Mean RH	55	59	65	70	74	83	85
8	Rainfall (m)	0.0	0.0	0.0	76.4	48.8	324.4	654.4
9	Rainy days	0	0	0	3	4	15	21
10	Tot. evaporation (mm)	144.8	143.4	148.4	142.1	122.5	84.4	73.8
11	Mean evaporation (mm/day)	4.7	5.1	4.8	4.7	4.0	2.8	2.4
12	Total sunshine (hours)	261.4	244.4	265.9	240.7	211.0	111.7	81.6
13	Mean sunshine (hours)	8.4	8.7	8.6	8.0	6.8	3.7	2.6
14	Mean wind speed (km/hr)	5.9	5.3	2.8	2.5	2.2	1.3	1.1

PHYSIOLOGICAL AND MOLECULAR STUDIES ON GENERA OF LORANTHACEAE AND THEIR MANAGEMENT

by

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ABSTRACT OF THE THESIS

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Abstract

Hemiparasitic plants belonging to the family Loranthaceae are major tree parasites. *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus* are major hemiparasites infecting perennial crops of the tropics. *Helixanthera wallichiana* and *Taxillus tomentosus* are two important hemiparasites infecting the fruit and timber trees of the high ranges. Apart from being a troublesome parasite they also serve as a linchpin reservoir supporting an extensive ecosystem. The prevailing investigation namely "Physiological and molecular studies on genera of Loranthaceae and their management" was carried out at Department of Plant Physiology, College of Agriculture, Vellanikkara, Thrissur, during the period from 2015 to 2020.

Morphological characters of representative species *viz.*, *Dendrophthoe falcata*, *Helicanthus elastica*, *Macrosolen capitellatus, Helixanthera wallichiana* and *Taxillus tomentosus* from five selected. Samples of these were collected from various locations from the plains and highranges of Thrissur. In all the selected species, leaves were oppositely arranged, *D. falcata* and *H. elastica* had oblong shaped leaves, leaves of *M. capitellatus* was lanceolate in shape, and *H. wallichiana* and *T. tomentosus* had narrow ovate shaped leaves. Fruit was berry in all the genera. Hemiparasites possess a physiological structure called haustoria through which they abstract water and minerals from the host. Haustorial branching pattern in all the genera was found to be basal epicortical root (ber). Haustorial portions of the collected samples were treated and prepared to permanent slides. Anatomical sections of the haustoria of the five selected genera of Loranthaceae revealed that there exists a transition zone between the host- parasite interphase region. This was observed as undifferentiated parenchymatous cells which aid in translocation of molecules. Presence of xylem elements were observed at the haustorial region. Haustorial anatomy of *M. capitellatus* was unique as there was complete merging of cells of host and parasite at the interphase region.

The lineage of five selected genera belonging to Loranthaceae family were studied by molecular assay. The results from the ISSR assay revealed that, *T. tomentosus* and *M. capitellatus* had maximum similarity compared to all the other genera, since it formed a separate cluster. Morphological characterization indicated

similarity between the two genera in the haustorial attachment pattern. Both the species have a single point of attachment to the host. *H. wallichiana* was observed to be more similar to the first cluster. *D. falcata* formed another branch close to *H. wallichiana*, where all these four genera formed the main group. *H. elastica* formed a separate lone group, which indicated genetical variance from other genera studied. Morphological observations supporting such a uniqueness in this parasite was noted in haustorial branching type, where basal epicortical root with lateral tendril like structures from nodes and internodes were observed. The parasite also showed other phylogenetically advanced characters according to Angiosperm Phylogeny Group (APG system) of classification like fused corolla and calyx.

To study the host parasite interaction, root feeding studies with labelled ^{32}P were undertaken. Cocoa plants infected with both parasites *D. falcata* and *H. elastica* was selected for the study. ^{32}P in carrier solution of 1000ppm orthophosphoric acid @ 2 mCi per plant was fed to the surface running roots of cocoa. Leaf samples of the host and parasite were collected at 7 and 14 days after treatment and assayed for radio activity. To understand the translocation from parasite to host, leaves of the parasitic species both *D. falcate* and *H. elastica* were also smeared with labelled ^{32}P and leaf samples were analysed from different parts of both the host and the parasite. These experiments were done on different trees. Leaf samples of the host and parasite were collected at 2, 7 and 14 DAT and assayed for radio activity. Results from the radio assay indicated that there is bidirectional movement of nutrients from host to parasite and parasite to host. There exists a prioritized partitioning pattern for nutrient transport (phosphorous) based on demand by the sink, regardless of the parent plant. The host and parasite were found to act as a single system indicating their co-existence.

Physiological parameters of the host and parasites were also observed using Infra-Red Gas Analyser (IRGA) for a period of six months. It was observed that stomatal conductivity of *D. falcata* and *H. elastica* was significantly higher than the host cocoa. Even though there was no much variation in the photosynthetically active radiation (PAR) received by the host and parasite during the period of observation, *D. falcata* and *H. elastica* had significantly higher rate of photosynthesis than cocoa. Transpiration rate of both parasites were significantly higher than their host. Carboxylation efficiency and light use efficiency of *H. elastica* were observed to be significantly higher than the host, cocoa**.**

Field experiment was conducted to improve the management strategy and control the spread of the parasite. Use of surfactant was found to improve the efficacy of ethephon in controlling the regrowth of parasite on host plant. Non- ionic surfactants *viz*., Nonyl phenol ethoxylate (NPE) and Organosilicone (OS) were selected for the study. A combination of OS (0.5 ml/L) surfactant with ethephon (25ml/L) was successful in suppressing the regrowth of both *D. falcata* and *H. elastica*.