RESOURCE ASSESSMENT AND SEED BIOLOGY OF *Clerodendrum colebrookianum* WALP. IN MIZORAM, INDIA

THESIS

SUBMITTED TO
MIZORAM UNIVERSITY, AIZAWL

FOR
THE DEGREE OF

Doctor of Philosophy

In
HORTICULTURE, AROMATIC AND MEDICINAL PLANTS

By

R. ZOTHANKIMA
(Reg. No. MZU/Ph.D/524 of 13.05.2013)

DEPARTMENT OF HORTICULTURE, AROMATIC AND MEDICINAL PLANTS
MIZORAM UNIVERSITY
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Dedicated

To

My Parents & my family

Mizoram University, Aizawl

(A Central University under the Act of Parliament)

Department of Horticulture, Aromatic & Medicinal Plants

उद्यानिकी, सगन्ध एवं औषधीय पादप विभाग

Dr. T. K. Hazarika
Head of the Department

No. 9/16/Ph.D. Prog./MZU-HAMP/22 **Dated 20.08.2018**

CERTIFICATE

This is to certify that **Mr R. Zothankima**, has prepared a Thesis under my Supervision on the topic "**Resource assessment and seed biology of *Clerodendrum colebrookianum* Walp. In Mizoram, India"** in partial fulfillment for the award of the Degree of Doctor of Philosophy (Ph. D.) in the department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl.

This thesis has been the outcome of his original work and it does not form a part of other thesis submitted for the award of any other degrees.

He is duly permitted to submit the Thesis.

Dr. T.K. Hazarika
Supervisor & Head

DECLARATION BY THE CANDIDATE

Mizoram University
August, 2018

I, **R. Zothankima**, hereby declare that the subject matter of the thesis entitled ***"Resource Assessment and Seed Biology of Clerodendrum colebrookianum Walp. in Mizoram, India"*** is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge, to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institutes.

This is being submitted to the Mizoram University for the Degree of Doctor of Philosophy in Horticulture, Aromatic and Medicinal Plants.

R. Zothankima

(Candidate)

(Head) **(Supervisor)**

Acknowledgements

The submission of this Ph.D. thesis gives me immense pleasure, satisfaction and unique sense of accomplishment despite many difficulties and troubles that came in its way. Of course it is a fact that such endeavour is never possible without the help of many persons in one way or the other. Naturally I cannot miss this opportunity to convey my sincere thanks and heart-felt gratitude to all those who helped me to make this task possible in its present fruitful form.

The first and foremost among the persons whom I convey my humble gratitude is Dr. T. K. Hazarika, Supervisor and Head, Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University who was most kind enough to me to be my Supervisor for this research study. With his systematic guidance, whole-hearted cooperation and support enabled me in carrying out and completing this research. His erudition and rich experience shaped the course of the research and sharpened its outcome. I thank him sincerely, for his consideration, support & confidence towards me. Sir, I am indebted to you for whatever you have done for me.

I am very much indebted to Prof. B.P. Nautiyal. Dean, VCSG College of Horticulture, Uttarakhand University of Horticulture & Forestry (UUHF), who supported me in every possible way for completion of this work.

Further, I would like to thank Dr. Chhungpuii Khawlhring, Assistant Professor; dept. of HAMP, MZU, who rendered help in each and every steps of my Ph.D work, Dr. Awadhesh Kumar, Dr. Debashis Mandal and Ms. Abigail Zothansiami, Assistant Professor, dept. of HAMP, MZU, for their encouragement and supports during the experimentation. My sincere gratitude also goes to the non-teaching staffs of the department of Horticulture, Aromatic and Medicinal Plants, Mizoram University for their invaluable help during my research work. I am also thankful to Ms. Laldingngheti Bawitlung and Nurpen Meitei Thangjam, my fellow research scholars for their valuable help during the research work. The thesis would not have come to a successful completion, without the help I received from them.

Last but not the least, I am also thankful to my parents Mr. R. Lalthanzauva and Mrs. Hrangthiangi and my wife Ms. Lalchhanhimi who are my constant and the best source of inspiration and fortitude to take this research work to its logical end and without their unflinching support and cooperation, I would not have been able to complete this work on time.

And finally, I thank the Almighty God for granting me the health and strength to undertake this research work and enabling me to its completion.

R. Zothankima
(Reg. No. MZU/Ph.D/524 of 13.05.2013)

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Abbreviations

%:	per cent
2-4-D:	2,4-Dichlorophenoxyacetic acid
A/F:	Abundance Frequency
cc:	Cubic centimeter
CD:	Critical Differences
cm:	Centimeter
CRD:	Complete randomized design
EVC:	Environmental coefficient of variation
FYM:	Farm Yard Manure
g:	Gram
GA:	Genetic Advance
GA_3:	Gibberellic acid
GCV:	Genotypic coefficient of variation
GI:	Germination Index
h^2:	Broad sense heritability
Ha:	Hectare
$HgCl_2$:	Mercuric Chloride
IAA:	Indole-3-acetic acid
IBA:	Indole-3-butyric acid
ISTA:	International Seed Testing Association
IVI:	Important Value Index
KNO_3:	Potassium nitrate
m:	Meter
MDG:	Mean Daily Germination
Me:	Mean sum of squares due to error
Mg:	Mean sum of squares due to genotypes
MGT:	Mean Germination Time
mm:	Millimeter
mM:	Millimolar

Mr:	Mean sum of squares due to replications
Mt:	Mean sum of square due to treatments/populations
NAA:	Naphthalene acetic acid
NaHClo3:	Sodium Hypochlorite
PGRs:	Plant Growth Regulators
ppm:	Parts per million
PV:	Peak Value
PVC:	Phenotypic coefficient of variation
r:	Radius
R:	Number of replications/Populations
RD:	Relative density
RF:	Relative Frequency
SE:	Standard Error
Se:	Sum of squares due to error
SG:	Speed of Germination
Sg:	Sum of squares due to genotypes
SGP:	Seed Germination Percentage
Sr:	Sum of Squares due to replications
TBC:	Total Basal Cover
TIBA:	Triiodobenzoic Acid
V:	Seed Volume
VC:	Vermicompost
Ve:	Environmental variance
VeXY:	Environmental covariance between X and Y
Vg:	Genotypic variance
VgX:	Genotypic variance of X
VgXY:	Genetic covariance between X and Y
VgY:	Genotypic variance of Y
Vp:	Phenotypic variance

Introduction

Plants have been used as an important source of food, medicines and other purposes since prehistoric period. According to World Health Organization (WHO) about four billion people (80%) still rely on herbal medicine for their primary health care. More than 15,000 species of higher plants occur in India of which about 9,000 are economically important. Of these about 7,500 are of medicinal value; 3,900 are of food value; 700 are culturally important; 525 are used for fibre; 400 for fodder; 300 for pesticides and insecticides; 300 for gum, resin and dyes and 100 provide incense and perfume (Anonymous, 1994). Out of the above, over 9,500 wild plant species are used by the tribal societies of India (Shankar, 2006). Over 4786 ecosystem specific species of plants are used by ethnic communities for human and veterinary health care, across the various ecosystems in India (Kaul, 2010).

The genus *Clerodendrum* (Verbenaceae, more recently placed in the Lamiaceae) is a diverse genus with about 560 (Moldenke, 1971) to 580 (Munir, 1989) species of small trees, shrubs or occasionally perennial herbs, mostly in the tropics and subtropics of the old world (Verdcourt, 1992). This genus was first described by Linnaeus in 1753 based on the species *Clerodendrum infortunatum* from India and later Adanson changed the Latinized form *"Clerodendrum*" to its Greek form "*Clerodendron*" in 1763. After almost two centuries, Moldenke adopted the Latinized word "Clerodendrum" in 1942 which is now commonly used by taxonomists for classification and description of the

genus (Hsiao and Lin, 1995). However, recent phylogenetic studies have reported that genus *Clerodendrum* traditionally classified in Verbenaceae has now been included in the Lamiaceae (Stevens, 2012). *Clerodendrum* species display a high degree of morphological, cytological (Steane *et al.,* 1997) and chemical variations. The genus has been found to contain terpenoids as the major secondary metabolites (Yang *et al*., 2000), neo-clerodane diterpenes (Kumari *et al*., 2003; Pandey *et al*., 2005), triterpenes (Ganapaty and Rao, 1985) and iridoids (Wei *et al.,* 2000). Phenolic compounds have been frequently reported with phenyl propanoids (Kim *et al*., 2001) and flavonoids as a predominant class (Sinha *et al*., 1981) and few of species have been reported to contain macrocyclic alkaloids (Lumbu and Hootele,1993) and cyanogenic glycosides (Miller *et al.,* 2006). Some of these compounds have been evaluated for a number of biological activities mainly anti-inflammatory (Park and Kim, 2007), antiasthmatic (Vincent *et al*., 2012), hepatoprotective (Gopal and Sengottuvelu, 2008), antioxidant (Chae *et al*., 2006), cytotoxicity (Cheng *et al*., 2001), antitumor (Shi *et al*., 1993) and for the effects on central nervous system (Zhu *et al.,* 1996).

A high degree of morphological and cytological variation (from 2n=24 to 2n=184) amongst the species, suggesting the paraphyletic or polyphyletic origin of the genus *Clerodendrum*. Molecular systematic studies based on chloroplast and nuclear DNA also indicate polyphyletic origin of the genus (Steane *et al.,* 1999). The genus *Clerodendrum* is widely distributed in the tropical and warm temperate regions of the world, with most of the species occurring in tropical and northern Africa, Asia, Egypt and Madagascar. *Clerodendrum* is the largest genus of the tribe Teucrieae (Steane *et al*., 1999). In India 23 species were recorded (Rajendran and

Daniel, 2002) of which 16 were recorded from Arunachal Pradesh (Srivastava and Choudhury 2008).

C. colebrookianum Walp commonly known as East Indian Glory Bower is a flowering shrub or small tree, characterized by a foetid smell. The species is found in tropical and subtropical regions of Asia including India, Myanmar, Bangladesh, Malaysia, Indonesia, Thailand, Bhutan and Nepal; and also in temperate China. It is erect, reaches up to 1.5-3.5 m in height and is evergreen. Branchlets are usually 4-angled when young. Leaves are simple, opposite or rarely whorled. Leaf base is wedge- shaped to heart-shaped, margin entire to slightly wavy, tip long-pointed to pointed. Flowers are white and borne in 4-6-branched corymbose cymes, at the end of branches. Inflorescences loosely cymose or capitate, in terminal or rarely axillary paniculatethyrses. Calyx is campanulate or cup-shaped, densely pubescent. Corolla with a slender tube; lobes 5, spreading. Fruit is a drupe with 4 1-seeded pyrenes, sometimes separating into 2 2-loculed or 4 1-locular mericarps. It flowers during post-monsoon, from August to December. *C. colebrookianum*, has been reported to have antidiabetic, antihypertensive and sedative properties (Cheng *et al*., 2001; Kang *et al*., 2003; Chae *et al*., 2004; Choi *et al*., 2004). The boiled leaves of *C. colebrookianum* is used for the treatment of high blood pressure of both the Mishing tribes of Arunachal Pradesh and Cachar District of Assam (Paul *et al*., 2010, Yonggam 2012). Naga tribes of North eastern India also use the leaf of this plant as anti-helminthic properties and to cure intestinal helminth parasitic infections (Temjenmongla and Yadav (2005). In Mizoram, north-east India, it is considered as anti- canceros herbal drug and also used to increase breast milk (Lalramnghinglova, 2003). Besides, it is one of the delicious vegetable recipes of local people.

For successful management of plant genetic resources, germplasm evaluation based on morphological characters, genetic variability and agronomic values of the species is very important (Sevile and Holle, 1995). Conservation policies for the economically important species are now being laid on the basis of their sustainable utilization and conservation through cultivation is one such potential option (Nautiyal *et al*., 2001). Furthermore, this also provides alternatives to improve the livelihood of people, to generate self-employment options and replacement of low yielding traditional crops with high beneficial cash crops. Therefore, efforts are also needed towards domestication. For successful domestication, information on natural habitats, regeneration status and propagation techniques are very much important.

Population assessment in natural habitats is considered necessary for developing *in-situ* conservation strategies. To develop appropriate conservation strategies for economically important species of wild occurrence or threatened species, details of occurrence, availability and distribution pattern in natural habitat are required. To date, the assessment of threat categories to species in India including Indian Red Data Book is mostly based on qualitative observations (Pangtey and Samant, 1988; Samant *et al*., 1996; 1998; Pandey and Well, 1997). Very few studies have used both qualitative and quantitative attributes for assessment of species status (Samant *et al*., 1996; Airi *et al*., 1997; Airi *et al*., 2000; Bhatt *et al*., 2005; Bhatt *et al*., 2006).

Nature sustains wide range of variability of germplasm which needs to be observed for the conservation point of view both *in situ* and *ex situ* including future cultivation and crop improvement study. Occurrence of natural variation within a species is a part of evolutionary strategy to survive against risk (Reich *et al*., 2003).

No two individuals in sexually reproducing populations are the same. A species regenerating in wild shows variation among populations at different levels i.e. morphological, genetically and biochemical variations. These variations may be environmentally or genetically induced. Morpho-biochemical and genetic variations within species are often associated with the difference in geographical features and climatic conditions. Variations in morphological features help to assess genetic variation with superior genotype (Kumar *et al*., 2008; Malsawmkimi *et al*., 2013).

Long term viability of plant populations is related to the extent of genetic diversity they maintain (Szczepaniak and Cieslak, 2009). Moreover, recovering ability of declining populations is also related to and determined by genetic variations (Schemske *et al*., 1994). In terms of setting priorities for conservation, especially with regard to successful reintroduction of the populations in the wild, studies on genetic diversity are being increasingly used. Supporting evidence from many theoretical and empirical studies show a positive relationship between genetic variation and fitness in animal and plant species (Ledig, 1986).

Seed is a unit of life developing from fertilized ovule. Botanically seeds are essentially young plants whose life activities are going on at a minimum rate. They represent the most critical phase of a plant's life cycle and are responsible for the evolutionary continuum of plant species (Desai *et al*., 1997). It is a means of multiplication, a vehicle of dispersal and a form of resistance to environmentally unfavourable periods and therefore needs to work out in detail. Seed characteristics, germination preferences and seed dormancy patterns have been proposed as tools for understanding evolutionary patterns (Baskin and Baskin, 2004). The time duration for which the seeds retain their viability varies with species and to some extent with

the prevailing environmental conditions. There are seeds which remain viable for hundreds of years and those which lose their viability within a week or a month. What brings about the state of non-viability in a seed has been the subject of extensive investigation.

In spite of immense medicinal values and local use of *C. colebrookianum* Walp, information on existing germplasm variability in Mizoram, north-east India and seed germination is lacking. An evaluation of the extent of variability is also of immense importance for the selection of superior types. Similarly, seeds lose their viability under normal storage condition within short period. Therefore, observations are required to work out effect of storage conditions on viability and germination potential of the species key to regenerate and survive in adverse condition. Keeping in view the above background information, the present investigation was carried out with the following objectives are set forth for present work:

1. To explore natural habitats of *Clerodendrum colebrookianum* for phytosociological analysis and threat status in Mizoram.
2. To observe variability within species and selection of elite germplasm.
3. To observe microscopic feature of plants/ part constitute the drug.
4. To observe seed biology with special reference to viability, dormancy (if any) and seed germinability under short and long storage conditions.

Review of Literature

The literature pertaining to recent research works conducted in India and abroad on the phytosociological analysis, germplasm variability, microscopic evaluation and seed biology of a number of medicinal plants related to *C. colebrookianum* Walp. have been reviewed in this chapter.

2.1 Phytosociological Analysis

Phytosociology is the branch of science which deals with plant communities, their composition and development, and the relationships between the species within them (Hennekens and Schaminee, 2001). Phytosociological studies envisage the existing vegetation structure, species diversity, soil plant relationship; generate data on seasonal and temporal variation in available nutrients. There has always been a need to analyze and interpret the plant communities on different exposures and together firsthand information of the vegetation of this unexplored and floristically rich area. A phytosociological system is a system for classifying these communities. The aim of phytosociology is to achieve a sufficient empirical model of vegetation using plant *taxa* combinations that characterize univocally vegetation units. It indicates species diversity which determines the distribution of individuals among the species in a particular habitat. Quantitative information on a species plays a vital role in formulating a conservation plan and in understanding the ecology of the species (Uniyal *et al*., 2002). Therefore, association of tree species (Dobrovi *et al*., 2006) as well as herbs (Bhatt *et al*., 2005; Bhatt *et al*., 2006) was also studied through phytosociology in India and abroad.

Manjunatha *et al.* (2001) studied the vegetation pattern of Bhadra wild life sanctuary, and they analyzed frequency, density, A/F ratio, IVI value. A total of 3,405 individuals were encountered from the sampling sites. *Helecteres isora* and *Terminalia paniculata* were the dominant species in all transects of sampling sites.

Ayyappan and Parthasarathy (2001) worked on the patterns of tree diversity within a large-scale permanent plot of tropical evergreen forest, Western Ghats. Their study depicted that extent of variation in tree diversity assemblages in relatively "homogeneous" forest (for the sample size considered), under the same prevailing climatic and soil conditions, except for the minimal topographic variations of which with or without streams. The spatio-temporal distribution of tree species assemblages in the forest stand is possibly governed by a multitude of factors.

Padalia *et al*. (2004) analyzed the pattern of tree species diversity, diameter class distribution, species versus girth class relationship, evenness characteristic and similarity parameters of tree populations for different forest types of Andaman, India. The tree layer in evergreen forest is dominated by *Myristica andamanica*, with the highest IVI of 16.42.

Supriya Devi and Yadava (2006) studied the floristic diversity of *Dipterocarpus tuberculatus,* dominated forest of Manipur, North-Eastern India. A total of 123 species belonging to 48 families were recorded. The quantitative features such as density and importance value index of species varied significantly. The diversity index of shrubs and herbs were found to be higher than the tree species. The concentration of dominance was recorded highest in the tree species. The presence of low number of higher girth class of tree species and higher number of the saplings and seedlings indicates that the present forest is young exhibiting frequent regeneration.

Reddy *et al.* (2008) studied the assessment of quantitative structure and floristic composition of tropical forest of Mudumalai wildlife sanctuary. Forest structure was

analyzed across girth classes and height intervals. Altogether 156 tree species were analyzed and vegetation type-wise importance value index, Shannon- Weiner index, Simpson index, Margalef's index and Pielou index were calculated.

Perveen *et al*. (2008) prepared an inventory of plant species of Dureji game reserve. A total of 79 plants species were collected belonging to 66 genera under 32 families. Three rare species were found and observed quantitative analyses on species diversity in addition to phytosociological attributes. Analysis was conducted for some other ecological parameters also such as life forms, density, relative density, cover, relative cover, frequency, and relative frequency.

Khurana (2009) analyzed phytosociological attributes of woody vegetation, along the disturbance gradient in tropical dry deciduous forest. Maximum numbers of trees, seedlings, saplings were recorded in protected area and minimum in hillocks. Shrub spp. was maximum because of open canopy.

Singh and Singh (2010) studied the biodiversity of macrophytes in drains and their embankment of Jaunpur, Uttar Pradesh, India. They reported that phytosociological characters such as frequency, density and abundance were influenced by the climatic, anthropogenic and biotic stresses prevailing at the three study sites. All the species present at the study sites have shown maximum values of frequency, density and abundance in rainy season in comparison to that of summer and winter seasons.

Deb *et al*. (2011) investigated tree species diversity, population, structure and species richness in a lowland tropical rain forest in the eastern Himalaya, with reference to three distance canopy covered. They recorded a total of 1,053 trees covering 130 species in 44 families. Lauraceae, Dipterocarpaceae, Euphorbiaceae, Fabaceae, and Leguminaceae were dominated in the flora.

Kumar *et al*. (2012) studied the vegetation diversity in degraded hills of bar conglomerate formation in Rajasthan and reveals that highest population, frequency, density and abundance was recorded in *Zizipus mauritiana* among trees and *Euphorbia caducifolia* among the shrubs.

Moktan and Das (2012) studied the floristic diversity, dominance and abundance to frequency ratio of tree, shrub and herb species in the sub-tropical vegetation in Darjiling Himalaya and the dominant species recorded from these three layers were *Schima wallichii* (IVI-37.64), *Eupatorium adenophorum* (IVI-34.93) and *Oplismenus compositus* (IVI-21.72) respectively. The maximum species diversity (Shannon-Wiener Index) was marked for herbs (3.357) followed by shrubs (3.130) and lowest for the trees (2.927). The highest concentration of dominance was recorded for tree species (0.085) and lowest for herbs (0.052). Most of the species were randomly distributed whereas some showed clumped distribution

Kour and Sharma (2012) studied the diversity and Phytosociological analysis of Tree species in Sacred Groves of Vijaypur Block, Samba (J&K), India. Their study enumerated altogether, 28 tree species. Based on the calculations of frequency, density and abundance, IVI of each species was calculated. The basal area of the trees varied between highest of 712.33 m^2/ha for *Ficus religiosa* followed by 472.16 m^2/ha for *Eucalyptus citridora* and a lowest of 3.16m^2/ha for *Morus alba*. The overall tree diversity in sacred groves calculated by Shannon Weiner index was found to be 2.62. Some important trees in the groves are *Mangifera indica*, *Syzygium cumini*, *Eucalyptus*, *Ficus religiosa* etc.

Sinha and Sinha (2013) studied the phytosociological analysis of vegetation of Koria district in Chhattisgarh. Their study revealed that plants having 100% frequency were *Alangium lamarckii, Diospyros melanoxylon, Lawsonia inermis,*

Vicia sativa and *Shorea robusta*. Maximum density and abundance was observed in case of *Vanda roxburghii* and *Cassia sophera* which was 200, 150 and 166.6 respectively. Minimum density and abundance was observed with *Curculigo orchioides* (0.25 and 5), *Cordia macleodii* (0.5 and 5) and *Tecomella undulata* (0.25 and 5).

Moktan and Das (2014) studied the species richness and phytosociology in cold temperate zone vegetation of Darjiling Himalaya. The diversity indices represented the dominant species like *Rhododendron arboreum* Smith, *Daphne bholua* var. glacialis (Smith and Cave) Burtt and *Fragaria nubicola* (Lindley ex Hooker f.) Lacaita and rare species like *Gamblea ciliata* C.B. Clarke, *Sambucus adnata* Wallich ex DC., *Treutlera insignis* Hooker f., *Arisaema concinuum* Schott and *Codonopsis affinis* Hooker f. and Thomson. The maximum species diversity was marked for herbs (4.332) followed by shrubs (3.577) and lowest for trees (3.131). The highest species richness (Menhinick's Index) was estimated for herb layer (3.568) and least for the canopy (1.799). The concentration of dominance was 0.056, 0.032 and 0.014 respectively for the three layers. The species evenness was greater for herb layer (0.980) and least for the canopy (0.911).

Rao *et al*. (2015) studied the population structure and tree species diversity of Khammam district, Telangana state, India. They reported a total of 110 species belongs to 82 genera and 40 families. Among these only one family belongs to monocots (Arecaceae). Highest important index value was reported for the species *Mangifera indica* (8.29) followed by *Tamarindus indica* (6.66), *Ficus religiosa* (5.23), *Xylia xylocarpa* (4.53), *Madhuca longifolia* (4.48), *Terminalia bellerica* (4.43) *Ficus benghalensis* (4.34), *Ficus hispida* (4.34), *Semecarpus anacardium* (4.34) and *Terminalia chebula* (4.23).

Ismail *et al.* (2015) analyzed the phytosociological characteristics and the diversity patterns of herbaceous plants in Rashad and Alabassia localities of Sudan. During the study period, a total of 48 species, representing 42 genera from 20 families, were recorded. The phytosociological characteristics revealed that *Tetrapogon cenchriformis* dominated herbaceous species in sites 1, 7, 10, 11 with IVI values 139.3, 113, 70.3 and 95.8, respectively, followed by *Spermacoce pusilla* dominating sites 3, 4, 5 and 6 with IVI values 65.1, 50.4, 104.2 and 133.5, respectively. The distribution pattern revealed that 87.5 per cent species showed aggregated distribution, while 12.5 per cent were randomly distributed.

Shahid and Joshi (2016) studied the phytosociological analysis for tree species in three forest ranges (Barkot, Lachchiwala and Thano) of Dehra Dun forest division, Uttarakhand, India. Their study revealed that species richness ranged between 6 species in Thano to 15 species in Barkot. Among all the species, *Shorea robusta* was the dominant species in the Barkot range with the IVI value 141.32. In Lachchiwala range, the maximum IVI (126.36) was for the *Shorea robusta. Mallotus philippensis* and *Syzygium cumini* has the IVI value of 33.28 and 29.84 respectively. Similarly, at Thano range also, the *Shorea robusta* has the maximum IVI (187).

Tikariha *et al*. (2016) studied the phytosociological analysis of weed species in Durg District of Chhattisgarh state, India. They reported that IVI ranges from 4.22 to 26.15, Mean 13.41, Median 9.99, Mode 25.77, SD were 36.58 while SE were 5.39 and coefficient of variance were 272.78. *Blumea lacera* shows maximum IVI 26.15 and minimum was in *Boerhaavia difussa*, (4.22) and other are present in between this range. Plants showing maximum frequency were *Cynodon dactylon, Cyperus rotundus* etc. the numerical strength and abundance was recorded as high in *Alternanthra paranychioides, Andropogan odoratus*, *Medicago denticulata*. Density

range is 0.2 to 2.3, and abundance range from 1.0 to 7.0. Plants showing minimum frequency were 0.5 to 5.6.

Singh and Shukla (2017) studied the ecological investigation of some selected medicinal plants of Anpara region of Sonebhadra district of India. In their study 15 medicinal plants has been studied during rainy season with special reference to their phytosociological aspects viz Relative Frequency, Relative Density, Relative Dominance as well as Importance Valve Index (IVI). Their results revealed that out of 15 selected medicinal plants *Cynodon dactylon* maximum value of RF, RD, RM, IVI followed by *Vernonia cinerea Abrus precatorius*. Plant *Scoparia dulcis* has minimum value of RF, RD, RM, IVI.

Hailu (2017) analysed the phyto diversity, distribution, herb biomass and physico-chemical conditions of the vegetation system in the context of communal continuous open grazing and enclosed grazing management practices in the Harishin rangelands of Eastern Ethiopia. A total of 58 herbaceous species and 11 woody species were recorded. Analysis of Importance Value Index for two management practices was represented by different combinations of species with varied dominance. The herbs' diversity–dominance curve revealed a lognormal distribution in both managements practices. The overview of distribution patterns for most of the species layer showed contiguous growth and a clumped distribution pattern. Species diversity, richness, herb biomass, basal cover and soil physico-chemical attributes showed a distinct separation in relation to grazing management practices.

2.2 Germplasm variability

2.2.1 Genetic variability

In plants, genetic diversity within a species is often correlated with its geographical and ecological ranges (Szczepaniak and Cieslak, 2009). Among various factors, population size and habitat distribution greatly affects the level and distribution of genetic variation. According to population genetic theory, the populations (i) that remain small for several generations (genetic drift), (ii) initiated from a small number of colonists (founder effect), or (iii) that suffer rapid decline in size (population bottleneck) leads to loss of genetic diversity (Barrett and Kohn, 1991). Such anticipated effects not only reduce the chances of population persistence but also have serious implications on possible species extinction. Therefore, understanding the genetic consequences of such changes in population structure and their effects on the conservation value are major research challenges. Furthermore, *ex situ* conservation and domestication of wild species requires screening of wild populations for adaptability and in case of economically important plants. It also requires identification of best strains in terms of best quality and quantity of chemical constituents including bioactive molecules. Thus, maintenance of genetic diversity in conservation planning has become a central theme for survival of species (Falk and Holsinger, 1991).

The most easily obtained assessment of genetic variation is that of measuring morphological or phenotypic variation. Advantage of studying morphological variation is that phenotypic characters are often ecologically adaptive. Such morphological variation is often assumed to indicate genotypic variation, local differentiation or ecotypes. In these cases, variation in phenotype indicates

underlying variation in genome. The classic studies of Clausen *et al*. (1948) on *Achillea lanulosa* ecotypes showed that the morphological differences of ecotypes are often indicative of genetic differences in many other genes. Several attempts have already been carried out regarding intra-population variations including Nautiyal *et al*. (2003); Vashistha *et al*. (2006) and Purohit *et al*. (2008). Observations on morphological characters of *Lilium* (Lykkegaard, 2006; Balode, 2007) and a number of medicinal plant species was performed by several workers (Cirak *et al*., 2006; Dusek *et al*., 2007; Shimono *et al*., 2009; Sharma *et al*., 2009). These studies reveal that variability in morphological trait was due to interaction between genotype and environment.

Nautiyal *et al*. (2003) studied the assessment of diversity among populations on *Nardostachys jatamansi.* Vashistha *et al*. (2006) also assessed the population of two species of *Angelica* viz. *A. glauca* and *A. archangelica*. Studies conducted on population assessment of *Swertia chirayita* (Bhatt *et al*., 2006) and *Swertia angustifolia* (Bhatt *et al*., 2005) indicated the poor availability of these species in their respective study area. A number of studies on population assessment of various medicinal plants were taken by a number of researchers (Kala, 2000; Uniyal *et al.*, 2002; Rawat and Uniyal, 2004; Kala, 2005; and Semwal *et al.*, 2007) to know the current status and nature of endangerment of the medicinal plant species in Himalayan region.

2.2.2 Heritability and Genetic Advance

Any crop improvement depends on the magnitude of genetic variability and the extent to which the desirable characters are heritable. The role played by environment in expression of economic characters also needs to be taken into account. No doubt that the efficiency of selection depends mainly on the extent of

genetic variability present in the population. However, Burton (1952) suggested that co-efficient of variability together with heritability estimate will give a good picture of amount of genetic advance to be expected from selection. Similarly, Thakur and Choudhary (1965) reported that high heritability estimates together with high genetic advance and high genotypic coefficient of variability lead to high genetic gain.

Phenotype of an individual is determined by genotype and environment in which it grows. Success of a breeder in changing and improving the heredity of a character depends upon the degree of correspondence between phenotypic and genotypic values. Heritability is a measure that provides this information (Dabholkar, 1992). Heritability in broad sense or degree of genetic determination is proportion of total hereditary variance to phenotypic variance. The more useful estimate i.e. narrow sense heritability or degree of resemblance between relatives is ratio of additive genetic variance to phenotypic variance (Falconer, 1989). The most important function of heritability in the genetic studies of metric characteristics is its predictive role in expressing the reliability of phenotypic value as a guide to breeding value (Falconer, 1989). Genetic advance means improvement in the performance of selected lines over original population.

Heritable variation can be determined with greater accuracy, when heritability is studied along with genetic advance (Swarup and Chaughale, 1962). High heritability with high genetic gain is associated with additive gene effects (Panse, 1957). On the contrary, non-additive gene effect (dominance or epistasis) is associated with characters exhibiting high heritability and low genetic advance.

Rajagopal and Kandhasamy (2009) studied the heritability (%) and genetic advance in 18 genotypes of glory lily (*Gloriosa superba* L.). They obtained the

higher estimates of heritability and genetic advance as per cent of mean were obtained per plant for number of leaves, fresh pod yield and fresh seed yield.

Singh and Kumar (2010) studied the heritability and genetic advance for yield and its component traits in tulsi (*Ocimum sanctum* L.). Both additive and non-additive gene effects were present. Mean square due to lines, testers were also found significant for all the characters. They obtained moderate to high heritability for almost all the characters under study. The characters with high heritability were dry herbage yield, spike length, plant height and Fresh herbage yield. The traits having high heritability coupled with high genetic advance was fresh herbage yield.

Dalkani *et al*. (2012) assessed the genetic diversity in 10 populations of Iranian Ajwain based on agronomical and morphological characteristics. Among the characteristics studied, a high coefficient of variation was observed for the number of seeds (197.58), single plant yield (57.56) and shoot dry matter (56.28). Broad-sense heritability for all of the characteristics studied was moderate to high, and varied from 0.41 to 91 per cent with the exception of the number of branches, length and ripening period, which had low broad-sense heritability.

Sundesha and Tank (2013) studied forty-six genotypes of ashwagandha for twelve morphological and biochemical traits. High heritability along with high genetic advance was observed for dry root yield per plant, total alkaloid content in roots and number of secondary branches. High GA indicates that variation for these characters is due to additive gene effects and consequently the scope is more for improving dry root yield per plant and total alkaloid content through selection.

Tuppad *et al*. (2017) studied the heritability and genetic advance for yield and yield contributing traits were studied on *Holostemma adakodien* accessions. Broad sense heritability values revealed high heritability for fruit length (97.75 %),

petiole length (94.87 %), pedicel length (94.12 %), number of fruits per plant (92.89 %), plant height (90.61 %), leaf length (87.80 %), fruit diameter (86.82%), leaf width (85.86 %) and thickness of mesocarp (62.50 %). The highest genetic advance was recorded for number of fruits per plant (78.73 %), petiole length (65.47 %), pedicel length (47.27%), leaf width (39.88 %), plant height (31.36 %), leaf length (29.80 %), fruit length (25.91 %), thickness of pericarp (22.90 %)

2.2.4. Correlation Studies

Usually more than one trait is measured on progenies evaluated either for a specific trait in cyclical selection programmes or in applied breeding programmes that require a combination of traits to satisfy growers. Although yield is the primary trait of interest, yield contributing traits are all other corollary traits that a breeder must consider for eventual usefulness of genotypes evaluated. It is natural that the attention is given to associations among traits during selection and testing of genotypes.

Correlation, measured by a correlation coefficient is important in plant breeding because it measures the degree of association, genetic or non-genetic between two or more characters. If genetic association exists, selection for one trait will cause changes in other traits called the correlated response. The cause of correlation can be genetic and/or environmental. Genetic causes may be attributed to pleiotropism and/or linkage disequilibrium. When genes are not closely linked, linkage disequilibrium is not an important cause of correlation between characters in random mating populations. In such cases the existence of genetic correlations is mostly attributable to pleiotropism (Hallauer and Miranda, 1982).

Rao *et al*. (2008) studied the correlation of morphological traits in a population of *Curcuma amada* Roxb. Finger weight had significant and positive

correlations with all the other characters except sheath length. The highest correlation was recorded with corm weight (r = 0.772), while partial and multiple correlations suggest that corm weight, herb yield and finger length are important components for increasing finger weight.

Raghu *et al*. (2011) analyzed the variability and correlation of characters among five accessions of *Gmelina arborea* collected from different geographical and agroclimatic regions of South India. All the five accessions showed different levels of morphological and phytochemical variability. Among the morphometric characters studied, leaf length, leaf breadth, leaf area and petiole length showed significant positive correlation towards each other.

Wani *et al*. (2012) Evaluated *Jatropha curcas* germplasm comprising seven accessions and their results indicated a wide range of variability in vegetative growth and other qualitative attributes. Seed yield/plant had a positive and significant correlation with number of branches/plant, oil yield, plant spread (r=0.806, 0.802, 0.782), plant spread had a highest correlation with plant height (r=0.840).

In order to generate information on character association, and influence of characters on rhizome yield of cultivated *Atractylodes macrocephala*, correlation analysis for 21 morphological characters were studied by Zheng *et al*. (2013) on 100 morphologically distinct accessions. The significant and positive correlation for dry rhizome yield per plant was observed with the largest diameter, number of buds, number of branches and shape of the rhizome, and closely followed by primary branches per plant, plant height, plant crown, and apical lobule length and width of the largest lower leaf .

Aguilera-Cauich *et al.*, (2015) conducted a study to determine the genetic variation and relationships between American accessions of *Jatropha curcas* from

different origins. The principal component analysis explained 75.99 per cent of the total variation in three components. Repeatability analysis showed a low effect of the environment on the characters associated with oil yield (R = 0.99), 100 seed weight (R = 0.98) and seed volume (R = 0.97), indicating a high level of diversity among accessions and the feasibility of finding desirable characters in each collection.

Metougui *et al*. (2017) studied the correlation analysis among 30 quantitative traits of *Argania spinosa* (L.) and their results revealed that vigor traits (leaf and shoot sizes) were positively correlated with fruit traits. Broad sense of heritability estimates were high for clustered leaf traits and thorn numbers (H^2>0.90) and for most of the fruit, stone and almond traits (H^2>0.70).

2.3 Microscopic Evaluation

Plant anatomy describes the physical form and external structure of plants. It is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy. Studies on anatomy of plants have much significance in different sectors investigation. Anatomical studies can explain where, what, when and how level chemical compounds deposited, cellular changed, cellular abnormalities are occurred. The anatomical studied can be clarified the qualities of the wood properties. Anatomical studies can be a potential tool of taxonomic studies, mainly where there is no reproductive organ (Solereder, 1908; Metcalfe and Chalk 1950).

Idu *et al*. (2009) undertaken a comparative study of the morphological and anatomical features of the leaves and stems of *Stachytarpheta jamaicensis* and *S. cayennensis*. The presence of angular stem and pubescent leaves in the latter distinguishes it morphologically from the former, which is characterized by smooth

circular stem and glabrous leaves. The presence of trichomes was there in the leaf of *S. caynnensis* but absent in *S. jamaicensis*.

The anatomical features of *Indigofera aspalthoides* reveals that the mesophyll is differentiated in to adaxial zone of palisade cells, median level of circular cells and adaxial zone of spongy parenchyma cells. The lamina possesses reticulate venation system with wide, irregularly shaped vein-islets and well defined vein terminations. Calcium oxalate crystals are fairly abundant in the leaf mesophyll, cortical cells of the stem and phloem parenchyma of the root (Tamilselvi *et al*., 2011)

Mubo and Osiyemi (2012) investigated the anatomy, morphology and trichome distribution of leaves of two medicinal plants, viz. *Harrisonia abyssinica* and *Spathodea campanulata*. from Nigeria. Both the species showed variations in stomata types, epidermal cell shape, size and trichomes morphology. Trichomes were mostly multicellular and glandular on adaxial surface of *H. abyssinica*, on the two surfaces of *S. campanulata*, they were glandular and non-glandular. Stellate hairs were observed in *S. campanulata*. Trichomes were well segmented in *H. abyssinica*. Trichomes of *S. campanulata* on abaxial surface had aggregates of 5 basal cells, in *H. abyssinica* there was just one basal cell.

Cali (2014) determined the anatomical characteristics of the root, stem, leaf, petiole, calyx and corolla of medicinal species of *A. orientalis* in Turkey. Their study revealed that the pith rays of the root composed 3 to 4 rowed cells and stem was quadrangular. The shape of pith cell in the stem was ovaidal-polygon. There were glandular and non-glandular hairs on the surface layers on stem, leaves, petiole, calyx and corolla. Starch particles were also detected in the cortex cells of stem. The stomata were diastatic and the leaf was bifacial.

Elkamali *et al*. (2016) investigated the anatomical characters of the stems and leaflets of *T. longipetalous*, *T. pentandrus* and *T. terrestris*. The anatomical structures of the three studied species are very similar. The stems are formed of one layer epidermis, parenchymatous cortex, pericyclic fibers above the collateral vascular bundles, primary and secondary xylems and phloems, narrow medullary rays and wide parenchymatous pith. The densities of the epidermal hairs in the stems are found to be larger in *T. pentandrus* followed by *T. longipetalous* and lastly *T. terrestris*. There are sclrenchymatous fibres in the pith of *T. longipetalous* and *T. pentandrus* but not in *T. terrestris*. The leaflets are dorsiventral, showing kranz structure and different types of hairs. The densities of the epidermal hairs in the leaflets are found to be larger in *T. longipetalous* followed by *T. pentandrus* and lastly *T. terrestris*.

Tekin and Eruygur (2016) studied the anatomy and histology of the vegetative and reproductive organs of *Haplophyllum telephioides* Boiss., The anatomy of plant parts such as stem, leaf, sepal, petal, filament and pistil, reveals that stem has incipient secondary growth. The leaf was amphistomatic and the mesophyll is equifacial. Stomata were anomocyticand sunken. The leaf was coated by a thick cuticle and above epicuticular wax. Schizogenous glands were found in all vegetative and reproductive organs.

Ketjarun *et al*. (2016) investeigated the micromorphology of *Evolvulus* taxa viz. *Evolvulus alsinoides*, *Evolvulus nummularius* and *Evolvulus glomeratus* and reported that all taxa share several common features, such as a single layer of epidermis on both sides of leaf surfaces, sinuous anticlinal epidermal cell walls, anomocytic, paracytic or laterocytic stomata, and capitate glandular trichomes. Y-shaped hairs were found in two species but not in *E. nummularius*. Similarly,

isobilateral mesophyll occurs in both *E. alsinoides* and *Evolvulus glomeratus*, but a dorsiventral mesophyll is present in *E. nummularius*. Stems consist of a single layer of epidermis, one to four chlorenchyma layers, one to seven layers of cortical cells and a bicollateral bundle with pith in the center. Pollens of all taxa are monads, spheroidally shaped with 28–47 μm diameter, and 15-pantocolpate apertures type with microechinate ornamentation.

Florence and Domettila (2016) investigated the anatomical features of the leaves and stems of the plant *Gmelina asiatica*. Their study revealed the anatomical characters of *Gmelina asiatica* such as echinate epidermal cells, glandular trichomes, anomocytic stomata, calcium oxalate crystals, periderm cylinder, phellem cells and vascular bundle of leaf and stem explains typical features of Verbenaceae. This study provides valuable information for reference and correct identification of the family Verbenaceae.

Macro and micro morphology of the leaves of *Abutilon figarianum* and *Abutilon pannosum* were compared by Mohammed Ali *et al*. (2017). Their study reveals that the epidermises formed of one layer of small polygonal cells covered by mucilage, large numbers of epidermal hairs appeared. The two leaves are dorsiventral. The vascular bundles arch shaped surrounded by collenchymas and ground parenchyma. The structures at the apex, middle and bases of the leaves were similar, trichomes are denser in the middle region followed by the upper and lastly the lower region.

Sultana (2017) studied the stem and leaf anatomical structure of *Euphorbia hirta* L. Their results revealed that cross section of the stem has a circular shape where epidermis was uniseriate and isodiametric. Cortex was distinctly formed with about 5-6 rows composed of chlorenchyma and found laticifers. Tracheary elements

were resembled by vessels and trachieds. In leaf, the epidermis was uniseriate, regular, thin walled, usually similar in diameters and covered with thin cuticle layer. Mesophyll was differentiated into palisade and spongy layers, was composed of parenchyma cells.

2.4 Seed Biology

Germination begins the life cycle. It usually requires suitable temperature and moisture. Germination consists of those processes which begin with water uptake and which successfully terminate with emergence of the radicle or hypocotyl through the seed coverings (Bewley and Black, 1982). Germination of seed represents a dynamic period in the life cycle of plants and as a seed, makes the transition from a metabolically quiescent to an active and growing entity (Kumar and Purohit, 2001). For seed germination to occur, the dry, quiescent seed must imbibe water and hydrated (Srivastava, 2002). If imbibition is prevented by impermeable seed coats, as with hard seeds, germination cannot occur. Seed imbibition includes two simultaneous processes: entry of water into the seed, and the swelling of seed polymers. The swelling of the seed, resulting from the expansion of polymers and the intercalation of water between polymers may be expected to create forces of deformation in the expanding tissues (Leopold, 1983). Seed germination phase of plants is considered critical for the cultivation of plants as its timing largely predetermines the chances of survival of a seedling up to maturity (Thompson,1973) and it indirectly determines the crop stand density and consequently the yield of resultant crop (Gelmond, 1978). Temperature is important environmental factor that regulates seed germination (Mayer and Poljakoff- Mayber, 1982). Alternate low and high moderate temperature treatments induce and enhance germination of dormant seeds (Dhyani *et al*., 2013).

Gopikumar and Moktan (1994) noticed the highest germination in *Cassia fistula* and *Bauhinia purpurea* with IBA at 300 ppm. Gibberellic acid and indole butyric acid at 300 ppm concentration yielded the best germination and seedling survival in *Atropha belladonna* (Bisht and Kediyal, 1995). According to Singh *et al.* (1995), seeds of *Quercus leucotrihophora* treated with GA_3 solution 500 ppm for 24 hours gave maximum germination. GA_3 400 ppm and 200 ppm recorded maximum collar diameter and above and below ground biomass. A maximum germination (89.5%) was obtained by treatment of *Atropa belladonna* seeds with $GA_3$1000ppm (Elena *et al.*, 1997).

Chauhan and Nautiyal (2007) noticed GA_3 at 100 ppm treatment seeds favoured highest germination (90%) to *Nardostachys jatamansi* species. Similarly, seeds of *Pedicularis* species treated with GA_3 solution 500 ppm for 24 hours gave maximum germination (96%) (Ali *et al.,* 2007), The seeds of *Hippophae salicifolia* treated with 200ppm GA_3 improve germination up to 83 per cent (Airi *et al.,* 2009). Mohammad *et al.,* (2010), reported that the soaking of *Andrographis* species seeds in 200 ppm GA_3 improved germination (89%). A maximum germination (85%) was obtained by treatment of *Polygonatum rumicifolium* seeds with $GA_3$100ppm (Prakash *et al.,* 2011). Seeds of *Ferula assafoetida* treated with GA_3 2000 ppm at 4º C obtained 91.6 per cent germination (Zare *et al.,* 2011).

Kandari *et al*. (2007) studied the effect of GA_3, (100, 200, 300 ppm), at 3 temperature regimes (15, 20, 25ºC) and 2 photoperiodic conditions (light and dark) for enhancing and synchronizing the uniform germination in two endangered and commercially important medicinal herbs of the Himalayan region namely *Angelica glauca* Edgew and *Pleurospermum angelicoides* (Wall. ex DC.) Benth. ex C.B. Clarke. The viability of freshly collected seeds was good but it declined under

storage conditions at 4ºC with time. In *A. glauca,* seeds treated with GA3 at 100 ppm enhanced germination significantly (P<0.05) under light conditions at 25ºC. However, in *P. angelicoides* GA_3 did not influence seed germination as compared to control at 25ºC under light conditions. Mean germination time was recorded lowest under all treatments at 15ºC for both the species.

Ramasubbu *et al*. (2012) analyzed the seed biological characters and germination rate *Coscinium fenestratum* (Gaertn.) Colebr in the natural as well as in the laboratory condition. The fresh seeds showed 65 per cent viability and 28 per cent germinability. But the seeds pretreated with GA3 (1000-4000 ppm) showed 55 to 70 per cent germinability and 79 per cent of the seed germination observed after 6 months.

Patil *et al*. (2012) investigated the seed viability and influence of plant growth regulators on *in vitro* seed germination and seedling development of *Digitalis purpurea* L., a medicinally important cardiac glycoside producing plant. Significantly higher seed germination (65.5 ± 1.2% and 63.1 ± 3.2%) was observed on MS medium containing 10.0 µM BA and Kin, respectively. Addition of 10.0 µM IAA in the MS medium was most effective for significantly highest (81.0 ± 3.1%) germination percentage. This was evident by significantly higher germination speed (GS; 2.70 ± 0.1), germination value (GV; 31.3 ± 2.4) and vigor index (VI; 259.1 ± 10.1) on MS medium fortified with 10.0 µM IAA.

Ferodousi *et al*. (2014) studied seed germination of six indigenous medicinal plants of Bangladesh, namely *Adenanthera pavonina* L., *Helicteres isora* L., *Murraya paniculata* (L.) Jack, *Psoralea corylifolia* L., *Uraria lagopodioides* (L.) Desv. and *U. picta* (Jacq.) Desv. *ex* DC. The minimum days taken to germinate seeds in *Adenanthera pavonina* L., *Murraya paniculata* (L.) Jack, *Psoralea corylifolia* L.,

Uraria lagopodioides (L.) Desv. and *U. picta* (Jacq.) Desv. *ex* DC. are 12, 36, 10, 39 and 14, respectively. Seeds were not germinated in *Helicteres isora* L., however, propagation through stem cutting in this species revealed that plants flowers and set fruits only six to seven months. Epigeal type of seed germination was observed in all cases.

Warakagoda and Subasinghe (2015) studied the seed germination of *Coscinium fenestratum,* a threatened medicinal plant and their study revealed that mature seeds of *Coscinium fenestratum* recorded 92.2 per cent germination on exposure of seeds to direct sunlight for 6 h (sun cracking) followed by dipping seeds in 2250 mg/L gibberelic Acid (GA_3) solution for 24 h. Seeds subjected to sun cracking followed by water soaking for 24 h started to germinate 4 months after sowing and continued up to 6 months while GA_3 pre-treatments significantly reduce the time taken for germination from 6 months to 3 months. Seeds of *C. fenestratum* coupled with exogenous (physical, chemical and mechanical) seed dormancy created by hard seed coat, inhibitory substances present in the seed coat and the endosperm and endogenous (physiological) dormancy created by some other physiological factors as high Abscisic acid (ABA)/ GA_3 ratio.

Tiwari and Dubey (2017) investigated the dormancy and germination requirement in one year old and fresh seeds of *Asparagus racemosus, Cassia angustifolia, Abelmoschus moschatus* subjected to 12 pretreatments. *Abelmoschus moschatus* seeds, water soaking for 24 hrs, Gibbrellic acid and sand paper scarification had higher per cent seed germination on fresh seed under *in vitro* and *in vivo* conditions. In *Asparagus racemosus* hot water treatment (70 0ºC) for 1hr had significant positive effect closely followed by Cow dung Water + 24 hrs water

Soaking (76.00). *Cassia angustifolia*, showed promising results with sand paper scarification + 24 hrs water soaking under *in vitro in vivo* conditions.

Mehalaine *et al.* (2017) studied the seed germination behaviour of three medicinal plants growing wild in Algeria, viz. *Thymus algeriensis* Boiss & Reut., *Rosmarinus officinalis* L., *Marrubium vulgare* L. and assessed the effect of gibberellic acid (GA3) on breaking seed dormancy. The seeds were subjected to two experiments. First, seed germination ability without pre-treatment by incubating the seeds at ambient temperature (23±2 °C) and continuous darkness. Secondly, seeds were treated with GA3 (125, 250, 500 mg/L) and incubated in thermoperiod (25°C/16h, 15°C/8h) and continuous darkness. From the experiment one, *T. algeriensis* seeds presented the highest germination percentage (94.43 %) followed by *M. vulgare* (57.76 %). However, *R. officinalis* seeds did not germinate. The GA3 treatment did not exhibit any significant effect ($P > 0.05$) on the germination of the three tested plants. The highest germination rates were observed in *T. algeriensis* seeds (80 and 100 %) and *M. vulgare* (53.3, 73.3 and 86.7 %). *R. officinalis* seeds presented very low germination rates with (3.3, 6.7 and 16.7 %).

Materials and Methods

3.1 STUDY AREA

3.1.1 Geographical location

3.1.1.1 Mizoram State

With a geographical area of over 21, 087 Sq km and perched on the high hills of the North Eastern part of the country, Mizoram possibly has the most difficult terrain, over 80% of the total geographical area being hilly and with steep hills separated by rivers flowing North to South, thus, creating innumerable hurdles in intra-state as well as inter- state communication. This landlocked area is bounded by foreign countries on all sides except for a small stretch that rubs shoulder with Assam, Manipur and Tripura. Its international border, which is about 722 km, is almost 3 times longer than its border with the mainland. Mizoram lies between 21° 30´ N to 23° 15´ N Latitudes and 92° 16´ E to 93° 26´ E longitudes (Pachuau, 1994). Mizoram is bounded on the North side by Cachar district of Assam and Manipur state on the East and South by Chin Hills of Myanmar; on the west by Chittagong hill tracts of Bangladesh and Tripura.

The topography of Mizoram is, by and large, mountainous with precipitous slopes forming deep gorges culminating into several streams and rivers. Almost all the hill ranges traverse in the North-South direction. The eastern part of Mizoram is at a higher elevation compared to the western part. The average height of hill ranges is around 920 m, although the highest peak, the Blue mountain (Phawngpui), goes upto 2165 m.

3.1.1.2 Aizawl District

Aizawl district is situated in the northern part of Mizoram, it lies between 24˚ 25′16.04" and 23˚ 18′ 17.78″ N latitudes and 92˚ 37′ 03.27″ and 93˚ 11′ 45.69″ E longitudes. The total geographical area of Aizawl district is 3576.31 sq. km. and accounts for 16.96 per cent of the total geographical area of the state. It is bounded on the east by Champhai district and Manipur state, on the west by Mamit district and Kolasib district, on the north by Assam state and on the south by Serchhip district.

3.1.1.3 Kolasib District

Kolasib district is situated in the northern part of Mizoram, it lies between 24.1670° N latitudes and 92.7382° E longitudes. The total geographical area of Kolasib district is 1382.51 sq. km. and accounts for 6.56 % of the total geographical area of the state. The district is bounded on the north and northwest by Hailakandi district of Assam state, on the west by Mamit district, on the south and east by Aizawl district and on the northeast by Cachar district of Assam state.

3.1.1.4 Mamit District

Mamit District is situated in the north-western part of Mizoram between 23°15' 21.25” and 24° 15‟ 16.80" N latitudes and 92° 15' 44.54" and 92° 40' 39.63" E longitudes. The total geographical area of Mamit district is 3025 sq. km. and accounts for 14.35 % of the total geographical area of the state. The district is bounded on the north by Hailakandi district of Assam state, on the west by North Tripura district of Tripura state and Bangladesh, on the south by Lunglei district and on the east by Kolasib and Aizawl districts.

3.2 CLIMATE AND WEATHER

3.2.1 Mizoram

Mizoram has a pleasant climate. The upper part of the hills are predictably cold, cool during the summer, while the lower reaches are relatively warm and humid. Storms break out during March-April, just before or around the summer. During winter, the mean air temperature varies from 11° C to 21° C and in the summer it varies between 20°C to 32° C. The entire state is under the direct influence of south west monsoon. The rainy season normally starts from June and continues upto September and the rainfall is more or less evenly distributed throughout the state excepting the south-western parts that generally receive slightly higher rainfall.

3.2.2 Aizawl district

Aizawl district, the north central part of the state enjoys a moderate climate owing to its tropical location. It is neither very hot nor too cold throughout the year. Aizawl district falls under the direct influence of south west monsoon. As such the area receives an adequate amount of rainfall which is responsible for a humid tropical climate characterized by short winter and long summer with heavy rainfall.

Aizawl district enjoys a pleasant and moderate climate. It is generally warm in summer and mild cold in winter. The climatic condition accorded to Aizawl can be called humid-tropical, sub-tropical and sub-temperate climate characterized by short winter and long summer with heavy rainfall.

The year may be divided into four seasons:

i) The winter lasts from December to February

ii) The spring lasts from March to May

iii) The summer lasts from June to August

iv) The autumn season lasts from September to November

The entire district is under the direct influence of maritime tropical air mass brought in by south-west monsoon. The rainy season lasts from May to October with an average rainfall of 2500mm per annum. July-August are the rainiest months, whereas December and January are the driest months of the year with almost no rainfall.

Humidity is relatively high nearly all the year round. The relative humidity is highest during monsoon rains (about 90%). The period from January to April is comparatively dry, whereas the relative humidity remains between 60 and 70 per cent (Pachuau, 1994).

3.2.3 Kolasib district

Kolasib district enjoy moderate climate due to their tropical location. The weather is neither very hot nor too cold throughout the year. The district falls under the direct influence of the south west monsoon that receives an adequate amount of rainfall during the monsoon season. The highest temperature is observed during the months of April and May. May and June are the warmest months with mean daily maximum of 36ºC and the mean daily minimum of 18.5ºC. The temperature falls down sharply from the month of November and it is minimized in December and January. The average rainfall of Kolasib district is 2703 mm per annum and highest rainfall during a particular month was 852 mm recorded during August and July. The salient thermo-characteristics of the district is that temperature do not fluctuate much

throughout the year. The highest temperature observed during past decade was 35° C in the month of July. The warmest months with mean daily maximum at about 26° C and mean daily minimum at about 23° C was observed during June and July. The temperature started to fall down from the month of November and it is minimize in December and January.

3.2.4 Mamit district

The District is under the influence of Sub-Tropical Monsoon and the climate is tempered to a great extent by the altitude of its terrain and therefore is pleasant and not subjected to extremes. According to the classification of the Department of Environment & Forest, Govt. of Mizoram, the year is characterized by four distinct seasons:

1. Summer - March to May
2. Rainy - June to August
3. Autumn - September to October
4. Winter - November to February

The temperature varies between 10° to 24° C in between winter and summer. The District receives abundant rainfall with an average of 2200 mm. It is heaviest during June, July & August. The winter is normally cold and dry.

3.3. SOIL

3.3.1 Mizoram

The soils of Mizoram are dominated by sedimentary formation. These are generally young, immature, morstly developed from parent materials such as fereginous sandstones and shale. The soils of Mizoram are classified into three orders such as ultisols, inceptosols and entisols (Sarkar and Nandy, 1976; Singh and

Datta, 1989). The soils in the foot hills are collocium deposit and in plain areas alluvial deposits are predominant. The soils as a whole are well drained except in few valley flat lands. The soils in general have low inherent fertility viz. bases and mineral reserves. The soils in the hills are strongly acidic in reaction, wheras the soils in alluvial deposits are less acidic in nature (Anon., 1991)

The surface soils of the hilly terrains of Mizoram are dark, highly leached and poor in bases, rich in iron and mostly acidic with pH values ranging from 4.5-6.0. The soils are well drained, deep to very deep, rich in organic carbon, low in available phosphporus content and high in available potash. The surface soil textures are loam to clay loam with clay content increasing with depth. The percentages of clay, silt and sand within 50 cm of the surface in most cases are 20-30 per cent and 25-45 per cent respectively. The pH and organic carbon contents decreases and clay increases with depth. The base saturation above a lithic or paralithic contact is mostly low which is below 35 per cent (Anon., 1991). They are capable of providing substantial oxygen supply for plant growth and have capability to retain moisture and maintain supply through the growing seasons of most crops.

3.3.2 Aizawl district

The soil of the district is acidic in nature due to heavy rainfall. It contains a high amount of organic carbon and is high in available nitrogen, low in phosphorus and potassium content. These are deep to very deep but moderately to poor drained. The texture of the soil is mostly sandy loam to sandy clay loam. The soils found at order level are - entisols, inceptisols and ultisols.

3.3.3 Kolasib district

The soils in the valley flat lands of Kolasib District are dominated mainly by loose sedimentary formations. The soils are brown to dark brown, poor in bases, moderately acidic to neutral with pH ranging from 6.5 to 7.5, medium to high in organic carbon content, low available phosphate and medium to high available potash. These are deep to very deep but moderately to poorly drained. The texture of the soil is mostly sandy loam to sandy clay loam. The percentage of clay, silt and sand in the upper 50 cm ranges 15-35 per cent, 5-34 per cent and 40-75 per cent respectively (Anon., 2010).

3.3.4 Mamit district

The major soils of Mamit district is colluvial type. The soils of the district, in general, have been derived from parent rock such as ferruginous sandstone, shale, alluvial and colluvial materials. In general, the soil formations have been categorized into following groups: Hills, which includes colluvial soil, formed along the steep sided slopes because of accumulation of soil forming materials on slope surface. Valleys soils occur as a mixture of colluvial and alluvial materials. It is restricted to the rolling valleys along the river cources. Terraces are the remnants of deposits of cobbles and pebbles.

3.4 FOREST AND VEGETATION

3.4.1 Mizoram

The state of Mizoram falls under the tropical semi- evergreen belt. However, due to reduced jhum cycles it is replaced by bamboo interspersed with secondary forests. Various authors have classified the vegetation into different classes. Based

on Champion and Seth's Classification (1968) the following types of forests are found to be present in the state: (a) Tropical wet evergreen forests (up to 900 m) (b) Tropical semi evergreen forests (900-1500 m) and (c) Montane sub-tropical pine forests (1500-2158 m).

Of the three types, the most important one is Tropical Wet Evergreen Forests and are found in the Southern and Western parts of Mizoram. The common timber species found in the se area are *Dipterocarpus turbinatus, Artocarpus chaplasa, Terminalia myriocarpa, Duabanga sonneratoides, Michelia champaka* (Anon, 2003).

Tropical semi evergreen forest covers the central bio-geographic zone and the coverage is approximately 50 per cent of the total geographical area. The common timber species are: *Michelia champaka, Scima wallichi, Gmelina arborea, castanopsis tribuloides etc.* The Montane sub-tropical pine forest covers in the eastern fringes bordering Myanmar and approximately extending from 1500-2158 msl and constitutes about 24 per cent of the total geographical area. The common tree species are *Pinus kesiya, Rhododendron arboretum, Quercus serrata, Quercuc griffithii* etc. (Anon., 2003).

3.4.2 Aizawl district

The forest type of Aizawl district is mainly tropical wet evergreen forest mixed with semi- evergreen and tropical moist deciduous forests comprising mainly of bamboo. There are also sub-tropical forest found at high altitude places. The vegetation consists of a mixture of several species. Depending on the density of the canopy cover, the forests have been divided into Dense/closed, Medium dense and less dense forest. The dominant species in the district are *Celtis tetrandra,*

Anthocephalus chinensis, Wendlandia grandis, Protium serratum, Phoebe lanceolata, Phoebe attenuate, Ficus benghalensis, Garuga pinnata, Callicarpa arborea, Albizzia chinensis, Oroxyllum indicum, Aporusa octandra, Erythrina stricta etc.

3.4.3. Kolasib district

Kolasib district falls under the Tropical Wet Evergreen Forests. The common tree species found in the district are *Acrocarpus fraxinifolius, Adina cordifolia, Albizzia lebbek, Areca catechu, Artocarpus chaplasa, Bauhnia variegata, Bombax ceiba, Butea parviflora, Callicarpa arborea, Duabanga grandiflora, Erythrina stricta, Emblica officinalis, Ficus hirsuta, Garuga pinnata, Gmelina arborea, Lagerstroemia parviflora, Parkia rouxburghii, Sapium baccatum, Schima wallichi, Sterculia villosa and Tectona grandis.* The dominant herb species growing around the experimental area are *Mikania micrantha, Euphatorium odoratum, Saccharum spontaneum and Imperata cylindrica. Thysanolaena maxima* is also found in abundance.

3.4.4. Mamit district

The forest cover type of Mamit district is mainly tropical wet evergreen forest associated with moist deciduous forests and semi evergreen forest. Semi evergreen forests are found in small pockets on the hill slopes. The vegetation consists of a mixture of several species. Depending on the density of the canopy cover, the forests have been divided into Dense, Medium Dense and Less Dense forests.

The dominant species in the dense forests are *Macaranga indica, Anthocephalus chinensis, Quercus dealbata, Phoebe lanceolata, Leea indica,*

Maniltoa polyandra, Dillenia indica, Leea compactiflora, Callicarpa arborea. The vegetation of this forest is more or less similar with those species found in dense forests. The only difference lies in the crown density of these forests. The common vegetation of less dense forests are *Croton hookeri, Phoebe hainesiana, Terminalia myriocarpa, Erythrina Variegata, Mesua ferrae, Ficus religiosa, Macaranga indica, Albizzia procera, Leea indica, Schima wallichii*. etc.

3.5 AGRICULTURAL/ HORTICULTURAL SCENARIO

3.5.1 Mizoram

Agriculture is the mainstay for about 60 per cent of the population of Mizoram. Out of the total area only 21 per cent is put on the paddy/seasonal crops. As high as 63 per cent of the total cropped area is under shifting cultivation. The crops grown in the jhum are mixed. The principal crop is paddy and others are maize, cucumber, beans, ginger, mustard, sesame, cotton etc. Paddy occupies almost 50 per cent of the total cropped area and more than 88 per cent of the total area under food grains.

The state occupies an area of 41270.6 hectares under horticultural crops. Being endowed with favourable agro-climate condition, Mizoram is suitable for growing tropical, sub-tropical and even some of temperature fruits. A total area of 22,644.5 ha land has been covered for fruit cultivation in the state. The major fruit crops are, Orange, Pineapple, passion Fruit, Banana, Mango, Papaya, Guava, jack fruit, Grapes, Pear, Litchi and Apple. The state is highly conducive for the growth of various commercial fruits including kiwi fruits. At present Banglore Blue variety of grape is grown in large scale in the district of Champhai areas of Mizoram. From the grape production of around 600 MT of fruit, wine/grape juice is produced for local

consumption. A number of vegetables like cabbage, brinjal, tomato, french bean, lady's finger, chow chow, cowpea, pumpkin, broccoli are also grown in the state. The mild climate of the hills in Mizoram has the unique advantage of growing almost all type of flowers round the year. Flowers like anthurium, roses, bird of paradise, gladiolus, chrysanthemum etc. are grown successfully round the year. Anthurium has been exported outside the state regularly.

3.5.2 Aizawl district

Majority of the population in Aizawl district are mostly shifting cultivators. Rice cultivation in lowland and traditional shifting cultivation in hill slopes is the main livelihood of the villagers. In general, the economic condition of the rural people is low. The crop productivity per unit area is low due to poor technical know how and biophysical causes associated with the land.

The district also houses a variety of horticultural plantations. The common fruit crops in the district are orange, lemon, banana, pineapple, papaya. Similarly vegetables like cucumber, cowpea, cabbage, french bean, chow chow are growing commercially in the district. Among flowers, anthuriam, bird of paradise cultivation started on commercial scale under greenhouses by the farmers of the district.

3.5.3 Kolasib district

In Kolasib District, farmers mainly grow Paddy in Jhum and flat lands whereas WRC generally practice in rainfed conditions. Next to rice, maize comes second as cereal crop which mainly grows in Jhum land during summer i.e. from April to August. Winter Maize is also cultivated successfully where irrigation facility is available. Among the oilseed crops sesamum, soyabean, groundnut, sunflower etc.

are grown during Kharif and mustard M-27 and T-59 varieties are successfully grown during winter. Pulses, beans, arhar, rice bean etc. are grown during Kharif. Depending on the soil moisture condition, field pea, green gram, blackgram are grown on the river side successfully during winter without irrigation. The total cultivated area of the district is 12747 ha. Approximately, out of the total cultivated area only 892 Ha. i.e. 7.2% area is irrigated by flow and lift irrigation. Most of the area under hill slope cultivation and Jhum cultivation are rainfed. The economy of the district centers around agriculture and paddy is predominant commodity which is cultivated during kharif season and even during winter season in some pockets of the district where assured irrigation exist. Maize is another important field crop and is cultivated both during kharif and rabi season.

Most of the horticultural crops are grown under rainfed condition. The fruit crops like mandarin orange, hatkora, banana, passion fruit, grape, and some vegetable crops like beans, potato, cole crops, squash etc. spices like ginger, bird‟s eye chillies, turmeric etc. are highly popular and have good economic bearing.

3.5.4 Mamit district

Agriculture land has been divided into Kharif crop land and Agricultural/Horticultural Plantation. Though Rabi crops are also grown in small areas in a scattered manner they are not given separate class because they are less in area. Major crop produced during the Kharif season is paddy and in the Rabi season, pulses, grams, and mustard and vegetables such as cabbage, radish, carrot, tomato, and potato are grown. Most of the area depends upon rain. Cropping intensity is less than 160 per cent of the cropped area. In most of the area single crop is grown. Most of the farmers follow Jhuming. The major cereal crops includes rice and maize,

whereas rice bean, arhar, field pea, cow pea and French bean are the common pulses of the district. Oil seed crops like soybean, seasamum and rapseed and mustard are also grown commercially in the district.

The district is famous for orange plantation especially hatkora and mandarin fruits. The great havoc of Citrus decline in the western belt of the state has brought the blooming orange orchard to a standstill. A large number of Teak plantations also exist. Few oil palm plantation areas have started coming up in the area while much of the land left out still suits best for coffee and rubber plantation.

3.6 METHODOLOGY

3.6. 1. Survey of *C. colebrokianum* Walp.

Extensive surveys were made to identifying the natural habitats of *C. colebrokianum* in different parts of Mizoram. Among the all locations, 10 locations have been selected for the present investigation. Six plants were selected in each location for the investigation. The details of the geographical coordinates of all selected locations are presented in Table 3.1. The selected habitats were observed for phytosociological observation and assessment of threat status and morpho-genetic variability.

Table 3.1: Different locations and their Geographical coordinates

Sl no	Location	Latitude	Longitude	Elevation (m)
1	Durtlang	N 23°46'30.0"	E 092°44'00.3"	1254
2	Reiek	N 23°41'45.7"	E 092°36'44.0"	1148
3	Luangmual	N23°45'39.1"	E 092°43'19.4"	1041
4	Lungdai	N 23°53'11.9"	E 092°44'34.5"	1132
5	Serkhan	N 23°54'25.2"	E 092°44'27.6"	1028
6	Zonuam	N 23°44'12.9"	E 092°42'11.1"	1109
7	Chawlhhmun	N 23°44'39.6"	E 092°41'32.1"	942
8	Tanhril	N 23°44'21.1"	E 092°40'59.5"	901
9	Sakawrtuichhun	N 23°76’11’’	E 92°67’11.5"	440
10	Lengpui	N 23° 84’05.99”	E 92°61’96.89"	426.11

3.6.2 Phytosociological Analysis

The phytosociological studies were done by quadrat method. It was done by laying ten quadrats of 5mx5m around the study sites. This was determined by species area curve method as given by Mishra, (1968) and running's mean method of Kershaw, (1973). Proper care was taken to sample the most representative sites belonging to the whole area. The whole study area was covered for the estimation of relative frequency, relative density and relative dominance of various species.

3.6.2.1 Percentage Frequency

Percentage Frequency denotes the homogeneity of distribution of various species ecosystem. It was calculated as per the methods of Curtis and Mcintosh (1950) and Misra (1968) as follows and expressed in percentage.

$$\text{Frequency} = \frac{\text{Total number of quadrates in which species occurred}}{\text{Total number of quadrates studied}} \times 100$$

3.6.2.2 Abundance

Abundance of a species is determined as the number of individuals per quadrat. Abundance was calculated as per the methods of Curtis and Mcintosh (1950) and Misra (1968) by the following equation.

$$\text{Abundance} = \frac{\text{Total Number of individuals of species occurring}}{\text{Total number of quadrates in which species occurred}} \times 100$$

3.6.2.3 Density

Density is defined as the number of individuals of a species in a unit area and is an expression of the numerical strength of a species in a community. From the sampling data the density was calculated as per the methods of Curtis and Mcintosh (1950) and Misra (1968) as follows

$$\text{Density} = \frac{\text{Total number of individuals of the species (per quadrate)}}{\text{Total number of quadrates studied}} \times 100$$

3.6.2.4 Average basal cover

The average basal cover of each individual was calculated by using the methods of Greig-Smith, (1983) as,

Average basal cover = πr^2 (cm^2)

Where, r (radius)

3.6.2.5 Total basal cover

The total basal cover (TBC cm^2m^{-2}) was obtained by using the methods of Greig-Smith, (1983) by multiplying the average basal cover of each species by the respective density to of each and every species.

3.6.2.6 Relative Frequency

The relative values of frequency of each species was calculated following Curtis and Mcintosh (1950) to identify the status of *C. colebrokianum* population as:

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Frequency of all species}} \times 100$$

3.6.2.7 Relative Density

The relative density of each species was calculated following Curtis and Mcintosh (1950) to identify the status of *C. colebrokianum* population as:

$$\text{Relative Density} = \frac{\text{Density of a species}}{\text{Density of all species}} \times 100$$

3.6.2.8 Relative Dominance

The relative dominance of each species was calculated following Curtis and Mcintosh (1950) to identify the status of *C. colebrokianum* population as:

$$\text{Relative dominance} = \frac{\text{Total basal area of a species}}{\text{Total basal area of all species}} \times 100$$

3.6.2.9 Importance Value Index (IVI)

In order to record the dominance and ecological success of selected species, Importance Value Index (IVI) was calculated. The index was calculated by summing the three relative values viz., relative frequency, relative density and relative dominance (Curtis and Cottom, 1956) as:

IVI = Relative frequency + Relative density + Relative dominance.

3.6.2.10 Abundance and frequency (A/F) ratio

The ratio and abundance of frequency (A/F) is a relative measure to present the distribution of species in a community. It was calculated as per the method of Whitford (1949) as: A/F < 0.025 regular; between 0.025 and 0.05 Random and > 0.05 contiguous distributions.

3.6.3 Threatened categories

For threat category assessment, two criteria, i.e. population estimation (density and number of mature individuals) and extent of occurrence (number of populations) were used as per IUCN Red List Categories (IUCN, 1993). During the study only seed producing plants was considered as mature individuals. Further species having matured individuals < 250 was considered as critically endangered, < 2500 as endangered and, < 10,000 as vulnerable. Similarly species having single

population was categorized as critically endangered, 5 populations as endangered and 10 populations as vulnerable.

3.6.4 Germplasm Variability :

3.6.4.1 Morphological features

Morphological features of plants from different habitats were observed as per the methods given by Airi *et al.* (2000) and Nautiyal *et al.* (2003). Different phenophases especially reproductive phase i.e. budding, flowering, fruiting, seed setting and fruit dispersal was observed over the period of time in nature. Parameters viz., plant height (cm), collar diameter (mm), number of leaves per plant, Biomass production, root length (cm), root/shoot length ratio, root fresh weight (g),, shoot fresh weight (g), leaf fresh weight (g), root and shoot dry weight (g), root/shoot dry weight ratio, flowering and fruiting parameters.

3.6.4.1.1 Plant height (cm)

The height the six numbers of tagged plants were marked with permanent marker at 15 cm above the ground level. Height of the plant was measured from the red mark to the tip of youngest leaf. Finally the 15 cm length was added and the total height was expressed in cm.

3.6.4.1.2 Collar diameter (mm)

The collar diameter of the tagged plants was measured at 15 cm above the ground level and was expressed in mm.

3.6.4.1.3 Number of leaves plant^{-1}

The total number of leaves produced by the six numbers of tagged plants during the entire growth period was counted from the first leaf emergence up to the shooting stage.

3.6.4.1.4 Biomass production

Total biomass production was determined by taking the weight of plant with roots, leaves and fruits and finally they were added and expressed in kilogram.

3.6.4.1.5 Root length (cm)

The root length was calculated by uprooting the roots and measuring the root length from the collar joint to the tip of the root and was expressed in cm.

3.6.4.1.6 Root/shoot length ratio

The root / shoot length ratio was calculated by uprooting the whole plant along with the roots and measuring the root length and shoot length separately and dividing the root length by the shoot length.

$$\text{Root/shoot length ratio} = \frac{\text{Root length}}{\text{Shoot length}}$$

3.6.4.1.7 Root fresh weight (g)

The root fresh weight was calculated by weighing the weight of the roots immediately after harvest in digital balance and was expressed in g.

3.6.4.1.8 Shoot fresh weight (g)

The shoot fresh weight was calculated by weighing the weight of the shoots immediately after harvest in digital balance and was expressed in g.

3.6.4.1.9 Leaf fresh weight (g)

The leaf fresh weight was calculated by weighing the weight of the leaves immediately after harvest in digital balance and was expressed in g.

3.6.4.1.10 Root dry weight (g)

The root dry weight was calculated by weighing the oven dry weight of the roots in digital balance and was expressed in g.

3.6.4.1.11 Shoot dry weight (g)

The shoot dry weight was calculated by weighing the oven dry weight of the shoots in digital balance and was expressed in g.

3.6.4.1.12 Root/shoot dry weight

The root/shoot dry weight was calculated by dividing the oven dry weight of roots by the oven dried weight of the shoots.

$$\text{Root/shoot dry weight} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

3.6.4.2 Flowering and fruiting characteristics

3.6.4.2.1 Days required from bud initiation to bud differentiation

The number of days required from initiation of flower bud to the differentiation of bud was counted in each population.

3.6.4.2.2 Days required from initiation of bud to flowering

The number of days required from the initiation of flower bud till the full bloom in each population was counted.

3.6.4.2.3 Number of flowers/inflorescence

The total number of flowers in each inflorescence in each population was counted.

3.6.4.2.4 Length of inflorescences (cm)

The length of each inflorescence was measured with the help of a digital vernier calliper and was expressed in cm.

3.6.4.2.5 Fruit setting percentage (%)

Per cent fruit set per plant was worked out with a formula given by Westwood (1979).

$$\text{Fruit setting percentage} = \frac{\text{No. of flowers able to set fruit}}{\text{No. of flowers present in each inflorescence}} \times 100$$

3.6.4.2.6 Number of fruits/inflorescence

The total number of fruits in each inflorescence of a plant in each location was counted.

3.6.4.2.7 Days required from setting of fruits to maturation

The number of days required from fruit formation till the full maturity of fruits was counted in each population.

3.6.4.2.8 Fruit retention percentage (%)

The fruit retention percentage was recorded by using the following formula:

$$\text{Fruit retention percentage} = \frac{\text{No. of fruits retained till harest}}{\text{No. of fruits set in each inflorescence}} \times 100$$

3.6.5 Statistical analysis

The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTAT and SPAR 1.0 packages. The mean values of data were subjected to analysis of variance as described by Gomez and Gomez (1983) for Randomized Block Design (RBD). For estimation of different statistical parameters, following procedure and formulae were adopted:

3.6.5.1 Analysis of variance

Source of Variance	Degree of Freedom	Sum of squares	Mean sum of squares	Variance ratio (V.R.)
Replication (r)	r-1	Sr	Sr/(r-1) = Mr	Mr/Me
Genotypes (g)	g-1	Sg	Sg/(g-1) = Mg	Mg/Me
Error (e)	(r-1) (g-1)	Se	Se/(r-1) (g-1) = Me	

Where,

r = Number of replications

g = Number of genotypes

Sr = Sum of squares due to replications

Sg = Sum of squares due to genotypes

Se = Sum of squares due to error

Mr = Mean sum of squares due to replications

Mg = Mean sum of squares due to genotypes

Me = Mean sum of squares due to error

The calculated F-value was compared with tabulated F-value. When F-test was found significant, critical difference was calculated to find out the superiority of one entry over the others.

The standard error and critical differences were calculated as follows:

SE (m) ± = $\sqrt{Me}/r$

SE (d) ± = $\sqrt{2Me}/r$

$CD_{0.05}$ = S.E. (d) x $t_{(0.05)}$ (r-1) (g-1) df

Where,

SE (m) ± = Standard error of mean

SE (d) ± = Standard error of difference

$CD_{0.05}$ = Critical difference at 5 per cent level of significance

All the traits, which differed significantly, were utilized further for estimation of following genetic parameters

3.6.5. 2 Variance

3.6.5.2.1 Genotypic variance

The Genotypic variance was calculated using the following equations (Burton, 1952) as:

Genotypic variance (Vg) = (Mt - Me) / R

Where, Mt=mean sum of square due to treatments/populations

Me = mean sum of square due to error and

R=number of replications/populations

3.6.5.2.2 Phenotypic variance

The phenotypic variance was calculated using the following equations (Burton, 1952) as:

Phenotypic variance (Vp) = Vg + Ve

Where, Vg= Genotypic variance

Ve= Environmental variance

3.6.5.2.3 Environmental variance

The environmental variances were calculated using the following equations (Burton, 1952) as:

Environmental variance (Ve) = Me

Where, Me = mean sum of square due to error

3.6.5.3 Coefficient of variation

3.6.5.3.1 Genotypic coefficient of variation (GCV)

GCV is a measure of total genetic variability existing in a particular character and was calculated by using the formula as suggested by Burton and Devane (1953).

$$GCV\ (\%) = \sqrt{Vg} / X \times 100$$

Where, X = General mean of population

Vg = genotypic variance

3.6.5.3.2 Phenotypic coefficient variation (PCV)

PCV is the measure of total variation existing in a particular character and was calculated as per the method of Burton and Devane (1953) as:

$$PCV\ (\%) = \sqrt{Vp} / X \times 100$$

Where, X = General mean of population

Vp= phenotypic variance

3.6.5.3.3 Environmental coefficient variation (ECV)

ECV is the measure of environmental variation existing in a particular character and was calculated as suggested by Burton (1952) as:

$$ECV\ (\%) = \sqrt{Ve} / X \times 100$$

Where, X = General mean of population

Ve= environmental variance

GCV, PCV and ECV were classified (Sivasubramanian and Menon 1973) as shown below.

0-10% = Low

10-20 = Moderate

21% and above = High

3.6.5.4 Heritability (in broad sense) (h^2)

Heritability is the ratio of genetic variance to the total phenotypic variance. It was was calculated by the formula as suggested by Allard (1960).

$$\text{Heritability } (\%) = \frac{Vp}{Vg} \times 100$$

where,

Vg = Genotypic variance [Vg = (Mt - Me) / r]

Vp = Phenotypic variance [Vg + Ve]

As suggested by Johnson et al. (1955), h^2 estimates were categorized as

Low = 0-30%

Medium = 31-60%

High = 61% and above

3.6.5.5 Genetic advance (GA)

Genetic advance is the expected increase in the magnitude of a particular character when a selection pressure of chosen intensity is applied. The expected genetic advance (GA) resulting from selection of five per cent superior individuals was worked out as suggested by Allard (1960).

$$GA = Vg. K.\sqrt{Vp} / Vp$$

Where, K = 2.06 (selection differential at 5 per cent selection index)

Vg = Genotypic variance [Vg = (Mt - Me) / r]

Vp = Phenotypic variance [Vg + Ve]

3.6.5.6 Genetic gain

It is expressed as per cent ratio of genetic advance and population mean was calculated by the method given by Johanson *et al.* (1955).

Genetic gain = GA / X x 100

The genetic advance as percent of mean was categorized as suggested by Johnson *et al.* (1955) and is mentioned below:

0-10% = Low

10-20% = Moderate

20% and above = High

3.6.5.7 Correlations

The genotypic and phenotypic correlations were calculated as per Al-Jibouri *et al.* (1958) by using analysis of variance and covariance matrix in which total variation has splitted into replications, genotypes and errors. All the components of variance were estimated from the analysis of covariance as given below:

3.6.5.7. 1 Analysis of Variance and Covariance

Source of variance	Degree of freedom	Mean sum of Squares		Mean sum of products	Variance
		X	Y		
Replications (r)	r-1				
Genotypes (g)	g-1	Mg X	Mg Y	Mg XY =MP_1	MP_1/MP_2
Error (e)	(r-1) (g-1)	Me X	Me Y	Me XY =MP_2	

Genotypic, phenotypic and environmental covariances between X and Y characters were worked out as under:

Ve XY = MP_2

Vg XY = (MP_1-MP_2) / r

Vp XY = Vg XY + Ve XY

Where,

Ve XY = Environmental covariance between X and Y

Vg XY = Genetic covariance between X and Y

Vp XY = Phenotypic covariance between X and Y

3.6.5.7.2 Coefficients of correlation

3.6.5.7.2.1 Genotypic correlation coefficient between X and Y

$$r_g = \frac{Vg\ XY}{\sqrt{Vg\ X \times Vg\ Y}}$$

Where,

Vg XY = Genotypic covariance between X and Y

Vg X = Genotypic variance of X

Vg Y = Genotypic variance of Y

3.6.5.7.2.2 Phenotypic correlation coefficient between X and Y

$$r_p = \frac{Vp\ XY}{\sqrt{Vp\ X \times Vp\ Y}}$$

Where,

Vp XY = Phenotypic covariance between X and Y

Vp X = Phenotypic variance of X

Vp Y = Phenotypic variance of Y

The calculated correlation coefficients (r) values were compared with 'r' tabulated values as given by Fisher and Yates (1963) at (n-2) degrees of freedom to test their significance, where 'n' denotes number of genotypes. If calculated 'r' value at 5 per cent level of significance was greater than tabulated value of 'r', the correlation was said to be significant.

3.6.5.8 Regression

Regression matrix and multiple regressions to see the effect and relation of different growth parameters on biomass was calculated by using software Windostat Version 9.2 from Indostat services, Hyderabad (Plant Breeding Division, Sugarcane Breeding Institute, Coimbatore).

3.6.6 Microscopic Evaluation

Microscopic evaluation studies were carried out by taking free hand sections of stem, leaf petiole and root of *C. colebrookianum* following Johansen (1940) and Wallis (1985) methods. Photographs were obtained by observing free hand section under microscope as well as methods described by dept. of AYUSH, Ministry of H&FW, GOI, New Delhi as 'Protocol for Testing of Ayurveda, Sidha and Unani Medicines'. Thereafter, tissues were identified and key characters were identified.

3.6.7 Seed biology

3.6.7.1 Physical parameters of the seeds

For physical parameters of the seeds 20 seeds were taken in each replication.

3.6.7.1.1 Seed Weight (g)

The weight of the seeds was measured with the help of digital weighing balance and expressed in g.

3.6.7.1.2 Seed Length (mm)

The seed length was measured with the help of digital vernier calipers and expressed in mm.

3.6.7.1.3 Seed diameter (mm)

The seed diameter was measured by measuring the broadest portion of the seeds with the help of digital vernier calipers and expressed in mm.

3.6.7.2 Moisture Loss (%)

The moisture loss was recorded immediately after collection and at 30 days interval up to 90 days. The moisture loss percentage was assessed at each level as:

$$\text{Moisture loss \%} = \frac{\text{Moisture loss}}{\text{Fresh weight}} \times 100$$

3.6.7.3 Seed volume (cc)

Volume of the seeds was measured by water displacement method for which seeds were dipped in a known volume of water in a measuring cylinder and after immersing the seeds the rise in water level was noted. Ten replicates with 10 seeds in each replicate were used for this purpose. Seed volume (V) was calculated using following formula:

$$V = V2 - V1$$

Where, V1 = initial water level

V2 = final level after dipping the seeds

3.6.7.4 Imbibition (%)

To determine the water uptake by the seeds, five replicates of five seeds each was randomly taken and weighed individually for their initial weight using the electric balance. Seeds was then soaked in distilled water and kept at room temperature (25+2ºC). Weight of these seeds was taken after every 24 hrs till the constant weight (Baskin and Baskin, 1998). Water Imbibition was estimated as percent increase in weight of seed using following formula.

Percent Imbibition = [Imbibed weight - Initial weight] / Initial weight x 100

3.6.7.5 Seed viability

The viability of the stored seeds was tested using tetrazolium (pH 6.0) according to the method of Moore (1962).

Viability test was conducted from two different storage conditions i.e. ambient conditions and refrigerated condition at one month interval by using 2,3,5 triphenyl tetrazolium chloride. The seeds were kept in 2,3,5 triphenyl tetrazolium chloride solution in a conical flask. The seeds containing 2,3,5 triphenyl tetrazolium chloride solution was kept in water bath maintaining the temperature of 35º C for 24 hours. Thereafter the embryo of the seeds was observed. The tissue which stains red color or pink color is a positive indicator of viability. Non-viable seed tissues do not react with triphenyl tetrazolium chloride, and consequently do not stain.

3.6.7.5.1 Seed viability at refrigerated condition

Seed viability was tested by using 2,3,5 triphenyl tetrazolium chloride as described above by storing the seeds in refrigerator at 4°C. The seeds were stored immediately after harvest, i.e. in the month of November and viability test was conducted at monthly interval i.e. from December to August and the results were expressed in percentage.

3.6.7.5.2 Seed viability at ambient condition

Seeds were stored at ambient condition and viability was tested by using 2,3,5 triphenyl tetrazolium chloride as described above. The viability was tested at monthly interval i.e. from December to August and the results were expressed in percentage.

3.6.7.6 Germination studies

3.6.7.6.1 Germination of the refrigerated seeds

Germination studies of the seeds were conducted by storing the seeds in refrigerator at 4°C. The seeds were stored immediately after harvest, i.e. in the month of November and germination was conducted at monthly interval i.e. from December to August and the results were expressed in percentage.

3.6.7.6.2 Germination of seeds stored at ambient condition

Seeds were stored at ambient condition and germination percentage was tested at monthly interval i.e. from December and the results were expressed in percentage.

3.6.8 Germination test of fresh seeds by using PGRs and chemicals and media composition

The germination test was conducted in the seed germinator at the laboratory of Dept. of Horticulture, Aromatic and Medicinal Plants. The experiment comprised of 3 replicates with 20 seeds each. The seeds were first surface sterilized with aqueous solution of Mercuric chloride ($HgCl_2$) 0.1 % for one minutes followed by ethanol (avoid microbial contamination) for two minutes and then rinsed thoroughly (3-4 times) with distilled water.

3.6.8. 1 Germination studies with different PGRs and chemicals

The treated seeds were kept in glass petri dishes on single layer of Whatman No.1 filter paper after pretreatments as describe below. The substratum was moistened regularly with distilled water to keep it moist. The petridishes were kept at different temperature regimes (10,15, 20 and 25+2^{o}C with humidity at 80%) in growth chamber with 16 /8 hours light and dark conditions in laboratory. The following pre-treatments were for the study. The different PGRs were selected by studying previous literatures.

Treatments	PGRs and chemicals
T_1	Control
T_2	GA3 50 PPM
T_3	GA3100 PPM
T_4	GA3200 PPM
T_5	GA3500 PPM
T_6	IAA 50 PPM
T_7	IAA 100 PPM
T_8	IAA 200 PPM
T_9	IAA 500 PPM
T_{10}	IBA 50 PPM
T_{11}	IBA 100 PPM
T_{12}	IBA 200 PPM
T_{13}	IBA 500 PPM
T_{14}	NAA 50 PPM
T_{15}	NAA 100 PPM
T_{16}	NAA 200 PPM
T_{17}	NAA 500 PPM
T_{18}	2,4-D 50 PPM
T_{19}	2,4-D 100 PPM
T_{20}	2,4-D 200 PPM
T_{21}	2,4-D 500 PPM
T_{22}	TIBA 50 PPM
T_{23}	TIBA 100 PPM
T_{24}	TIBA 200 PPM
T_{25}	TIBA 500 PPM
T_{26}	KNO_3 100 mM
T_{27}	KNO_3 150 mM
T_{28}	$NaHClO_3$100 mM
T_{29}	$NaHClO_3$150 mM

3.6.8. 2 Germination studies with different media composition

For conducting germination test with various compositions of media in the nursery, a bed size of $1x1m^2$ area was taken for each replication under each treatment. Experimental set consisting of 3 replications with 20 seeds in each replication. The different growth media were selected by studying previous literatures. The different germinating media *i.e.* various composition of FYM, vermicompost with nursery soil and sand was used as different treatments.

T1= FYM+Vermicompost (VC)+ Soil + Sand (1:1:1:1)

T2= FYM+Vermicompost (VC)+ Soil + Sand (1:1:2:1)

T3= FYM+Vermicompost (VC)+ Soil + Sand (1:1:1:2)

T4= FYM+Vermicompost (VC)+ Soil + Sand (2:1:1:1)

T5= FYM+Vermicompost (VC)+ Soil + Sand (2:2:1:1)

T6= FYM+Vermicompost (VC)+ Soil + Sand (2:2:2:1)

T7= FYM+Vermicompost (VC)+ Soil + Sand (2:2:2:1)

T8= FYM+Vermicompost (VC)+ Soil + Sand (2:2:1:2)

T9= Nursery soil alone

T10= Sand alone

3.6.9 Acquisition of germinability

To determine the acquisition of germinability, seeds were harvested at three stages i.e. immature seeds (green fruit), mature seeds and dehisced seeds. If seed germinability found low or delayed even at favourable conditions the type of

dormancy were also be observed as per the method suggested by Baskin *et al*. (2006). The following parameters were observed for all the seeds.

3.6.9.1 Germination Percentage

Emergence of radical was considered as germination. Seed germination percentage (% germination, SGP) was calculated following the ISTA (1999) methods. Percentage germination was calculated as:

Germination %= [Number of germinated seeds / Total number of seeds] x100

3.6.9.2 Speed of germination

Speed of germination (SG) was calculated by using the methods of Magurie (1962) as follows:

$$\text{Speed of germination (SG)} = \frac{A1}{N1} + \frac{A2}{N2} + \frac{A3}{N3} \quad \frac{}{} \quad \frac{An}{Nn}$$

Where, A1, A2, A3,---------- ----An is number of seeds newly germinated on N1, N2, N3 ----------N ^{nth} day, respectively.

3.6.9.3 Mean Germination Time

Mean germination time (MGT) was calculated by using the methods of Eliss and Roberts (1981) as follows:

$MGT = \Sigma dn / \Sigma n$

Where, n = number of seeds germinated; d = number of days

3.6.9.4 Peak Value

Peak value (PV) was calculated as per the methods of Czabator (1962);

PV = highest seed germinated/d

3.6.9.5 Mean Daily Germination

Mean daily germination (MDG) was calculated by following the methods of Czabator (1962) as follows:

MDG = total number of germinated seed/total number of days

3.6.9.6 Germination Index

The germination index (GI) was calculated as per the methods of Kendrik and Frankland (1969) as follows:

GI =Total % germination/time taken to reach 50% germination

3.6.10 Nursery parameters

3.6.10.1 Percent survival

Surviving plants in each bed was counted in the nursery and the survival percent was worked out as follows:

$$\text{Survival percent} = \frac{\text{Plant count in a bed}}{\text{Number of seeds germinated in that bed}} \times 100$$

3.6.11 Seedlings growth

Ten seedlings of an individual seed lot per replication were separately selected and tagged. The following parameters were recorded.

3.6.11.1 Plant height

The height of the tagged plants were marked with permanent marker at 15 cm above the ground level. Height of the plant was measured from the red mark to the tip of youngest leaf. Finally the 15 cm length was added and the total height was expressed in cm.

3.6.11.2 Collar diameter

The collar diameter was measured at 15 cm above the ground level and was expressed in mm.

3.6.11.3 Number of leaves/plant

The total number of leaves produced by plants during the entire growth period was counted from the first leaf emergence up to the shooting stage.

3.6.11.4 Biomass Production (g)

Total biomass production was determined by taking the weight of plant with roots, leaves and fruits and finally they were added and expressed in gram.

3.6.11.5 Root Length (cm)

The root length was calculated by uprooting the roots and measuring the root length from the collar joint to the tip of the root and was expressed in cm.

3.6.11.6 Root /Shoot length ratio

The root / shoot length ratio was calculated by uprooting the whole plant along with the roots and measuring the root length and shoot length separately and dividing the root length by the shoot length.

$$\text{Root/shoot length ratio} = \frac{\text{Root length}}{\text{Shoot length}}$$

3.6.11.7 Root fresh weight (g)

The root fresh weight was calculated by weighing the weight of the roots immediately after harvest in digital balance and was expressed in g.

3.6.11.8 Shoot fresh weight (g)

The shoot fresh weight was calculated by weighing the weight of the shoots immediately after harvest in digital balance and was expressed in g.

3.6.11.9 Leaf fresh weight (g)

The leaf fresh weight was calculated by weighing the weight of the leaves in digital balance and was expressed in g.

3.6.11.10 Root dry weight

The root dry weight was calculated by weighing the oven dry weight of the roots in digital balance and was expressed in g.

3.6.11.11 Shoot dry weight

The shoot dry weight was calculated by weighing the oven dry weight of the shoots in digital balance and was expressed in g.

3.6.11.12 Root/shoot dry weight ratio

The root/shoot dry weight was calculated by dividing the oven dry weight of roots by the oven dried weight of the shoots.

$$\text{Root/shoot dry weight} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}$$

Results

4.1 Phytosociology

4.1.1. Percentage frequency

Table 4.1 displays percentage frequency of *Clerodendrum colebrookianum* among the different sites in Mizoram. The data presented in table reveals that there was variation in percentage frequency of *C. colebrookianum* Walp. in the range of 40 – 80 per cent among the different sites. The maximum frequency (80%) was obtained in Luangmual, which was followed by Durtlang and Chawlhhmun (70%) respectively whereas, the lowest was obtained in the germplasm of Lengpui (40%).

4.1.2 Abundance

There was variation among the germplasm with respect to abundance pattern of the germplasms. The data furnished in Table 4.1 reveals that among the different sites in our study, the highest value of abundance was obtained in Sakawrtuichhun (15.40%), followed by Lengpuii (12.50%) and Chawlhhmun (10.57%) respectively. Among all the studied locations, the lowest value of abundance was obtained in Lungdai (7.5%).

Table 4.1: Phytosociological parameters of *C. colebrookianum* in different locations

Sl No.	Location/ Population	Percentage Frequency	Abundance	Density (Plants $ha^{-1)}$	Average basal cover (cm^2)	TBC (cm^2m^{-2})	Associated Species
1.	Durtlang	70	9.85	276.00	22.38	154.42	*Ageratum conizoids, Eupatorium odoratum, Thysanolaina maxima, Colocasia spp, C ajanus cajans, Bidens pilosa*
2.	Reiek	60	8.16	196.00	16.82	82.41	*Achyranthes aspera L. Acmella oleraceae, Ageratum conizoids, Mimisa pudica L., Manihota esculenta, Solanum torvum*
3.	Luangmual	80	8.87	284.00	28.22	200.36	*Cuscuta reflexa Roxb, Mikania micrantha, Eupatorium odoratum, Centela asiatica, Ageratum conizoids, Bidens biternata*
4.	Lungdai	60	7.50	180.00	19.94	89.73	*Mimisa pudica L., Ageratum conizoids, Blumea lanceolaria, Mikania micranth, Trevesia palmat, Thysanolaina maxima*
5.	Serkhan	50	7.60	152.00	16.32	62.01	*Solanum indicum L. Acmella oleraceae Pteridium acquilinum Adhatoda vesica Mill Ageratum conizoids Cuscuta reflexa Roxb.*
6.	Zonuam	60	8.50	204.00	9.39	47.88	*Adenostemma lavenia Adhatoda vesica Mill Eupatorium odoratum Pteridium acquilinum Artemisia vulgaris*
7.	Chawlhhmun	70	10.57	296.00	16.84	124.61	*Acmella oleraceae Centela asiatica Colocasia spp Thysanolaina maxima Mikania micrantha Cuscuta reflexa Roxb.*
8.	Tanhril	60	7.83	188.00	12.06	56.68	*Ageratum conizoids Oroxylum indicum Eupatorium odoratum Ageratum conizoids Mikania micrantha*
9.	Sakawrtuichhun	50	15.40	308.00	13.84	106.56	*Solanum indicum L. Acmella oleraceae Adenostemma lavenia Solanum indicum L. Acmella oleraceae Bidens pilosa Cuscuta reflexa Roxb.*
10.	Lengpui	40	12.50	200.00	11.09	55.45	*Trevesia palmate Ageratum conizoids Eupatorium odoratum Blumea lanceolaria Centela asiatica Thysanolaina maxima Imperata cylindrical*

4.1.3 Density

The plant density of *Clerodendrum colebrookianum* in the studied locations ranged from 152.00-308.00 plants ha^{-1}. The data presented in Table 4.1. reveals that the maximum density of *Clerodendrum colebrookianum* Walp. was obtained in the location Sakawrtuichhun (308.00 plants ha^{-1}), followed by Chawlhhmun (296.00 plants ha^{-1}). However, among the studied geographical locations, the lowest density was obtained in Serkhan village (152.00 plants ha^{-1}).

4.1.4 Average basal cover (cm^2)

The average basal cover of *C. colebrookianum* Walp. in the different geographical locations are displayed the in Table 4.1. The data presented in the Table reveals that there was variation among the plants with respect to this parameter. The maximum average basal cover was found in Luangmual (28.22 cm^2), followed by Durtlang (22.38 cm^2), Lungdai (19.94 cm^2) and Chawlhhmun (16.84 cm^2). The minimum average basal cover among all the studied geographical locations was observed in Zonuam (9.39 cm^2).

4.1.5 Total Basal Cover (cm^2m^{-2})

The data presented in Table 4.1. reveals that the total basal cover (cm^2m^{-2}) of *C. colebrookianum* Walp. varied with respect to different geographical locations. Among all the locations, the highest Total Basal Cover was reported in Luangmual (200.36 cm^2m^{-2}), followed by Durtlang (154.42 cm^2m^{-2}), Chawlhhmun (124.61 cm^2m^{-2}) and Sakawrtuichhun (106.56 cm^2m^{-2}). Among the studied populations, the lowest total basal cover of *C. colebrookianum* Walp. was observed in Zonuam (47.88 cm^2m^{-2}).

4.1.6. Associated Species

Table 4.1. displays the associated species found with *Clerodendrum colebrookianum* Walp. in different locations. The data presented in the table reveals that a number of associated species are grown naturally in the vicinity of *C. colebrookianum* Walp. Most commonly found species in association with *C. colebrookianum* Walp. are herbs and shrubs. These includes *Achyranthes aspera L. Acmella oleraceae, Adhatoda vesica Mill, Adenostemma lavenia, Ageratum conizoids, Artemisia vulgaris, Bidens pilosa, Blumea lanceolaria, Cajanus cajans, Centela asiatica, Colocasia spp, Cuscuta reflexa Roxb, Eupatorium odoratum, Imperata cylindrical, Manihot esculenta, Mikania micrantha, Mimosa pudica L., Oroxylum indicum, Pteridium acquilinum, Solanum indicum L., Solanum torvum, Thysanolaina maxima* and *Trevesia palmate.*

4.1.7. Relative frequency

The relative values of frequency of each species was calculated to identify the status of *C. colebrookianum* population. It is evident from the data presented in table 4.2 and Fig. 4.1, that relative frequency of the germplasms varied among the different locations in the ranged of 6.66- 13.33 per cent. Among the studied locations, the highest relative frequency was obtained in Luangmual (13.33%), followed by Chawlhhmun and Durtlang (11.66%) and Reiek, Zonuam and Tanhril (10.00%). The lowest value of relative frequency was observed in Lengpui (6.66).

Table 4.2: Relative frequency, density, dominance, Importance Value Index (IVI), A/F Ratio and distribution pattern of *C. colebrookianum* Walp.

Sl No.	Place	Relative frequency	Relative Density	Relative Dominance	IVI	A/F ratio	Distribution pattern
1.	Durtlang	11.66	12.08	15.75	39.49	0.14	Contagious
2.	Reiek	10.00	8.57	8.40	26.97	0.13	Contagious
3.	Luangmual	13.33	12.44	20.44	46.21	0.11	Contagious
4.	Lungdai	10.00	7.88	9.15	27.03	0.12	Contagious
5.	Serkhan	8.33	6.65	6.33	21.31	0.12	Contagious
6.	Zonuam	10.00	8.92	4.88	23.83	0.14	Contagious
7.	Chawlhhmun	11.66	12.95	12.71	37.32	0.15	Contagious
8.	Tanhril	10.00	8.23	5.78	24.01	0.13	Contagious
9.	Sakawrtuichhun	8.33	13.48	10.87	32.68	0.30	Contagious
10.	Lengpui	6.66	8.75	5.65	21.06	0.31	Contagious

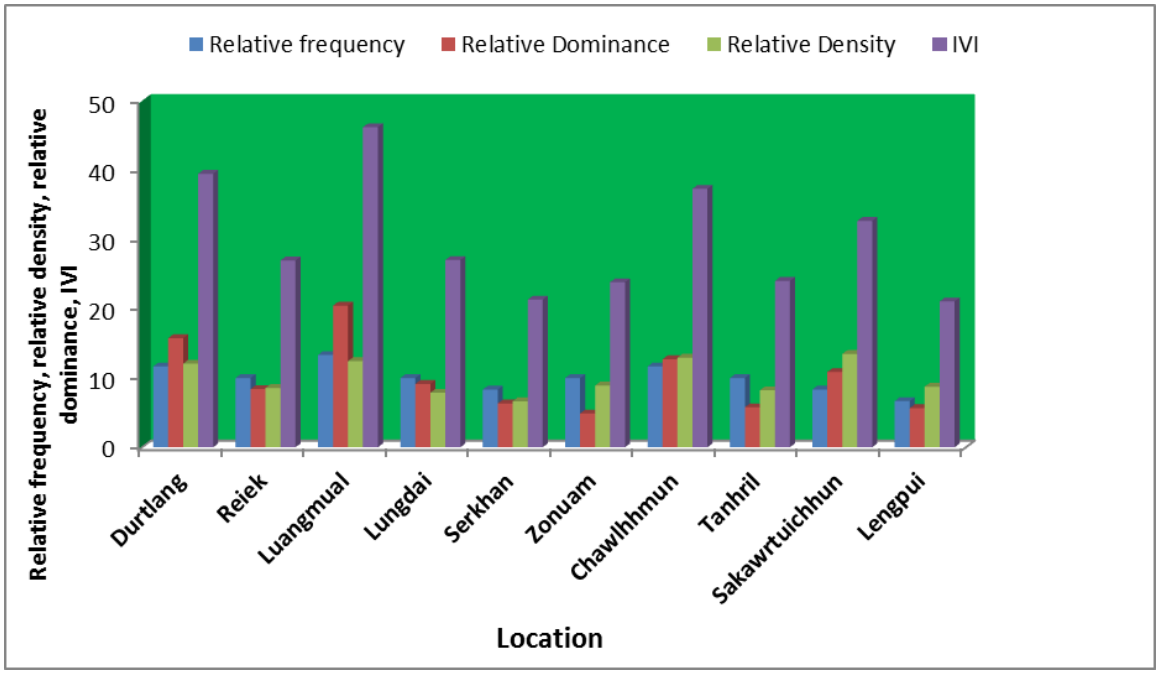


Fig. 4.1: Relative frequency, density, dominance and IVI over selected locations

4.1.8 Relative Density

Table 4.2 and Fig. 4.1 displays the data on relative density of *Clerodendrum colebrookianum* Walp.. From the data it is reveals that the relative frequency of the germplasms ranged between 6.65-13.48 per cent. Among the studied locations, the maximum relative density was found in Sakawrtuichhun (13.48%) followed by Chawlhhmun (12.95%), Luangmual (12.44%) and Durtlang (12.08%). The lowest relative density was observed in in Serkhan (6.65%*)*.

4.1.9. Relative Dominance

Similarly, the relative dominance of *Clerodendrum colebrookianum* Walp. are shown in Table 4.2 and Fig. 4.1. It is evident from the data presented in Table 4.2 that the relative dominance of the particular species varied in the range of 4.88-20.44 per cent. The highest relative dominance was obtained in Luangmual (20.44%*)* followed by Durtlang (15.75%), Chawlhhmun (12.71%) and Sakawrtuichhun (10.87%), whereas the lowest relative dominance among all the locations was observed in Zonuam (4.88%).

4.1.10. Importance Value Index (IVI)

The Importance Value Index (IVI) was calculated in order to record the dominance and ecological success of selected species. The data presented in Table 4.2 and Fig. 4.1 revealed that the IVI among the different geographical locations varied from 21.06 - 46.21. Among the studied locations, the highest IVI was observed in Luangmual (46.21), It was followed by Durtlang (39.49), Chawlhhmun (37.32) and Sakawrtuichhun (32.68) respectively. The lowest value with respect to IVI was

observed in Lengpui (21.06*)*. Since, the *C.colebrookianum* mainly grown as kitchen garden crop mostly nearby human settlements, jhum field and fallow land it was dominant shrub as only few trees / shrubs in some locations found and mostly associated with herbs was co-dominant species.

4.1.11 Abundance and frequency Ratio (A/F Ratio)

A/F is a relative measure to present the distribution of species in a community. The data presented in Table 4.2 indicated that there was variation among the different locations with respect to abundance of frequency (A/F). In the present investigation, the highest A/F ratio was observed in Lengpui (0.31). It was followed by Sakawrtuichhun with a A/F ratio of 0.30. Among all the studied locations, the lowest A/F ratio was obtained in Luangmual (0.11), followed by Lungdai and Serkhan (0.12).

4.1. 12 Distribution pattern

The Table 4.2 displays the distribution pattern of the species among different locations. The data presented in the table revealed that in the present study, in all the locations, the distribution pattern of the species was found contagious, since the A/F ratio was found > 0.05 in all the locations.

4.2 Threatened categories

4.2.1 Threat identified

The data displayed in Table 4.3. revealed the major threat identified with respect to *Clerodendrum colebrookianum* Walp. The following threats have been identified with respect to this highly medicinal species:

- **No wild, few plants cultivated**: From the present study, it have been found that there was no plant found in the wild state in the forests of Mizoram. Mostly the plant was found in the semi wild state as well as cultivated state.
- **Kitchen garden crop**: The plant is commonly grown as kitchen garden crop in the homested gardens at the backyard of most of the farmers as well as cultivated in the jhum lands.
- **Habitat destruction:** From the present study, it has noticed that since last few years there was destruction of the habit of *Clerodendrum colebrookianum* Walp. in the natural populations. That is the reason of non-availability of the species in the wild forests.
- **Low seed viability and germination**: From the present study, it has observed that, the seeds of *Clerodendrum colebrookianum* Walp. is recalcitrant seed. The seeds lost the viability very soon. If they are not sown within a specified time, they fail to germinate. The percentage of germination is also very poor. That is also another reason for decreasing the population of *Clerodendrum colebrookianum* Walp.
- **Slow growth rate**: The initial growth rate of *Clerodendrum colebrookianum* Walp. is very slow.
- **Over exploitation:** The rural people are aware about the medicinal values of this species. They harvest the leaves, seeds as well as stems to sell in the market is also another threat identified for this species.

Table 4. 3: Major threats identified and Threatened Status as per IUCN Red List Categories, 2012

Threat identified	No of Mature individuals	Area of Occurrence / endemism/ No of locations	Threat status
• No wild, few plants cultivated. • Grown as kitchen garden crop • Habitat destruction. • Low seed viability and germination, slow growth rate. • Over exploitation	152-308 in different populations	- • Endemic to the region • Severely Fragmented • No of locations ≤ 10 • Area of Occurrence <5000km^2 • Area of occupancy <500km^2	Critically endangered to endangered * Vulnerable** Endangered** Endangered**

*on the basis of no of mature individuals; ** on the basis of occurrence

4.2.2 No. of matured individuals

Table 4.3 displays the number of matured individuals of *Clerodendrum colebrookianum* Walp. in different locations. From the data presented in Table it has been observed that the number of matured individual in different locations ranged between 152-308.

4.2.3 Area of Occurrence / endemism/ number of locations

The present investigation also studied the area of occurrence, endemism and number of locations of *Clerodendrum colebrookianum* Walp. The results of these revealed that the species is endemic to the region. The population is severely fragmented in different parts of the state. The number of locations found was ≤ 10. Similarly, the area of Occurrence was $<5000km^2$ and area of occupancy was $<500km^2$.

4.2.4 Threat status

Table 4.3 displays the threat status of *Clerodendrum colebrookianum* Walp. in the state of Mizoram. The data presented in the Table indicates it as critically endangered to endangered species on the basis of no of mature individuals. Since in the present study the number of matured individual was observed between 152-308, hence, according to IUCN, red list categories, this species falls in critically endangered to endangered species In addition, on the basis of area of occurrence and occupancy the species have been identified as endangered to vulnerable.

4.3 Germplasm Variability

4.3.1 Morphological features

Data furnished in Table 4.4 indicated that there was significant variation in morphological characters of *Clerodendrum colebrookianum* Walp. among the different locations.

4.3.1.1 Plant height (cm)

There was significant variation among the locations with respect to plant height. The data presented in Table 4.4 reveals that the plant height of the germplasm ranged between 189.80±37.69-303.53±41.09 cm. Among all the germplasm, the highest plant height was obtained in Sakawrtuichhun (303.53±41.09 cm), which was significantly higher than other germplasm except in Reiek (300.49±43.93 cm) and Serkhan (276.34±16.79 cm), with which it was found statistically *at par*. The significantly lowest plant height were recorded in Lengpui(189.80±37.69 cm) which was significantly lower than most of the germplasms.

4.3.1.2 Collar diameter (mm)

Table 4.4 displays the data regarding the collar diameter. Among all the location, the significantly highest collar diameter was obtained in Sakawrtuichhun (45.67±3.92 mm) which was significantly higher than all other locations except Reiek (42.73±9.45 mm), Serkhan (42.58±1.66 mm) and Chawlhhmun (35.49±4.26 mm) respectively. The significantly lowest collar diameter among the different locations were obtained in Luangmual (27.89±10.33 mm) which was significantly lower than

most of the locations. It was followed by Durtlang (28.06±11.15mm), Lengpui (29.30±7.86 mm) and Tanhril (29.90±7.46 mm).

4.3.1.3 Number of leaves

The data pertaining to number of leaves are presented in Table 4.4. Analysis of variance revealed significant difference in number of leaves among the different locations. The maximum number of leaves was recorded in Sakawrtuichhun (211.00±5.29) which was significantly higher than all other locations. However, the minimum number of leaves among all the locations was recorded in Tanhril (33.33±2.52) which was significantly lower than all other locations.

4.3.1.4 Root length (cm)

Data presented in Table 4.4 reveals that there was significant difference in root length among the different locations. The highest root length was also obtained in Sakawrtuichhun (94.11±2.44 cm) which was significantly higher than all other locations. It was followed by Zonuam (79.24±3.55 cm). The lowest root length was found in Luangmual (36.84±6.38 cm) which was significantly lower than most of the locations except Chawlhhmun (38.61±7.67 cm), Lengpui (41.82±3.28 cm) and Reiek (44.30±2.79 cm) which was found statistically *at par*.

Table 4.4 : Morphological features of the plant of *Clerodendrum colebrookianum* among different locations

Location	Plant height (cm)	Collar Diameter (mm)	No. of leaves	Root length (cm)	Root/Shoot length ratio	Root fresh Weight (g)	Shoot fresh weight (g)	Leaf Fresh weight (g)	Root dry wt (g)	Shoot dry wt (g)	Root/Shoot dry weight ratio	Biomass Production (Kg)
Sakawrtuichhun	303.53±41.09	45.67±3.92	211.00±5.29	94.11±2.44	0.31±0.04	1273.33±37.86	2086.67±70.24	840.00±13.23	596.67±20.82	886.67±37.86	0.66±0.08	3.36±0.12
Reiek	300.49±43.93	42.73±9.45	113.67±3.21	44.30±2.79	0.14±0.03	1136.67±32.15	1663.33±72.34	460.00±31.22	276.67±32.15	690.00±30.00	0.86±0.07	2.74±0.13
Chawlhhmun	210.01±21.79	35.49±4.26	80.67±4.04	38.61±7.67	0.18±0.02	413.33±32.15	1226.67±64.29	430.00±45.83	206.67±20.82	473.33±20.82	0.44±0.05	1.64±0.10
Luangmual	215.37±42.51	27.89±10.33	45.33±3.51	36.84±6.38	0.18±0.07	280.00±40.00	713.33±32.15	323.33±9.61	143.33±5.77	230.00±22.91	0.58±0.15	1.00±0.04
Tanhril	219.46±42.34	29.90±7.46	33.33±2.52	45.34±3.30	0.23±0.17	266.67±35.12	608.33±38.19	170.00±10.00	110.00±20.00	220.00±10.00	0.51±0.05	0.88±0.03
Lungdai	242.82±29.61	32.30±2.65	82.33±3.21	51.90±5.52	0.22±0.10	626.67±68.07	826.67±15.28	246.67±25.66	303.33±15.28	336.67±20.21	0.91±0.13	1.45±0.16
Zonuam	206.24±12.69	30.45±1.13	97.67±1.53	79.24±3.55	0.39±0.05	748.33±35.47	770.00±43.59	220.00±26.46	350.00±10.00	303.33±20.82	1.13±0.43	1.52±0.15
Lengpui	189.80±37.69	29.30±7.86	74.67±5.69	41.82±3.28	0.24±0.16	506.67±62.27	763.33±32.15	370.00±26.46	266.67±20.82	356.67±25.17	0.92±0.28	1.27±0.16
Durtlang	197.09±40.57	28.06±11.15	49.40±1.37	53.39±6.58	0.27±0.06	443.33±55.08	616.67±37.86	173.33±20.82	220.00±20.00	250.00±20.00	0.92±0.13	1.06±0.11
Serkhan	276.34±16.79	42.58±1.66	107.73±2.05	63.50±5.77	0.23±0.03	1160.00±72.11	1203.33±50.08	483.33±40.41	583.33±15.28	550.00±17.32	1.06±0.26	2.37±0.12
S Ed(±)	29.18	5.68	2.53	4.31	-	12.04	40.68	23.64	16.24	17.96	0.17	0.10
$CD_{0.05}$	61.31	11.93	5.31	9.05	NS	88.33	85.48	49.67	34.12	37.73	0.35	0.21

Fig: 4.2: Root fresh weight, shoot fresh weight, leaf fresh weight, root dry weight and shoot dry weight along the selected locations

4.3.1.5 Root shoot length ratio

There was no significant difference among the locations with respect to root/shoot length ratio. However, Zonuam and Reiek recorded the highest (0.39±0.05) and lowest (0.14±0.03) root/shoot length ratio.

4.3.1.6 Root fresh weight (g)

It is evident from the data presented in Table 4.4 and Fig. 4.2 that the root fresh weight was significantly influenced by different locations. Among the different locations, the highest root fresh weight was observed in Sakawrtuichhun (1273.33±37.86 g) which was significantly higher than all other germplasm. The lowest root fresh weight was recorded in Tanhril (266.67±35.12 g) which was found significantly lower than other locations except Luangmual (280.00±40.00 g) with which was found statistically *at par*.

4.3.1.7 Shoot fresh weight (g)

A highly significant difference was observed in shoot fresh weight of the plant among the different locations (Table 4.4 and Fig. 4.2). The highest shoot fresh weight was observed in the germplasm from Sakawrtuichhun (2086.67±70.24 g) which was significantly higher than all other locations. The lowest shoot fresh weight was obtained in Tanhril (608.33±38.19 g) which was found significantly lower than other locations except in Durtlang (616.67±37.86g) which was found statistically *at par*.

4.3.1.8 Leaf fresh weight (g)

The effect of different locations with respect to leaf fresh weight was found significant (Table 4.4 and Fig. 4.2). The highest leaf fresh weight was obtained in Sakawrtuichhun (840.00±13.23 g) which was significantly higher over than rest of the locations, while the lowest was obtained in Tanhril (170.00±10.00 g) which it was found statistically *at par* with Durtlang (173.33±20.82 g).

4.3.1.9 Root dry weight (g)

The data recorded on root dry weight was observed to have significantly varied among the locations (Table 4.4 and Fig. 4.2). Among the locations, the maximum root dry weight was observed in Sakawrtuichhun (596.67±20.82 g) which was significantly higher than rest of the germplasm except Serkhan (583.33±15.28 g). The lowest root dry weight was observed in Tanhril (110.00±20.00 g) which was significantly lower than the rest of the germplam.

4.3.1.10 Shoot dry weight (g)

The effect of different locations on shoot dry weight is depicted in Table 4.4 and Fig. 4.2 and the results were found to be varied significantly. The highest shoot dry weight was obtained in Sakawrtuichhun (886.67±37.86 g) which was significantly higher than all other locations. It was followed by Reiek (690.00±30.00 g) and Serkhan (550.00±17.32 g) respectively. The lowest shoot dry weight was recorded in Tanhril (220.00±10.00 g) which was significantly lower than all other locations except

Luangmual (230.00±22.91 g) and Durtlang (250.00±20.00 g) with which it was found statistically *at par*.

4.3.1.11 Root / shoot dry weight ratio

Observations pertaining to root shoot dry weight ratio were found to be varied significantly among the different locations as shown in Table 4.4. Among all the locations, the germplasm from Zonuam (1.13±0.43) showed the maximum root shoot dry weight ratio which was significantly higher than most of the locations. It was followed by Serkhan (1.06±0.26). The lowest root shoot dry weight ratio was obtained in Chawlhhmun (0.44±0.05) which was significantly lower than most of the locations.

4.3.1.12 Biomass Production (kg)

The data on biomass production of the plants are furnished in Table 4.4. Comparison of data on this parameter revealed that the biomass production varied significantly among the different locations. The highest biomass production was recorded in Sakawrtuichhun (3.36±0.12 kg) which was significantly higher among the rest of the locations. The lowest biomass production was recorded in Tanhril (0.88±0.03 kg) which was significantly lower than all other germplasm except Luangmual (1.00±0.04 kg) and Durtlang (1.06±0.01 kg).

Table 4.5: **Flowering and fruiting characteristics of *Clerodendrum colebrookianum* Walp. among different locations**

Location	Days required from initiation of bud to bud differentiation	Days required from initiation of bud to flowering	No of flowers/inflorescence	Length of inflorescence (cm)	Fruit setting %	No.s of fruits/inflorescence	Days required from setting of fruits to maturation	Fruit retention percentage
Sakawrtuichhun	11.33±0.58	40.33±0.58	109.33±5.86	21.90±1.85	98.90±3.10	38.00±3.00	47.00±1.00	44.33±3.79
Reiek	9.67±0.58	39.33±0.58	112.67±8.08	24.10±1.82	93.50±4.33	42.67±4.73	45.67±1.53	45.67±1.53
Chawlhhmun	11.00±0.00	41.50±0.50	104.33±8.33	19.90±0.85	87.03±3.23	26.33±11.02	48.00±1.00	37.33±2.08
Luangmual	11.33±0.58	42.33±0.58	107.67±6.03	18.30±1.47	93.90±6.68	28.67±10.02	48.67±0.58	29.33±1.53
Tanhril	10.67±0.58	42.33±1.15	96.33±7.57	23.20±0.75	96.67±1.15	18.33±6.11	48.67±1.15	28.67±3.51
Lungdai	11.00±0.00	42.67±0.58	98.67±3.06	15.03±1.05	92.33±3.51	34.67±16.77	49.00±1.00	41.67±2.08
Zonuam	12.00±1.00	42.00±1.00	113.33±6.66	15.50±0.72	90.50±1.80	29.67±14.36	49.33±0.58	31.33±6.81
Lengpui	11.67±1.15	42.30±0.61	116.67±5.51	19.20±1.31	87.20±2.99	31.00±6.08	48.33±2.31	35.67±4.16
Durtlang	11.33±0.58	43.00±1.00	105.00±8.89	16.70±0.92	90.50±2.36	31.33±7.23	49.33±1.15	39.33±9.02
Serkhan	10.67±0.58	40.67±0.58	99.67±9.71	17.20±0.44	90.60±2.20	28.00±4.58	49.67±0.58	30.33±1.53
SEd±	0.54	0.59	5.37	0.99	2.94	5.67	0.81	3.60
$CD_{0.05}$	1.13	1.24	11.29	2.08	6.17	11.91	1.71	7.56

4.3.2 Flowering and fruiting characteristics

4.3.2.1 Days required from bud initiation to bud differentiation

A significant influence of locations on days required from bud initiation to bud differentiation was observed (Table 4.5). Among the different locations, the maximum days required from bud initiation to bud differentiation was recorded in Zonuam (12.00±1.00*)* which was significantly higher than most of the locations except Lengpui (11.67±1.15) and Durtlang, Luangmual and Sakawrtuichhun (11.33±0.58), Lungdai and Chawlhhmun (11.00±0.00) The minimum days required from initiation of bud to bud differentiation was obtained in Reiek (9.67±0.58) which was significantly lower than rest of the locations.

4.3.2.2 Days required from initiation of bud to flowering

Data presented in Table 4.5 indicated highly significant variation with respect to days required from initiation of bud to flowering of *Clerodendrum colebrookianum* Walp. The germplasm in Durtlang required maximum days (43.00±1.00) from initiation of bud to flowering, which was significantly higher than most of the locations, whereas, the germplasm in Reiek required the lowest days (39.33±0.58) for flowering. It was followed bv Sakawrtuichhun (40.33±0.58) and Serkhan (40.67±0.58) respectively.

4.3.2.3 Number of flowers/inflorescence

The data pertaining to number of flowers/inflorescence are presented in Table 4.5 Analysis of variance revealed significant difference in this parameter due to different

locations. The maximum number of flowers/inflorescence was observed in Lengpui (116.67±5.51) which was significantly higher than most of the locations except Zonuam (113.33±6.66), Reiek (112.67±8.08), Sakawrtuichhun (109.33±5.86), and Luangmual (107.67±6.03) with which it was found statistically *at par*. However, the minimum number of flowers/inflorescence was observed in Tanhril (96.33±7.57).

4.3.2.4 Length of inflorescences (cm)

It is evident from the data presented in Table 4.5 that the length of inflorescences was influenced by different locations. Among all the locations, the maximum length of inflorescence was observed in Reiek (24.10±1.82 cm) which was significantly higher than all other locations except Tanhril (23.20±0.75 cm). The lowest was observed in Lungdai (15.03±1.05 cm) which was significantly lower than most of the locations .

4.3.2.5 Fruit setting percentage (%)

A highly significant difference was observed among the locations with respect to fruit setting percentage (Table 4.5). Among the locations, the highest fruit setting percentage was found in Sakawrtuichhun (98.90±3.10 %), which was found statistically *at par* with Tanhril (96.67±1.15%), Luangmual (93.90±6.68%), and Reiek (93.50±4.33%). The lowest fruit setting percentage was obtained in Chawlhhmun (87.03±3.23%) which was also significantly lower than most of the locations.

4.3.2.6 Number of fruits/inflorescence

Data furnished in Table 4.5 showed a highly significant difference in number of fruit/inflorescence among different locations. The highest number of fruits/inflorescence

was found in Reiek (42.67±4.73) which was followed by Sakawrtuichhun (38.00±3.00) Lungdai (34.67±16.77), Durtlang (31.33±7.23), and Lengpui (31.00±6.08), while, the lowest was obtained in Tanhril (18.33±6.11) which was significantly lower than most of the locations.

4.3.2.7 Days required from setting of fruits to maturation

It reveals from the Table 4.5 that days required from setting of fruits to maturation was significant among the different locations. Among all the locations, Serkhan (49.67±0.58) required the maximum days for fruit maturation, which was significantly higher than all other locations except and Durtlang (49.33±1.15), Zonuam (49.33±0.58), Lungdai (49.00±1.00), Tanhril (48.67±1.15), Luangmual (48.67±0.58), Lengpui (48.33±2.31), and Chawlhhmun (48.00±1.00), with which it was found statistically *at par* . The minimum days required from setting of fruits to maturation was found in Reiek (45.67±1.53) which was also significantly lower than rest of the locations.

4.3.2.8 Fruit retention percentage (%)

Significant differences were observed with respect to fruit retention percentage among the different locations (Table 4.5). Among the different locations, the highest fruit retention percentage was observed in Reiek (45.67±1.53) which was significantly higher than rest of the locations. It was followed by Sakawrtuichhun (44.33±3.79) and Lungdai (41.67±2.08). However, the significantly lowest fruit retention percentage was obtained in Tanhril (28.67±3.51).

Table 4.6:.Estimates of phenotypic and genotypic coefficient of variation, heritability, expected genetic advance and genetic Advance as percent of mean for different traits in *Clerodendrum colebrookianum* Walp.

Sl. No	Characters	Range	Mean	Variance		Coefficient of variation		Broad sense of heritability	Genetic Advance	Genetic gain
				Genotypic	Phenotypic	Genotypic	Phenotypic			
1.	Plant Height (cm)	189.80-303.53	236.12	1385.56	2662.86	15.76	21.85	52.03	55.31	23.43
2.	No of Leaves	33.33-211.00	89.57	1674.50	4254.70	45.69	72.83	39.35	52.88	59.04
3.	Fresh Weight of Leaves (gm)	170.00-840.00	372.00	24840.37	71898.52	42.37	72.08	34.55	190.84	51.30
4.	Collar Diameter (mm)	27.89-45.67	34.44	29.78	78.12	15.85	25.66	38.12	6.94	20.16
5.	Root Length (cm)	36.84-94.11	54.99	258.37	538.43	29.23	42.19	47.99	22.94	41.71
6.	Fresh Weight of Root (gm)	266.67-1273.33	685.67	104354.07	218395.92	47.11	68.16	47.78	460.00	67.09
7.	Dry Weight of Root (gm)	110.00-596.67	305.67	20102.22	42425.55	46.38	67.39	47.38	201.05	65.77
8.	Shoot Fresh Weight (gm)	608.33-2086.67	1044.83	156884.27	411584.16	37.91	61.40	38.12	503.75	48.21
9.	Shoot Dry Weight (gm)	220.00-886.67	429.67	37655.55	71040.37	45.16	62.03	53.00	291.03	67.73
10.	Root: Shoot Length Ratio	0.14-0.39	0.24	0.0039	0.065	26.06	33.88	59.18	0.10	41.30
11.	Root: Shoot Dry Weight Ratio	0.44-1.13	0.80	0.04	0.08	25.44	36.26	50.00	0.29	36.77
12.	Biomass Production (kg/Plant)	0.88-3.36	1.73	0.46	1.12	39.39	61.21	41.07	0.90	52.23

4.3.3 Variability parameters

The estimates of variability *viz.* Genotypic, and phenotypic variances, coefficients of variation (genotypic, and phenotypic), broad sense of heritability, genetic advance and genetic gain as per cent of mean were worked out for selection of various characters (Table 4.6).

4.3.3. 1. Variance

4.3.3.1.1 Genotypic variance

Genotypic variance ranged from 0.0039-156884.27. The maximum genotypic variance was obtained in shoot fresh weight (156884.27) followed by fresh weight of root (104354.07), shoot dry weight (37655.55), fresh weight of leaves (24840.37), dry weight of root (20102.22), number of leaves (1674.50), plant height (1385.56), root length (258.37), collar diameter (29.78), biomass production (0.46), root/shoot dry weight ratio (0.04) and the minimum was obtained in root /shoot length ratio (0.0039).

4.3.3.1.2 Phenotypic variance

The highest phenotypic variance was obtained in shoot fresh weight (411584.16). It was followed by fresh weight of root (218395.92), fresh weight of leaves (71898.52), shoot dry weight (71040.37), dry weight of root (42425.55), number of leaves (4254.70), plant height (2662.86), root length (538.43), collar diameter (78.12), biomass production (1.12), root shoot dry weight ratio (0.08). The lowest phenotypic variance was observed in root shoot length ratio (0.065).

4.3.3.2 Coefficients of variation

The variations observed in the characters among all the genotypes are due to effect of genotype and environment. Environmental variations are not fixable. For determining the magnitude of genotypic and phenotypic variability, the genotypic and phenotypic coefficients of variations were calculated (Table 4.6).

For all the characters studied, phenotypic coefficients of variability were higher in magnitude than genotypic coefficients of variability, though difference was very less in majority of cases. Thus implying that the influence of environment on the expression of these traits were negligible hence selection based on phenotypic values is feasible. Coefficients of variability varied in magnitude from character to character, either low or moderate or high. Therefore, it indicated that there is a great diversity present among the genotypes.

4.3.3.2.1 Genotypic coefficient variation

The genotypic coefficients of variability (GCV) were high for fresh weight of root (47.11), dry weight of root (46.38), no. of leaves (45.69), shoot dry weight (45.16), fresh weight of leaves (42.37), biomass production (39.39), shoot fresh weight (37.91), root length (29.23), root/shoot length ratio (26.06), root/shoot dry weight ratio (25.44). A moderate genotypic coefficient of variability was recorded for collar diameter (15.85) and plant height (15.76).

4.3.3.2.2 Phenotypic coefficient variation

The phenotypic coefficients of variability (PCV) were high for all the characters viz. number of leaves (72.83), fresh weight of leaves (72.08), fresh weight of root (68.16), dry weight of root (67.39), shoot dry weight (62.03), shoot fresh weight (61.40), biomass production (61.21), root length (42.19), root/shoot dry weight ratio (36.26), root shoot length ratio (33.88), collar diameter (25.66) and plant height (21.85).

4.3.3.3 Heritability (h^2)

The estimates of heritability (broad sense) varied from 34.55 –59.18 per cent for different characters under study (Table 4.6). It was found moderate for all the characters viz., root/shoot length ratio (59.18%), shoot dry weight (53.00%), plant height cm (52.03 %), root/shoot dry weight ratio (50.00%), root length (47.99%), fresh weight of root (47.78), dry weight of root (47.38%), biomass production (41.07%), no. of leaves (39.35%), collar diameter (38.12%), shoot fresh weight (38.12%) and fresh weight of leaves (34.55 %).

4.3.3.4 Genetic advance (GA)

Table 4.6 showed expected genetic advance was high in nature and ranged from 0.10 - 503.75 per cent for different characters under study. It was found high for shoot fresh weight (503.75%), fresh weight of root (460.00 %), shoot dry weight (291.03 %), dry weight of root (201.05 %), fresh weight of leaves (190.84), plant height (55.31 %), number of leaves (52.88 %), root length (22.94 %). It was low for collar diameter (6.94 %), biomass production (0.90 %), root/shoot dry weight ratio (0.29 %) and root shoot length ratio (0.10 %).

4.3.3.5 Genetic gain

The genetic gain as percent of mean was also varied from 20.16-67.73 per cent for different characters under study (Table 4.6). It was observed high in all the parameters viz., shoot dry weight (67.73 %), fresh weight of root (67.09 %), dry weight of root (65.77 %), number of leaves (59.04 %), biomass production (52.23 %), fresh weight of leaves (51.30 %), shoot fresh weight (48.21 %), root length (41.71 %), root shoot length ratio (41.30 %), root shoot dry weight ratio (36.77 %) and plant height (23.43 %), collar diameter (20.16%).

4.3.4 Correlation studies

Correlation studies provide information regarding any two parameters, which are under consideration whether the increase in one variable causes either increase or decrease in another variable. Thereby, this definite relationship ascertains their inter-dependence. Correlation coefficients were worked out at phenotypic, genotypic and environmental levels for all possible combinations of yield and its attributing characters.

The results of phenotypic correlation coefficient have been discussed only as the genotypic and environmental correlation were mostly influenced by the environmental conditions, hence phenotypic correlation will give the correct idea about the association between two variables. The phenotypic and genotypic correlation between morphological and yield parameters are presented in Table 4.7 and 4.8.

Table 4.7 :**Phenotypic Correlation coefficients among different traits in *Clerodendrum colebrookianum* Walp.**

Characters	**Plant Height (cm)**	**No of Leaves**	**Fresh Weight of Leaves (gm)**	**Collar Diameter (mm)**	**Root Length (cm)**	**Fresh Weight of Root (gm)**	**Dry Weight of Root (gm)**	**Shoot Fresh Weight (gm)**	**Shoot Dry Weight (gm)**	**Root: Shoot Length Ratio**	**Root: Shoot Dry Weight Ratio**	**Biomass Production (kg/Plant)**
Plant Height (cm)	1.0000											
No of Leaves	0.7666***	1.0000										
Fresh Weight of Leaves (gm)	0.7577***	0.8388***	1.0000									
Collar Diameter (mm)	0.8654***	0.6883***	0.7898***	1.0000								
Root Length (cm)	0.3332	0.5972***	0.4102*	0.2863	1.0000							
Fresh Weight of Root (gm)	0.8416***	0.8139***	0.7385***	0.7778***	0.6133***	1.0000						
Dry Weight of Root (gm)	0.4419*	0.4328*	0.3996*	0.6121***	0.3856*	0.5550**	1.0000					
Shoot Fresh Weight (gm)	0.8618***	0.8644***	0.9084***	0.8554***	0.4172*	0.8194***	0.3517	1.0000				
Shoot Dry Weight (gm)	0.7858***	0.7056***	0.8410***	0.8989***	0.3768*	0.7634***	0.5721***	0.8853***	1.0000			
Root: Shoot Length Ratio	-0.2182	0.1799	-0.0418	-0.1796	0.8169***	0.1761	0.1800	-0.0777	-0.0804	1.0000		
Root: Shoot Dry Weight Ratio	-0.0479	0.0294	-0.1653	-0.0073	0.3173	0.3335	0.3706*	-0.1571	-0.0888	0.4521	1.0000	
Biomass Production (kg/Plant)	0.8921***	0.8830	0.8786***	0.8603***	0.5273	0.9378***	0.4575*	0.9673***	0.8731***	0.0359	0.0530	1.0000

Table 4.7 estimated the phenotypic correlation coefficients among the different traits in *Clerodendrum colebrookianum*. This study indicated the plant height is significant and positively correlated with number of leaves (0.7666**), fresh weight of leaves (0.7577**), collar diameter (0.8654**), root length (0.3332), fresh weight of root (0.8416**), dry weight of root (0.4419*), shoot fresh weight (0.8618**), shoot dry weight (0.7858**), and biomass production (0.8921**). Association of number of leaves was exhibited a positive and significant correlation with fresh weight of leaves (0.8388**), collar diameter (0.6883**), root length (0.5972), fresh weight of root (0.8139**), dry weight of root (0.4328*), shoot fresh weight (0.8644**), shoot dry weight (0.7056**), root: shoot length ratio (0.1799), root: shoot dry weight ratio (0.0294) and biomass production (0.8830**). The fresh weight of leaves expressed a significant and positive association with collar diameter (0.7898**), root length (0.4102), fresh weight of root (0.7385**), dry weight of root (0.3996*), shoot fresh weight (0.9084**), shoot dry weight (0.8410**) and biomass production (0.8786**). The collar diameter showed a significant and positive correlation with root length (0.2863), fresh weight of root (0.7778**), dry weight of root (0.6121*), shoot fresh weight (0.8554**), shoot dry weight (0.8989**) and biomass production (0.8603**). The root length showed a significant and positive correlation with fresh weight of root (0.6133**), dry weight of root (0.3856*), shoot fresh weight (0.4172**), shoot dry weight (0.3768**), root: shoot length ratio (0.8169), root: shoot dry weight ratio (0.3173) and biomass production (0.5273**). A significant and positive correlation was observed in fresh weight of root with dry weight of root (0.5550), shoot fresh weight (0.8194**), shoot dry weight

(0.7634**), root: shoot length ratio (0.1761), root: shoot dry weight ratio (0.3335) and biomass production (0.9378**). Dry weight of root showed a significant and positive correlation with shoot fresh weight (0.3517**), shoot dry weight (0.5721**), root: shoot length ratio (0.1800), root: shoot dry weight ratio (0.3706) and biomass production (0.4575**). Shoot fresh weight was significant and positive correlated with shoot dry weight (0.8853**), and biomass production (0.9673**). Shoot dry weight was positively and significantly correlated with biomass production(0.8731). root: shoot length ratio was positively and significantly correlated with root: shoot dry weight ratio (0.4521) and biomass production(0.0359). Root: shoot dry weight ratio was positive and significantly correlated with biomass production(0.0530).

Plant height is negatively correlated with root: shoot length ratio (-0.2182) and root: shoot dry weight ratio (-0.0479). Negative association of fresh weight of leaves with root: shoot length ratio (-0.0418) and root: shoot dry weight ratio (-0.1653). The collar diameter showed negative correlation with root: shoot length ratio (-0.1796) and root: shoot dry weight ratio (-0.0073). Shoot fresh weight was negatively correlated with root: shoot length ratio (-0.0777) and root: shoot dry weight ratio (-0.1571). Shoot dry weight was negatively correlated with root: shoot length ratio (-0.0804) and root: shoot dry weight ratio (-0.0888).

Table 4.8 : Genotypic Correlation coefficients among different traits in *Clerodendrum colebrookianum* Walp.

Characters	Plant Height (cm)	No of Leaves	Fresh Weight of Leaves (gm)	Collar Diameter (mm)	Root Length (cm)	Fresh Weight of Root (gm)	Dry Weight of Root (gm)	Shoot Fresh Weight (gm)	Shoot Dry Weight (gm)	Root: Shoot Length Ratio	Root: Shoot Dry Weight Ratio	Biomass Production (kg/Plant)
Plant Height (cm)	1.0000											
No of Leaves	0.7420**	1.0000										
Fresh Weight of Leaves (gm)	0.7040**	0.9744**	1.0000									
Collar Diameter (mm)	0.9922**	1.0236**	0.9202**	1.0000								
Root Length (cm)	0.5493	0.9771**	0.6207	0.7081	1.0000							
Fresh Weight of Root (gm)	0.8979**	0.9247**	0.7410	1.0537**	0.7065	1.0000						
Dry Weight of Root (gm)	0.8795*	1.3125**	1.1307*	0.9402*	1.2100**	1.2146**	1.0000					
Shoot Fresh Weight (gm)	0.8456**	0.9606**	0.9567**	1.0327**	0.5876	0.8236**	1.0325**	1.0000				
Shoot Dry Weight (gm)	0.9235**	1.1617**	1.0440**	1.0140**	0.6597	0.9792**	0.8880**	1.1111**	1.0000			
Root: Shoot Length Ratio	-0.1464	0.4316	0.0138	-0.0402	0.7612	0.1441	0.5974	-0.0729	-0.0092	1.0000		
Root: Shoot Dry Weight Ratio	0.1580	0.2560	-0.1434	0.1016	0.4147	0.5292	0.5403	-0.0791	0.0604	0.4625	1.0000	
Biomass Production (kg/Plant)	0.9082**	0.9950**	0.9022**	1.0930**	0.6799	0.9459**	1.1814**	0.9632**	1.1024**	0.0337	0.2041	1.0000

The estimates of genotypic correlation coefficients among the different traits in *Clerodendrum colebrookianum* are presented in Table 4.8. This study indicated the plant height is significant and positively correlated with number of leaves (0.7420**), fresh weight of leaves (0.7040**), collar diameter (0.9922**), root length (0.5493), fresh weight of root (0.8979**), dry weight of root (0.8795*), shoot fresh weight (0.8456**), shoot dry weight (0.9235**), root: shoot dry weight ratio (0.1580), and biomass production (0.9082**). Association of number of leaves was exhibited a positive and significant correlation with fresh weight of leaves (0.9744**), collar diameter (1.0236**), root length (0.9771**), fresh weight of root (0.9247**), dry weight of root (1.3125**), shoot fresh weight (0.9606**), shoot dry weight (1.1617**), root: shoot length ratio (0.4316), root: shoot dry weight ratio (0.2560) and biomass production (0.9950**). The fresh weight of leaves expressed a significant and positive association with collar diameter (0.9202**), root length (0.6207), fresh weight of root (0.7410), dry weight of root (1.1307*), shoot fresh weight (0.9567**), shoot dry weight (1.0440**), root: shoot length ratio (0.0138) and biomass production (0.9022**). The collar diameter showed a significant and positive correlation with root length (0.7081), fresh weight of root (1.0537**), dry weight of root (0.9402*), shoot fresh weight (1.0327**), shoot dry weight (1.0140**), root: shoot dry weight ratio (0.1016) and biomass production (1.0930**). The root length showed a significant and positive correlation with fresh weight of root (0.7065), dry weight of root (1.2100**), shoot fresh weight (0.5876), shoot dry weight (0.6597), root: shoot length ratio (0.7612), root: shoot dry weight ratio (0.4147) and biomass production (0.6799). A significant and positive correlation was

observed in fresh weight of root with dry weight of root (1.2146**), shoot fresh weight (0.8236**), shoot dry weight (0.9792**), root: shoot length ratio (0.1441), root: shoot dry weight ratio (0.5292) and biomass production (0.9457**). Dry weight of root showed a significant and positive correlation with shoot fresh weight (1.0325**), shoot dry weight (0.8880**), root: shoot length ratio (0.5974), root: shoot dry weight ratio (0.5403) and biomass production (1.1814**). Shoot fresh weight was significant and positive correlated with shoot dry weight (1.1111**), and biomass production (0.9632**). Shoot dry weight was positively and significantly correlated with root: shoot dry weight ratio (0.0604) and biomass production(1.1024**). Root: shoot length ratio was positively and significantly correlated with root: shoot dry weight ratio (0.4625) and biomass production (0.0337). Root: shoot dry weight ratio was positive and significantly correlated with biomass production (0.2041).

Plant height is negatively correlated with root: shoot length ratio (-0.1464). Negative association of fresh weight of leaves with root: shoot dry weight ratio (-0.1434). The collar diameter showed negative correlation with root: shoot length ratio (-0.0402). Shoot fresh weight was negatively correlated with root: shoot length ratio (-0.0729) and root: shoot dry weight ratio (-0.0791).

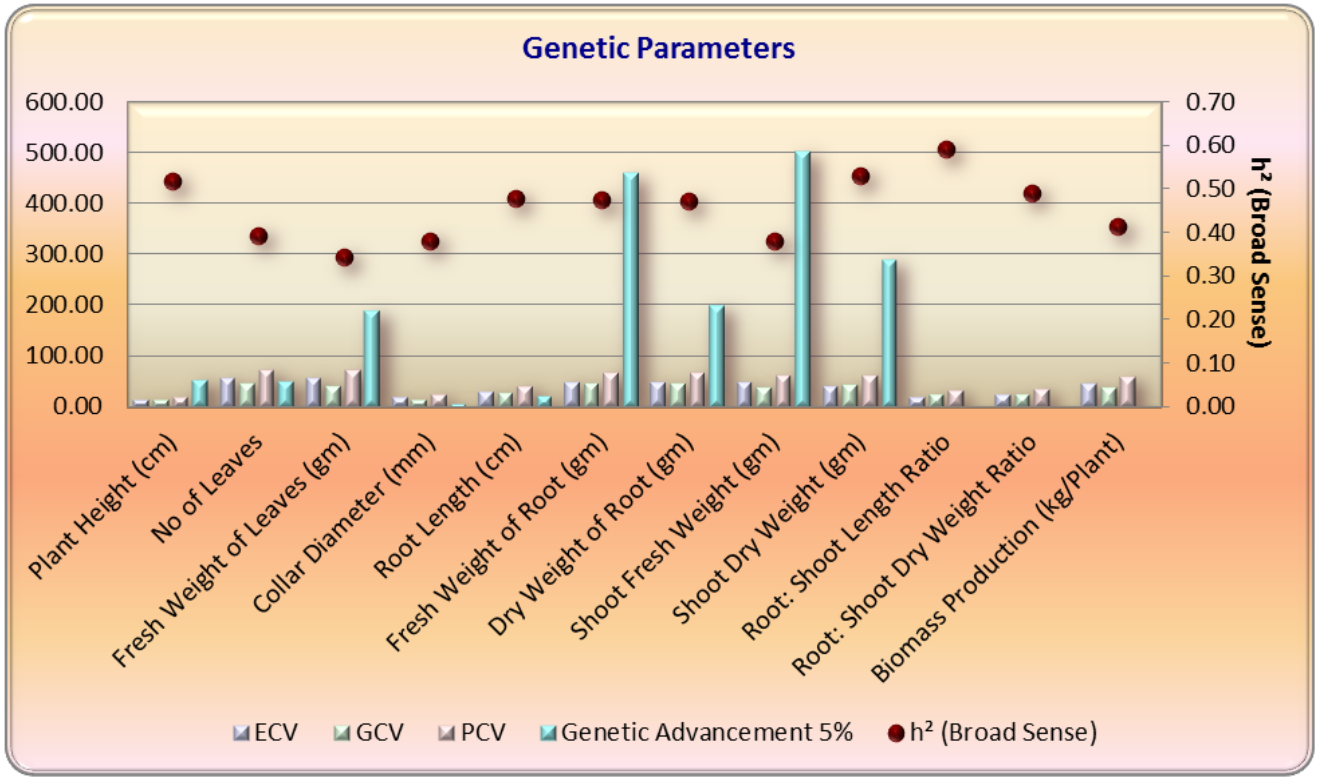


Fig 4.3 : Environmental, phenotypic and genotypic coefficient of variation, heritability, expected genetic advance and genetic advance as per cent of mean for different characters of *Clerodendrum colebrookianum* Walp.

Table 4.9 : Pearson's Correlation coefficients among different traits in *Clerodendrum colebrookianum* Walp.

Characters	Plant Height (cm)	No of Leaves	Fresh Weight of Leaves (gm)	Collar Diameter (mm)	Root Length (cm)	Fresh Weight of Root (gm)	Dry Weight of Root (gm)	Shoot Fresh Weight (gm)	Shoot Dry Weight (gm)	Root: Shoot Length Ratio	Root: Shoot Dry Weight Ratio	Biomass Production (kg/Plant)
Plant Height (cm)	1.0000											
No of Leaves	0.69107***	1.0000										
Fresh Weight of Leaves (gm)	0.75598***	0.78101***	1.0000									
Collar Diameter (mm)	0.86427***	0.58406***	0.78483***	1.0000								
Root Length (cm)	0.31247	0.60069***	0.3893*	0.24559	1.0000							
Fresh Weight of Root (gm)	0.80116***	0.78762***	0.72339***	0.73256***	0.58171***	1.0000						
Dry Weight of Root (gm)	0.43972*	0.40076*	0.40285*	0.60716***	0.36486*	0.55150**	1.0000					
Shoot Fresh Weight (gm)	0.85482***	0.82482***	0.90668***	0.83843***	0.40781*	0.80512***	0.35212*	1.0000				
Shoot Dry Weight (gm)	0.77161***	0.61616***	0.83358***	0.89077***	0.32289*	0.74843***	0.57409***	0.86639***	1.0000			
Root: Shoot Length Ratio	-0.22457	0.19488	-0.04832	-0.19271	0.81608***	0.17202	0.17272	-0.07851	-0.09578	1.0000		
Root: Shoot Dry Weight Ratio	-0.04003	0.00443	-0.15337	0.01276	0.28731	0.33600	0.37709*	-0.15310	-0.05554	0.43603*	1.0000	
Biomass Production (kg/Plant)	0.87233***	0.85103***	0.87287***	0.83192***	0.51028**	0.93443***	0.45912*	0.96335***	0.85767***	0.03510	0.05956	1.0000

The estimates of Pearson's correlation coefficients among the different traits in *Clerodendrum colebrookianum* are presented in Table 4.9. This study indicated the plant height is significant and positively correlated with number of leaves (0.69107), fresh weight of leaves (0.75598), collar diameter (0.86427**), root length (0.31247), fresh weight of root (0.80116**), dry weight of root (0.43972*), shoot fresh weight (0.85482**), shoot dry weight (0.77161**), and biomass production (0.87233**). Association of number of leaves was exhibited a positive and significant correlation with fresh weight of leaves (0.78101**), collar diameter (0.58406), root length (0.60069), fresh weight of root (0.78762), dry weight of root (0.40076), shoot fresh weight (0.82482**), shoot dry weight (0.61616**), root: shoot length ratio (0.19488), root: shoot dry weight ratio (0.00443) and biomass production (0.85103**). The fresh weight of leaves expressed a significant and positive association with collar diameter (0.78483**), root length (0.3893), fresh weight of root (0.72339), dry weight of root (0.40285), shoot fresh weight (0.90668**), shoot dry weight (0.83358**), and biomass production (0.87287**). The collar diameter showed a significant and positive correlation with root length (0.24559), fresh weight of root (0.73256), dry weight of root (0.60716), shoot fresh weight (0.83843**), shoot dry weight (0.89077**), root: shoot dry weight ratio (0.01276) and biomass production (0.83192**). The root length showed a significant and positive correlation with fresh weight of root (0.58171), dry weight of root (0.36486), shoot fresh weight (0.40781), shoot dry weight (0.32289), root: shoot length ratio (0.81608), root: shoot dry weight ratio (0.28731) and biomass production (0.51028). A significant and positive correlation was observed in fresh weight of root with dry weight

of root (0.55150), shoot fresh weight (0.80512**), shoot dry weight (0.74843**), root: shoot length ratio (0.17202), root: shoot dry weight ratio (0.33600) and biomass production (0.93443**). Dry weight of root showed a significant and positive correlation with shoot fresh weight (0.35212), shoot dry weight (0.57409), root: shoot length ratio (0.17272), root: shoot dry weight ratio (0.37709) and biomass production (0.45912). Shoot fresh weight was significant and positive correlated with shoot dry weight (0.86639**), and biomass production (0.96335**). Shoot dry weight was positively and significantly correlated with biomass production(0.85767**). Root: shoot length ratio was positively and significantly correlated with root: shoot dry weight ratio (0.43603) and biomass production (0.03510). Root: shoot dry weight ratio was positive and significantly correlated with biomass production (0.05956).

Plant height is negatively correlated with root: shoot length ratio (-0.22457) and root: shoot dry weight ratio (-0.04003). Negative association of fresh weight of leaves with root: shoot length ratio (-0.04832) and root: shoot dry weight ratio (-0.15337). The collar diameter showed negative correlation with root: shoot length ratio (-0.19271). Shoot fresh weight was negatively correlated with root: shoot length ratio (-0.07851) and root: shoot dry weight ratio (-0.15310). Shoot dry weight was negatively correlated with root: shoot length ratio (-0.09578) and root: shoot dry weight ratio (-0.05554).

Table 4.10*:* Regression equation for growth parameters of *C. cloebrookianum*

Yi Variable = Biomass Production (kg/Plant)

	Intercept a	Bx		t-Value	Probability	
Y1=	-2.484	+0.01784 x	Plant Height (cm)	9.441	0.000	***
Y1=	0.539	+0.01327 x	No of Leaves	8.576	0.000	***
Y1=	0.444	+0.00345 x	Fresh Weight of Leav	9.466	0.000	***
Y1=	-1.645	+0.09796 x	Collar Diameter (mm)	7.933	0.000	***
Y1=	0.461	+0.02304 x	Root Length (cm)	3.140	0.004	**
Y1=	0.301	+0.00208 x	Fresh Weight of Root	13.884	0.000	***
Y1=	1.006	+0.00236 x	Dry Weight of Root (	2.735	0.011	*
Y1=	0.060	+0.00160 x	Shoot Fresh Weight (	19.002	0.000	***
Y1=	0.296	+0.00333 x	Shoot Dry Weight (gm	8.826	0.000	***
Y1=	1.618	+0.45952 x	Root: Shoot Length R	0.186	0.854	
Y1=	1.555	+0.21684 x	Root: Shoot Dry Weig	0.316	0.755	

Table 4.11*:* Descriptive Statistics showing variability among growth parameters of *C. cloebrookianum*

Pooled n = 30		Lowest	Highest	Kurtosis	Skewness	Mean	Std. Dev.	Std. Error	C. V.	JarqueBera	□ Prob	
Plant Height (cm)	X1	146.304	350.520	0.003	0.401	236.116	50.194	9.164	21.258	0.804	0.669	
No of Leaves	X2	8.000	353.000	6.631	2.176	89.567	65.818	12.017	73.485	78.638	0.000	***
Fresh Weight of Leaves (gm)	X3	40.000	1070.000	0.340	0.983	372.000	259.527	47.383	69.765	4.980	0.083	
Collar Diameter (mm)	X4	17.740	52.380	-0.484	-0.056	34.437	8.717	1.592	25.313	0.309	0.857	
Root Length (cm)	X5	30.480	124.030	0.756	0.872	54.994	22.731	4.150	41.334	4.516	0.105	
Fresh Weight of Root (gm)	X6	110.000	1990.000	1.341	1.248	685.667	460.754	84.122	67.198	10.036	0.007	**
Dry Weight of Root (gm)	X7	40.000	700.000	-0.616	0.592	305.667	199.459	36.416	65.254	2.225	0.329	
Shoot Fresh Weight (gm)	X8	180.000	2790.000	1.657	1.245	1044.833	619.171	113.045	59.260	11.188	0.004	**
Shoot Dry Weight (gm)	X9	60.000	1110.000	0.380	0.919	429.667	264.086	48.215	61.463	4.402	0.111	
Root: Shoot Length Ratio	X10	0.123	0.429	-0.318	0.684	0.239	0.078	0.014	32.816	2.466	0.291	
Root: Shoot Dry Weight Ratio	X11	0.400	1.620	0.697	0.790	0.799	0.282	0.052	35.299	3.724	0.155	
Biomass Production (kg/Plant)	Y1	0.290	4.780	2.376	1.403	1.728	1.027	0.187	59.398	16.897	0.000	***

Table 4.12*:* Multiple Regression on Biomass Production (kg/Plant)

	Beta Wt.	Simple R^2	Reg. Coeff.	Std. Err.	t-Value	t-Prob.		Partial R^2
		0.000	-0.010	0.050	0.193	0.849		0.002
Plant Height (cm)	0.002	0.002	0.000	0.000	0.189	0.853		0.002
No of Leaves	-0.004	-0.003	0.000	0.000	0.423	0.678		0.010
Fresh Weight of Leaves (gm)	0.015	0.013	0.000	0.000	1.772	0.093		0.149
Collar Diameter (mm)	-0.005	-0.004	-0.001	0.001	0.443	0.663		0.011
Root Length (cm)	-0.009	-0.005	0.000	0.001	0.655	0.521		0.023
Fresh Weight of Root (gm)	0.456	0.426	0.001	0.000	39.881	0.000	***	0.989
Dry Weight of Root (gm)	-0.002	-0.001	0.000	0.000	0.245	0.809		0.003
Shoot Fresh Weight (gm)	0.592	0.571	0.001	0.000	34.338	0.000	***	0.985
Shoot Dry Weight (gm)	0.001	0.001	0.000	0.000	0.120	0.906		0.001
Root: Shoot Length Ratio	0.013	0.000	0.174	0.178	0.976	0.342		0.050
Root: Shoot Dry Weight Ratio	-0.003	0.000	-0.011	0.023	0.465	0.647		0.012
bu = Shoot Dry weight (gm)		**0.014**						
		R^2	0.9998		R^2adj	0.9997		
		F	8667.018	11, 18	Probability	0		
	RMS Error		0.0179		AIC	-7.7566		

4.3.5 Regression Coefficient

4.3.5.1 Regression equation of biomass with other growth parameters

As depicted in Table 4.10 that, the value of regression coefficient of biomass with plant height (0.01784), no of leaves (0.01327), fresh weight of leaves (0.00345), collar diameter (0.09796), fresh weight of root (0.00208), shoot fresh weight (0.00160) and shoot dry weight (0.00333) was highly significant whereas regression coefficient of independent variable biomass production with dependent variables root length (0.02304) and dry weight of root (0.00236) was significant. Regression coefficient of biomass production with shoot length (0.45952) and shoot dry weight (0.21684) was not significant.

4.3.5.2 Descriptive statistics

It is evident from the Table 4.11 that, for variable X2 (no. of leaves) data was normally distributed and therefore show flatness of the curve which is designated as leptokurtic. For all other variables (X1, Y1, X3-X11) data were platykurtic i.e. not bell shaped and departed from normal curve and therefore asymmetrical either positively or negatively. All variables were positively skewed in the side their tail exists except X4. Highest variability among different growth parameters of plant collected from different populations exist in number of leaves (73.4852 %) followed by fresh weight of leaves (69.7654 %), dry weight of root (65.2359), shoot dry weight (61.4629 %) biomass production (59.3984 %), shoot fresh weight (59.2603 %). This may be concluded that in

the above parameters, greater variability is present in all parameters and the plant height is considered as most consistent data among themselves.

4.3.5.3 Multiple regressions

Multiple regression equation models can explain the variation up to 99%. Maximum positive contribution was made by shoot fresh weight (59.25 %) followed by fresh weight of root 45.59 % and highest negative contribution made by dry weight of root (0.16 %). These parameters therefore showed relationship with biomass production.

4.4 Microscopic Evaluation

Fig. 4.4, 4.5 and 4.6 shows the results of the microscopic evaluation i.e. T.S. of stem and leaf petioles. The transverse section of the stem under microscopic study as depicted in the fig. 4.4 reveals that the stem of the plant is tender and soft. There was a fine outgrowths or appendages on plants called as Trichomes or plant hairs. The cell walls of trichomes are commonly of cellulose and are covered with a cuticle. They are lignified. The main function of trichomes is to aid in the protection of plant body from outer injurious agencies different in plants. Just beneath the tracheids, a single layer epidermis, compactly arranged square cells are present and acts as a boundary between the plant and the external environment. The epidermis serves several functions to protect against water loss, regulates gas exchange, secretes metabolic compounds, and absorbs water and mineral nutrients. This is followed by 5-6 layered schlerenchyma cells which are almost circular in shape. Immediately after schlerenchyma cells 3-4 layered parenchymatous cells are present. Below parenchymatous cells, ground tissues are distributed throughout and composed of circular cells. Vascular bundles are present along with the pith. The function of xylem is to collect water and minerals from the roots and transport to the leaves and phloem is to transport food from the leaves to rest of the plant parts. And lastly, pith was present in the middle of the stem and it is composed of soft, spongy parenchyma cells, and its importance function is to store and transport nutrients throughout the plant.

The leaves *C. colebrookianum* are simple, opposite or rarely whorled. Leaves are 10-20 cm in length and 6-12 cm in breadth. Leaf base is wedge- shaped to heart-shaped,

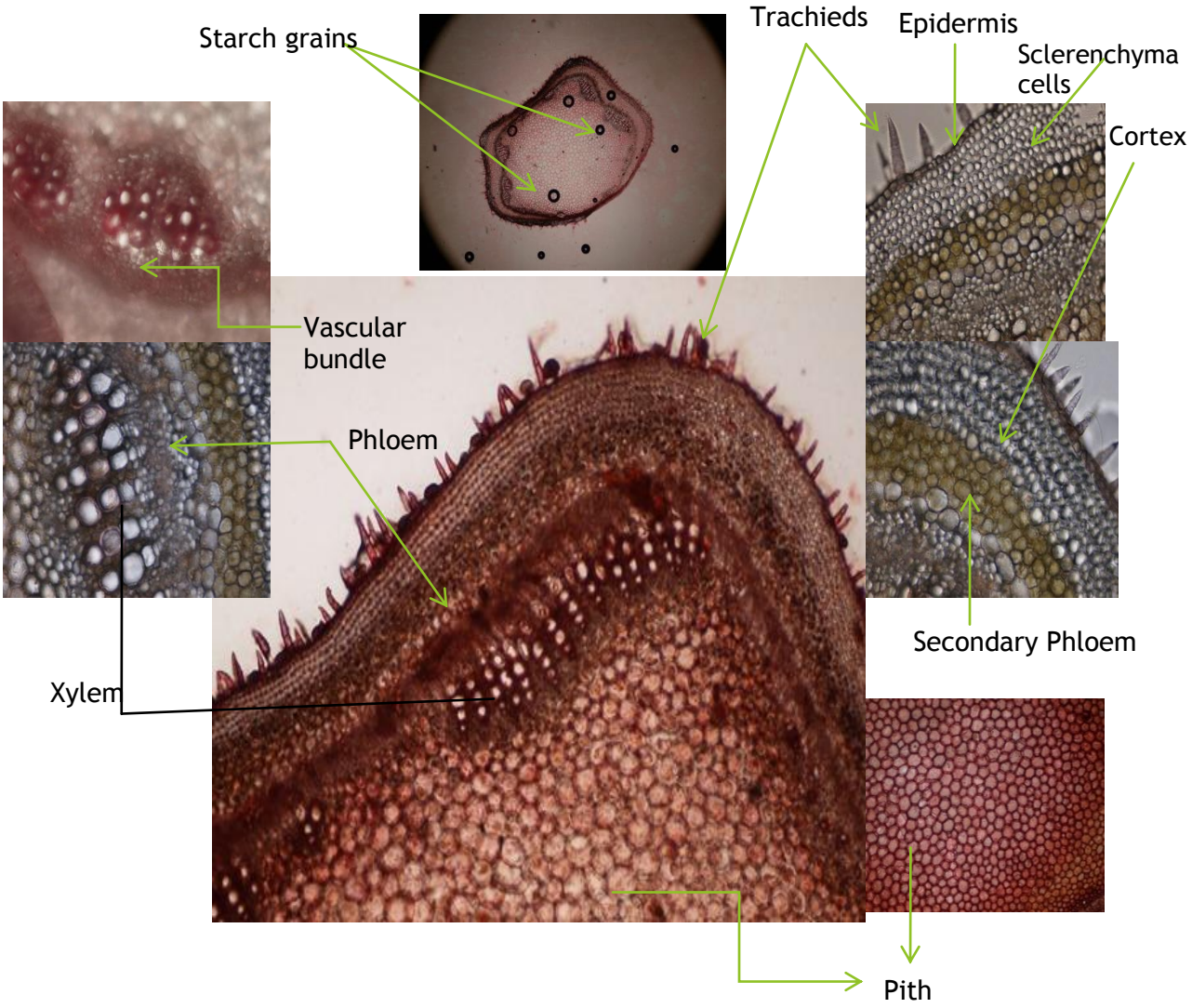


Fig. 4. 4: T.S. of Stem of *C. colebrookianum*

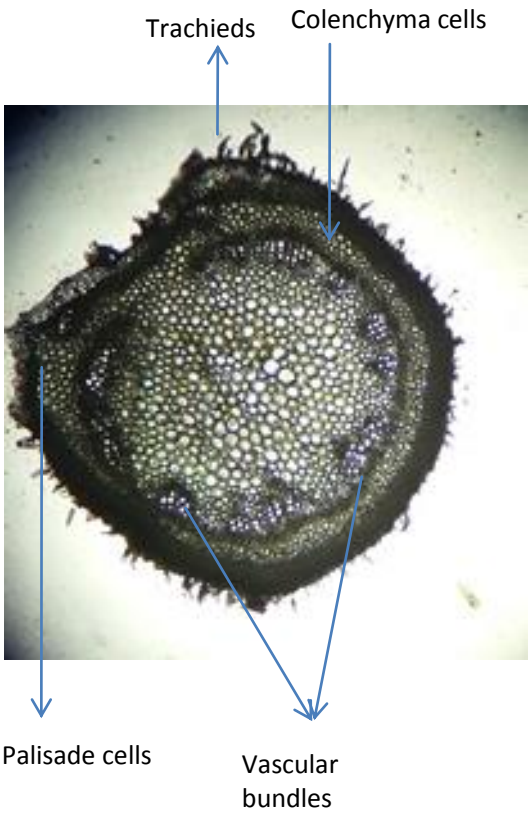


Fig. 4. 5: T.S. of Leaf petiole of *C. colebrookianum*

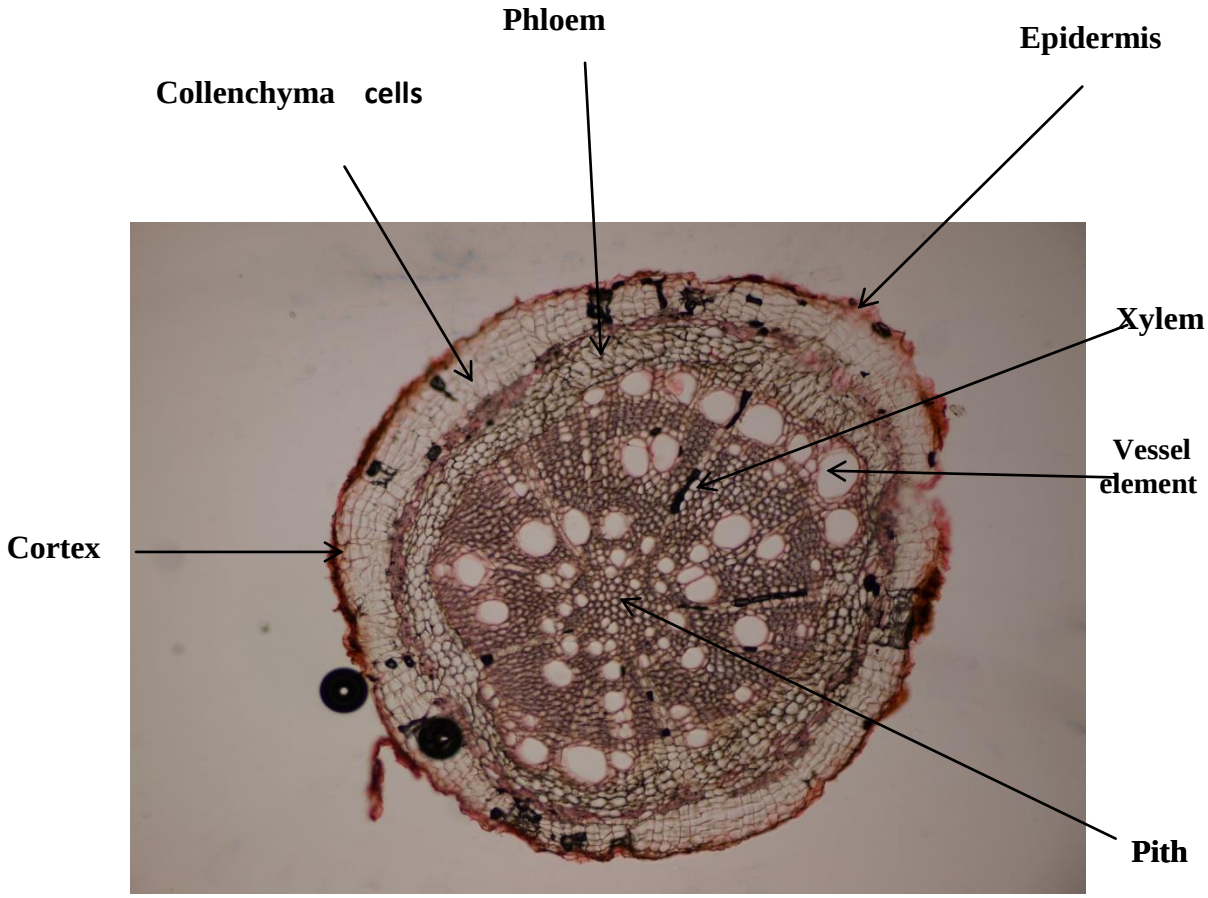


Fig. 4. 6: T.S. of root of *C. colebrookianum*

margin entire to slightly wavy, tip pointed. The Fig 4.5 showed the transverse section of leaf petiole of *C. colebrookianum.* It is clear from the Fig. that the outer cell wall was covered by Trichomes. It was followed by multiple layers of palisade cells. The palisade cells are round in shape. Just beneath the palisade cell, collenchyma cells are present. The vascular bundles are scattered in the collenchymatous cells around the pith.

The Fig. 4.6 shows the transverse section of roots of *C. colebrookianum*. It is clear from the Fig. that the root of *C. colebrookianum* comprises of a number of layers of different cells. The outermost layer of the root is called epidermis. It is immediately followed by cortex. Just beneath the cortex, 5-6 layers of collenchymatous cells are compactly arranged. Vascular bundles, xylem and phloem occupy the central region of the root. Xylem transports the water and minerals absorbed by the root up to the stems, leaves, and flowers. The phloem transports the sugars and other nutrients made by the leaves down to the root for immediate use or for storage during periods of dormancy.

4.5 Seed Biology

4.5.1 Physical parameters of the seeds

The data pertaining to physical parameters of the seeds are presented in Table 4.13. Analysis of variance revealed significant difference in physical parameters of the seeds with respect to locations.

4.5.1.1 Seed Weight (g)

It is evident from the data presented in Table 4.13 that the seed weight was influenced by different locations. The highest seed weight was obtained in Reiek (0.40±0.02 g) which was significantly higher than rest of the locations except seeds collected from Zonuam (0.38±0.03 g) with which it was found statistically *at par*. Among all the locations, the lowest seed weight was recorded in Chawlhhmun (0.21±0.01 g) which was significantly lower than other types of seeds except in Durtlang (0.22±0.02), Lengpui (0.23±0.02 g), Lungdai (0.24±0.02 g), and Sakawrtuichhun (0.24±0.03 g).

4.5.1.2 Seed Length (mm)

A highly significant difference in seed length was observed among the different locations (Table 4.13). Among the different locations, the highest seed length was obtained from the seeds collected from Reiek (7.38 ±0.46 mm) which was significantly higher than rest of the seeds except Zonuam (7.02±0.28 mm) and Chawlhhmun (6.92±0.41 mm). Among all the locations, the significantly lowest length was obtained from the seeds collected from Durtlang (6.43±0.20 mm).

4.5.1.3 Seed diameter (mm)

There was significant variation among the seeds collected from various locations with respect to diameter of the seeds. The highest diameter of the seeds was observed in Reiek (5.86±0.48 mm) which was significantly higher than rest of the locations except in Zonuam(5.65±0.13 mm), Chawlhhmun (5.50±0.19 mm) and Lungdai (5.47±0.21 mm)

with which it was found statistically *at par*. Among all the locations, the lowest seed diameter was obtained in Durtlang (5.02±0.30 mm).

4.5.2 Moisture Loss (%)

It reveals from the Table 4.13 and Fig. 4.7 that variation in moisture loss among the seeds collected from different locations was significant. At 30 days after storage of seeds, the highest moisture loss was observed from the seeds collected from Tanhril (17.65±0.01) which was significantly higher than all other locations. It was followed by the seeds collected from Durtlang (17.01±0.02). Among all the locations, the lowest was recorded in Serkhan (10.05±0.03) which was significantly lower than other seeds.

At 60 days after storage, the maximum moisture loss was obtained in Tanhril (18.26±0.02) which was significantly higher than all other locations. The mimimum moisture loss was obtained in Serkhan (13.51±0.01) which was significantly lower than seeds collected from all other locations.

At 90 days after storage of the seeds, the maximum moisture loss was observed in the seeds collected from Durtlang (19.13±0.02 %), which was significantly higher than all other locations, while lowest was observed in Sakawrtuichhun (14.13±0.00 %).

Table 4. 13: Physical parameters of the seeds of *Clerodendrum colebrookianum* Walp. from different locations

Sl no	Location	Weight (g)	Length (mm)	Diameter (mm)	Moisture loss (%)			Seed Volume (cc)	Imbibition (%)
					30 days	60 days	90 days		
1	Durtlang	0.22±0.02	6.43±0.20	5.02±0.30	17.01±0.02	17.92±0.02	19.13±0.02	0.55±0.07	34.82±3.79
2	Reiek	0.40±0.02	7.38±0.46	5.86±0.48	15.22±0.02	15.92±0.01	15.92±0.01	0.86±0.12	30.70±2.67
3	Luangmual	0.26±0.02	6.81±0.15	5.45±0.18	15.41±0.01	16.76±0.01	16.77±0.01	0.69±0.11	39.71±6.94
4	Lungdai	0.24±0.02	6.84±0.45	5.47±0.21	13.95±0.03	14.32±0.15	14.25±0.01	0.61±0.08	43.91±1.98
5	Serkhan	0.27±0.04	6.64±0.24	5.15±0.18	10.05±0.03	13.51±0.01	18.70±0.01	0.70±0.12	34.79±2.42
6	Zonuam	0.38±0.03	7.02±0.28	5.65±0.13	14.08±0.02	14.80±0.01	15.47±0.01	0.73±0.08	38.98±7.60
7	Chawlhhmun	0.21±0.01	6.92±0.41	5.50±0.19	13.06±0.01	14.31±0.02	16.04±0.01	0.68±0.08	36.29±9.82
8	Tanhril	0.25±0.01	6.71±0.38	5.22±0.20	17.65±0.01	18.26±0.02	18.28±0.01	0.64±0.11	33.83±5.11
9	Sakawrtuichhun	0.24±0.03	6.46±0.24	5.10±0.35	12.63±0.02	14.12±0.01	14.13±0.00	0.76±0.08	32.48±1.48
10	Lengpui	0.23±0.02	6.66±0.21	5.37±0.24	14.17±0.01	15.08±0.02	15.85±0.01	0.74±0.12	31.09±2.10
S Ed(±)		0.02	0.23	0.19	0.012	0.041	0.008	0.07	3.42
$CD_{0.05}$		0.04	0.49	0.40	0.026	0.087	0.019	0.15	7.19

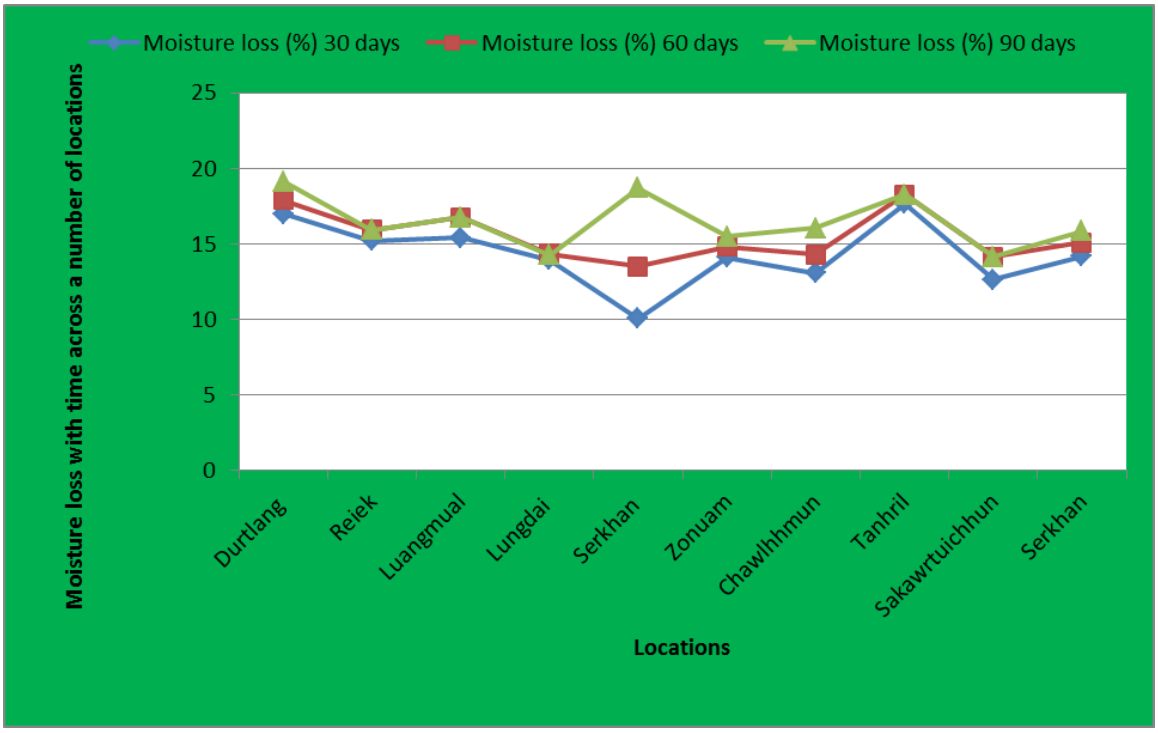


Fig. 4.7: Moisture loss at different days after storage across different locations

4.5.3 Seed volume (cc)

Significant differences were observed among the seeds collected from different locations with respect to seed volume (Table 4.13). Among all the locations, the highest seed volume was recorded in Reiek (0.86±0.12 cc), but it was statistically *at par* with Sakawrtuichhun (0.76±0.08cc) Lengpui (0.74±0.12 cc) and Zonuam (0.73±0.08 cc). However, the lowest volume of the seed was found in Durtlang (0.55±0.07cc) which was significantly lower than most of the seeds.

4.5.4 Imbibition (%)

The data recorded on imbibition of the seeds was observed to have significantly varied among the different locations (Table 4.13). Among the different locations, the seeds collected from Lungdai (43.91±1.98 %) recorded the maximum imbibition which was significantly higher than rest of the locations, except Luangmual (39.71±6.94 %) while the lowest imbibition was obtained in Reiek (30.70±2.67 %) which was significantly lower than most of the locations.

4.5.5 Seed viability

4.5.5.1 Seed viability at refrigerated condition

The data pertaining to seed viability tested by using tetrazolium at refrigerated condition are presented in Table 4.14. Analysis of variance revealed significant difference in seed viability at refrigerated condition with respect to different locations

At one month of storage, i.e. in December, the highest viability was observed in Reiek (100.00±0.00 %) which was significantly higher than all other locations except in Chawlhhmun (96.67±5.77 %). The lowest viability was observed in Lengpui, Luangmual and Zonuam (83.33±5.77 %).

At two months of storage, i.e. in January the maximum seed viability was obtained in Reiek (95.00±5.00 %) significantly higher than rest of the locations except Durtlang (91.67±2.89 %), Lungdai and Tanhril (86.67±5.77 %) with which it was found statistically *at par*, however, among all the locations, the lowest viability was observed in Lengpui (76.67±5.77 %).

The seeds collected from Reiek recorded the highest seed viability (93.33±5.77 %) at three months of storage i.e. February, which was significantly higher than rest of the seeds except, Durtlang (85.00±5.00 %). Among all the seeds, the lowest viability was observed in the seeds collected from Lengpui (73.33±5.77 %).

At four months, i.e. March, the maximum viability was recorded in Reiek (86.67±5.77 %) which was significantly higher than other locations except Durtlang (83.33±5.77 %) and Serkhan (80.00±0.00 %). The lowest viability at this storage was obtained in Chawlhhmun (70.00±0.00 %) which was significantly lower than the most of the locations.

The maximum viability was found in the seeds collected from Reiek (86.67±5.77 %) at five months of storage, i.e. April, which was significantly higher than all other

locations except Durtlang (80.00±0.00 %), while, the lowest was observed in Chawlhhmun and Sakawrtuichhun (66.67±5.77 %).

At six months of storage, i.e. in the month of May, among all the locations, Reiek (76.67±5.77 **%**) showed maximum viability which was significantly higher than all other locations, while, the lowest was recorded in Lengpui, Serkhan and Tanhril (50.00±10.00 **%**).

Reiek (53.33±5.77 %) showed maximum viability at 7 months of storage, i.e. June. It was followed by the seeds collected from Luangmual (50.00±10.00 %). Among all the locations, Lengpui (30.00±5.00 %) recorded the lowest viability but it was at par with Tanhril (30.00±10.00 %).

The seed viability varied significantly at 8 months of storage, and among all the locations, seeds collected from Reiek and Lungdai recorded the highest viability (20.00±10.00 %) which was significantly higher than all other locations, while Chawlhhmun and Sakawrtuichhun recorded the lowest seed viability (6.67±5.77 %).

There was no significant variation among the seeds collected from different locations with respect to seed viability at 9 months onwards, i.e. August. But Reiek and Lungdai recorded the highest viability of the seeds.

Table 4. 14: Viability of the seeds of *Clerodendrum colebrookianum* at refrigerated condition

Sl. N	Location	December	January	February	March	April	May	June	July	August
1	Durtlang	93.33±5.77	91.67±2.89	85.00±5.00	83.33±5.77	80.00±0.00	60.00±10.00	40.00±10.00	16.67±5.77	6.67±5.77
2	Reiek	100.00±0.00	95.00±5.00	93.33±5.77	86.67±5.77	86.67±5.77	76.67±5.77	53.33±5.77	20.00±10.00	10.00±10.00
3	Luangmual	83.33±5.77	80.00±0.00	76.67±5.77	75.00±5.00	70.00±0.00	60.00±10.00	50.00±10.00	10.00±10.00	6.67±5.77
4	Lungdai	90.00±0.00	86.67±5.77	83.33±5.77	76.67±5.77	73.33±5.77	53.33±5.77	40.00±10.00	20.00±10.00	10.00±0.00
5	Serkhan	86.67±5.77	83.33±5.77	83.33±5.77	80.00±0.00	73.33±5.77	50.00±10.00	46.67±5.77	6.67±5.77	6.67±5.77
6	Zonuam	83.33±5.77	80.00±10.00	76.67±15.28	73.33±5.77	70.00±10.00	60.00±5.77	40.00±10.00	10.00±0.00	6.67±5.77
7	Chawlhhmun	96.67±5.77	80.00±0.00	73.33±5.77	70.00±0.00	66.67±5.77	56.67±10.00	33.33±5.77	6.67±5.77	3.33±5.77
8	Tanhril	86.67±5.77	86.67±5.77	80.00±0.00	76.67±5.77	70.00±10.00	50.00±10.00	30.00±10.00	16.67±5.77	6.67±11.55
9	Sakawrtuichhun	86.67±5.77	83.33±5.77	80.00±10.00	76.67±5.77	66.67±5.77	53.33±5.77	36.67±5.77	6.67±5.77	3.33±5.77
10	Lengpui	83.33±5.77	76.67±5.77	73.33±5.77	73.33±5.77	70.00±0.00	50.00±10.00	30.00±5.00	10.00±0.00	3.33±5.77
	S Ed(±)	2.96	4.59	4.18	3.92	5.19	5.86	6.81	4.07	-
	$CD_{0.05}$	4.19	9.64	8.78	8.24	10.9	12.31	14.31	8.55	NS

4.5.5.2 Seed viability at ambient condition

Significant to highly significant variation was observed among the seeds collected from different locations with respect to seed viability at ambient conditions (Table 4.15).

The viability of the seeds collected from different locations was found to be varied significantly at one month of storage i.e. December at ambient conditions (Table 4.15). The highest seed viability at this stage was found in Reiek (96.67±5.77 %) which was significantly higher among other locations except Chawlhhmun (93.33±5.77 %) and Lungdai (90.00±10.00 %). Among all the locations, the lowest seed viability was obtained in Lengpui (80.00±5.00 %) which was found statistically *at par* with Luangmual (80.00±10.00 %) and Zonuam (81.67±10.41 %).

At two months of storage, i.e. January, the highest viability was obtained in Reiek (91.67±2.89 %) which was significantly higher than rest of the locations except Durtlang (85.00±8.66 %), Lungdai (85.00±5.00 %), and Tanhril (83.33±5.77 %). However, the lowest was found in Zonuam (66.67±5.77 %) which was significantly lower than other locations.

The significantly highest viability at three months of storage i.e. February was observed in Reiek (86.67±2.89 %) followed by Durtlang (83.33±5.77 %) and the lowest was observed in Zonuam (63.33±2.89 %) which was significantly lower than other seeds.

At four months of storage, i.e. March, the highest viability was recorded in Reiek (83.33±5.77) which was significantly higher than most of the locations. It was followed

by Durtlang (80.00±5.00 %). Among all the locations, the lowest viability at this stage was obtained in Zonuam (61.67±2.89 %) which was significantly lower than the most of the locations.

Maximum viability at five months of storage, i.e. April was observed in Reiek (78.33±7.64 %) which was significantly higher than all other locations except Durtlang (75.00±5.00 %) and Serkhan (73.33±5.77 %). At this stage, the significantly lowest seed viability was observed in Zonuam (58.33±2.89 %).

The viability of the seeds was very poor at 7 months of storage. Among all the locations, Lungdai (33.33±2.89 %) recorded the highest viability but it was found statistically *at par* with Luangmual (30.00±10.00 %), Durtlang (23.33±5.77 %) and Chawlhhmun(25.00±5.00 %). Among the storage seeds, Sakawrtuichhun (16.67±2.89 %) exhibited the lowest viability.

From 8 months onwards, no germination was observed among the seeds collected from different locations.

Table 4.15 : Viability of the seeds of *Clerodendrum colebrookianum* at ambient condition

Sl. No.	Location	December	January	February	March	April	May	June	July	August
1	Durtlang	86.67±5.77	85.00±8.66	83.33±5.77	80.00±5.00	75.00±5.00	58.33±7.64	23.33±5.77	0.00±0.00	0.00±0.00
2	Reiek	96.67±5.77	91.67±2.89	86.67±2.89	83.33±5.77	78.33±7.64	56.67±10.41	18.33±7.64	0.00±0.00	0.00±0.00
3	Luangmual	80.00±10.00	78.33±2.89	75.00±5.00	73.33±5.77	68.33±7.64	48.33±7.64	30.00±10.00	0.00±0.00	0.00±0.00
4	Lungdai	90.00±10.00	85.00±5.00	81.67±7.64	76.67±5.77	65.00±5.00	38.33±2.89	33.33±2.89	0.00±0.00	0.00±0.00
5	Serkhan	83.33±7.64	81.67±7.64	80.00±5.00	76.67±5.77	73.33±5.77	30.00±10.00	21.67±2.89	0.00±0.00	0.00±0.00
6	Zonuam	81.67±10.41	66.67±5.77	63.33±2.89	61.67±2.89	58.33±2.89	25.00±5.00	18.33±2.89	0.00±0.00	0.00±0.00
7	Chawlhhmun	93.33±5.77	76.67±5.77	73.33±5.77	70.00±5.00	66.67±2.89	38.33±2.89	25.00±5.00	0.00±0.00	0.00±0.00
8	Tanhril	86.67±5.77	83.33±5.77	80.00±0.00	76.67±2.89	65.00±5.00	23.33±5.77	18.33±2.89	0.00±0.00	0.00±0.00
9	Sakawrtuichhun	81.67±2.89	78.33±7.64	76.67±7.64	73.33±5.77	66.67±5.77	30.00±10.00	16.67±2.89	0.00±0.00	0.00±0.00
10	Lengpui	80.00±5.00	75.00±5.00	73.33±5.77	70.00±10.00	65.00±5.00	28.33±10.41	20.00±5.00	0.00±0.00	0.00±0.00
	S Ed(±)	4.76	4.77	4.57	4.94	4.59	5.99	4.44	0.00	0.00
	$CD_{0.05}$	10.00	10.03	9.61	10.39	9.64	12.59	9.32	0.00	0.00

4.5.6 Seed Germination

4.5.6.1 Seed germination at refrigerated condition (%)

Data presented in Table 4.16 revealed that there was significant difference among the seeds obtained from different locations in germination of the seeds under refrigerated conditions.

The highest germination of the seeds at one month after storage, i.e. December, was found in Reiek (66.67±5.77 %) which was significantly higher among other germplams except Durtlang (63.33±5.77 %), Lengpui (60.00±20.00 %) Tanhril (53.33±15.28 %) and Serkhan (53.33±5.77 %). However, the significantly lowest germination was obtained in Lungdai (40.00±14.14 %).

At two months of storage, i.e. in January, the maximum germination percentage of the seeds was obtained in Luangmual (93.33±5.77 %) which was significantly higher than most of the locations. It was followed by Lungdai (90.00±10.00 %). At this stage, the lowest germination was found in Reiek (66.67±5.77 %).

The highest germination percentage at three months of storage, i.e. February was observed in Reiek (96.67±5.77 %) which was significantly higher than most of the locations, while the significantly least was obtained in Chawlhhmun (63.33±11.55 %).

At four months of storage, i.e. March, the significantly highest germination percentage was recorded in Lungdai (83.33±15.28 %). It was followed by Tanhril (80.00±10.00 %) which was significantly higher than other locations. The lowest

germination percentage was obtained in Serkhan (63.33±15.28 %) which was significantly lower than the rest of the locations.

The significantly highest germination percentage was found in Luangmual (83.33±15.28 %) at five months of storage, i.e. April, which was significantly higher than most of the locations, while, the lowest was observed in Lengpui and Reiek (56.67±5.77 %).

At six months of storage, i.e. May, among all the locations, Durtlang (43.33±15.28 %) showed maximum germination percentage which was significantly higher than most of the locations. The significantly lowest germination percentage was recorded in Zonuam (26.67±5.77 %).

Durtlang (43.33±5.77 %) showed maximum germination percentage at 7 months of storage, but it was found statistically *at par* with Reiek (40.00±17.32 %) and Zonuam (40.00±10.00 %). Among all the locations, Lengpui (20.00±10.00 %) exhibited the lowest germination percentage.

At 8 months of storage, i.e. July, the germination was very poor. The maximum was reported in Reiek (6.67±5.77 %), and Zonuam (6.67±11.55 %), while the lowest (0.00±0.00 %) was recorded in Luangmual and Serkhan.

The germination was very poor at 9 months of storage, i.e., August, the seeds collected from 3 locations only germinated i.e. Durtlang, Serkhan and Sakawrtuichhun (3.33±5.77 %), while the rest fail to germinate (0.00±0.00 %) and there was no germination beyond 9 months.

Table 4.16: Month wise germination per cent of seeds at refrigerated condition

Sl. No.	Location	December	January	February	March	April	May	June	July	August
1	Durtlang	63.33±5.77	76.67±11.55	86.67±5.77	73.33±11.55	66.67±5.77	43.33±15.28	43.33±5.77	3.33±5.77	3.33±5.77
2	Reiek	66.67±5.77	66.67±5.77	96.67±5.77	76.67±15.28	56.67±5.77	30.00±10.00	40.00±17.32	6.67±5.77	0.00±0.00
3	Luangmual	46.67±5.77	93.33±5.77	76.67±5.77	66.67±5.77	83.33±15.28	36.67±15.28	26.67±5.77	0.00±0.00	0.00±0.00
4	Lungdai	40.00±14.14	90.00±10.00	73.33±11.55	83.33±15.28	76.67±5.77	40.00±17.32	30.00±20.00	3.33±5.77	0.00±0.00
5	Serkhan	53.33±5.77	83.33±15.28	86.67±5.77	63.33±15.28	83.33±11.55	30.00±10.00	30.00±17.32	0.00±0.00	3.33±5.77
6	Zonuam	43.33±15.28	83.33±5.77	76.67±15.28	70.00±20.00	70.00±10.00	26.67±5.77	40.00±10.00	6.67±11.55	0.00±0.00
7	Chawlhhmun	50.00±10.00	73.33±11.55	63.33±11.55	66.67±5.77	70.00±10.00	33.33±25.17	26.67±5.77	3.33±5.77	0.00±0.00
8	Tanhril	53.33±15.28	76.67±11.55	83.33±15.28	80.00±10.00	73.33±15.28	40.00±20.00	23.33±5.77	3.33±5.77	0.00±0.00
9	Sakawrtuichhun	56.70±5.77	86.67±5.77	86.67±15.28	66.67±15.28	63.33±25.17	30.00±20.00	26.67±11.55	3.33±5.77	3.33±5.77
10	Lengpui	60.00±20.00	80.00±10.00	66.67±5.77	66.67±30.55	56.67±5.77	33.33±20.82	20.00±10.00	0.00±0.00	0.00±0.00
	S Ed(±)	7.22	6.83	6.50	5.74	7.95	4.65	6.02	0.08	0.08
	$CD_{0.05}$	15.16	14.35	13.65	12.06	16.71	9.77	12.64	0.16	0.16

4.5.6.2 Seed germination at ambient condition

It is evident from the data represented in Table 4.17 that there was significant difference in germination among the seeds at ambient conditions. The petridishes were kept at different temperature regimes (10,15, 20 and 25+2^{o}C with humidity at 80%) in growth chamber with 16 /8 hours light and dark conditions in laboratory, but except 25+2^{o}C, in other temperature, there was no germination at all.

Tanhril (53.33±15.28 %) recorded the significantly highest germination percentage at one month of storage i.e. December, followed by Durtlang and Reiek (50.00±10.00 %), while the lowest was obtained in Zonuam (33.33±5.77 %).

The highest germination percentage at two months of storage i.e. January was observed in Reiek (90.00±10.00 %) which was significantly higher than rest of the locations. However, the minimum was found in Lengpui (46.67±15.28 %) which was significantly lower than most of the locations.

At three months of storage, i.e., February, the highest germination was observed in Durtlang, Chawlhhmun (86.67±5.77 %) and Tanhril (86.67±23.09 %) which was significantly higher than most of the seeds and the least was obtained in Serkhan (60.00±10.00 %).

The highest germination percentage at four months of storage, i.e. March was recorded in Serkhan (86.67±15.28 %), which was significantly higher than most of the locations. It was followed by Zonuam (80.00±20.00 %). Among all the locations, the lowest germination percentage was obtained in Lungdai (56.67±5.77 %) which was significantly lower than most of the locations.

Table 4.17: Month wise germination per cent of seeds at ambient condition

Sl. No.	Location	December	January	February	March	April	May	June	July	August
1	Durtlang	50.00±10.00	70.00±10.00	86.67±5.77	70.00±26.46	66.67±11.55	26.67±5.77	0.00±0.00	0.0±0.0	0.0±0.0
2	Reiek	50.00±10.00	90.00±10.00	80.00±0.00	66.67±15.28	60.00±10.00	23.33±15.28	3.33±5.77	0.0±0.0	0.0±0.0
3	Luangmual	46.67±15.28	66.67±5.77	70.00±10.00	63.33±5.77	53.33±5.77	16.67±5.77	6.67±11.55	0.0±0.0	0.0±0.0
4	Lungdai	43.33±15.28	56.67±5.77	63.33±5.77	56.67±5.77	50.00±10.00	23.33±20.82	0.00±0.00	0.0±0.0	0.0±0.0
5	Serkhan	43.33±5.77	76.67±5.77	60.00±10.00	86.67±15.28	70.00±10.00	23.33±15.28	3.33±5.77	0.0±0.0	0.0±0.0
6	Zonuam	33.33±5.77	70.00±10.00	70.00±10.00	80.00±20.00	50.00±10.00	13.33±5.77	6.67±11.55	0.0±0.0	0.0±0.0
7	Chawlhhmun	46.67±15.28	56.67±15.28	86.67±5.77	63.33±15.28	43.33±15.28	26.67±23.09	0.00±0.00	0.0±0.0	0.0±0.0
8	Tanhril	53.33±15.28	76.67±15.28	86.67±23.09	70.00±10.00	40.00±20.00	13.33±11.55	0.00±0.00	0.0±0.0	0.0±0.0
9	Sakawrtuichhun	46.67±5.77	53.33±11.55	73.33±5.77	70.00±17.32	36.67±11.55	26.67±15.28	6.67±11.55	0.0±0.0	0.0±0.0
10	Lengpui	36.67±5.77	46.67±15.28	73.33±15.28	76.67±5.77	40.00±20.00	30.00±26.46	0.00±0.00	0.0±0.0	0.0±0.0
	S Ed(±)	4.77	6.61	6.71	7.08	8.66	4.86	2.58	-	-
	$CD_{0.05}$	10.01	13.89	14.10	14.87	18.20	10.22	5.42	-	-

Maximum germination percentage at five months of storage i.e. April, was observed in Serkhan (70.00±10.00 %). It was followed by Durtlang (66.67±11.55 %) and Reiek (60.00±10.00 %), while, the lowest was observed in Sakawrtuichhun (36.67±11.55 %) which was significantly lower than most of the locations. Tanhril (40.00±20.00 %) and Lengpui (40.00±20.00 %) immediately followed it.

Lengpui (30.00±26.46 %) showed maximum germination percentage at six months of storage, i.e. May, which was significantly higher than the seeds collected from most of the locations. The lowest germination percentage was recorded in Zonuam (13.33±5.77 %) which was found statistically *at par* with Tanhril (13.33±11.55 %).

At seven months of storage, i.e. June, the maximum germination percentage was found in Luangmual, Zonuam and Sakawrtuichhun (6.67±11.55 %). While, the seeds collected from Durtlang, Lungdai, Chawlhhmun, Tanhril and Lengpui exhibited no germination (0.00±0.00 %).

There was no germination at eight and ninth months of storage irrespective of locations of collection of the seeds.

4.6. Germination studies

4.6.1 Germination studies with different PGRs and chemicals

Data presented in Table 4.18 showed that there was significant difference among the seeds treated with different PGRs and Chemicals with respect to the germination parameters.

Table 4.18: Germination of *Clerodendrum colebrookianum* Walp by using different PGRs and chemicals

Treatment	Germination%	Days required for onset of germination	Days required for completion of germination	Days required for initiation of true leaves
Control	23.27±5.87	17.33±1.15	28.33±1.53	18.33±2.31
GA_3 50 PPM	9.67±0.58	14.00±1.00	26.33±0.58	17.33±0.58
GA_3 100 PPM	30.00±10.00	14.00±1.73	21.00±2.83	17.00±1.00
GA_3 200 PPM	26.67±2.30	13.33±2.89	20.00±2.65	15.67±3.21
GA_3 500 PPM	53.33±11.55	15.33±0.58	20.00±1.00	16.00±0.00
IAA 50 PPM	17.67±1.53	16.00±1.73	18.00±1.00	16.67±0.58
IAA 100 PPM	33.33±2.89	12.67±2.52	19.67±1.53	13.00±1.00
IAA 200 PPM	20.00±0.00	12.33±2.52	18.33±1.15	14.00±2.65
IAA 500 PPM	6.67±0.23	9.33±0.58	19.00±2.00	10.00±1.00
IBA 50 PPM	33.33±5.77	11.00±1.00	16.00±1.00	11.33±0.58
IBA 100 PPM	33.33±11.55	13.33±1.53	20.67±1.53	14.33±2.31
IBA 200 PPM	26.67±5.77	15.33±4.73	23.33±0.58	16.00±3.46
IBA 500 PPM	7.33±2.08	13.00±2.00	20.00±1.00	13.33±3.06
NAA 50 PPM	33.33±11.55	15.00±0.00	20.33±0.58	17.00±1.00
NAA 100 PPM	51.67±12.58	17.33±2.08	19.67±3.06	18.00±1.00
NAA 200 PPM	5.67±1.15	12.33±2.52	25.67±2.08	17.00±1.00
NAA 500 PPM	13.33±2.89	15.00±0.00	22.00±1.00	16.67±3.06
2,4-D 50 PPM	26.67±0.38	12.33±2.52	19.00±2.65	13.00±1.00
2,4-D 100 PPM	10.00±2.29	9.67±2.08	24.00±2.65	11.67±1.15
2,4-D 200 PPM	13.33±5.77	11.67±2.89	22.00±2.65	13.00±2.65
2,4-D 500 PPM	10.00±0.00	11.33±1.15	21.00±3.00	14.00±2.00
TIBA 50 PPM	11.33±1.15	10.67±2.31	24.67±1.53	12.33±3.79
TIBA 100 PPM	13.33±2.89	15.67±1.15	25.33±2.08	16.00±2.00
TIBA 200 PPM	12.67±0.58	14.33±3.21	22.67±2.52	15.33±2.52
TIBA 500 PPM	20.00±8.66	13.33±2.89	23.33±2.52	15.33±2.08
KNO_3 100 mM	85.67±5.13	7.00±1.00	11.33±1.15	7.33±0.58
KNO_3 150 mM	88.67±1.53	6.33±0.58	12.00±2.00	6.67±0.58
$NaHClO_3$ 100 mM	56.67±11.55	8.00±1.00	15.33±1.53	8.67±0.58
$NaHClO_3$ 150 mM	84.00±3.61	7.33±0.58	10.00±1.00	8.00±1.00
SEd(±)	4.92	1.62	1.52	1.56
$CD_{0.05}$	8.22	2.71	2.54	2.61

It is evident from the data presented in Table 4.18 that the germination of the seeds was influenced by different PGRs and chemicals. Among all the PGRs and chemicals, the highest germination was obtained in KNO_3 150 mM (88.67±1.53%) which was significantly higher than other treatments, except in KNO_3 100 mM (85.67±5.13%), $NaHClO_3$ 150 mM (84.00±3.61%) with which it was found statistically *at par*. However, the minimum germination percentage was recorded in NAA 200 ppm (5.67±1.15) which was significantly lower than most of the treatments.

A highly significant difference was observed among the different treatments in days required for onset of germination (Table 4.18). Control (17.33±1.15) required the maximum days for onset of germination, which was significantly higher than other treatments, except NAA 100 ppm (17.33±2.08), IAA 50 ppm (16.00±1.73), and TIBA 100 ppm (15.67±1.15) NAA 200 ppm (12.33±2.52), and NAA 500 ppm (15.00±0.00), which it was found statistically *at par*. However, the minimum days was recorded in KNO_3 150 mM (6.33±0.58) which was significantly lower than other treatments, except in KNO_3 100 mM (7.00±1.00), $NaHClO_3$ 150 mM (7.33±0.58) $NaHClO_3$ 100 mM (8.00±1.00), with which it was found statistically *at par*.

Among all the treatments, control required the maximum days for completion of germination (28.33±1.53), which was significantly higher than all other treatments, except GA_3 50 ppm (26.33±0.58**)** with which it was found statistically *at par*. $NaHClO_3$ 150 mM required the minimum days for completion of germination (10.00±1.00) which

was significantly lower than all other treatments except KNO_3 100 mM (11.33±1.15**)** and KNO_3 150 mM (12.00±2.00**)** with which it was found statistically *at par*.

Similarly, the maximum days for initiation of true leaves was observed in control (18.33±2.31**)** which was significantly higher than most of the treatments. It was followed by NAA 100 ppm (18.00±1.00), GA_3 50 ppm (17.33±0.58), NAA 50 ppm**,** NAA 200 ppm and GA_3 100 ppm **(**17.00±1.00), NAA 500 ppm (16.67±3.06), IBA 200 ppm (16.00±3.46) respectively. The minimum days for initiation of true leaves was recorded in KNO_3 150 mM (6.67±0.58) which was significantly lower than all other treatments except in KNO_3 100 mM (7.33±0.58), $NaHClO_3$ 150 mM (8.00±1.00**)**, and $NaHClO_3$ 100 mM (8.67±0.58) with which it was found statistically *at par*.

4.6.2 Germination studies with different media

It reveals from the Table 4.19 and Fig. 4.8 that variation in germination per cent of the seeds among the different media was significant. Among the various media composition, the maximum germination percentage (88.33±2.89 %) was recorded in FYM + vermicompost (VC) + soil + sand (2:2:1:2) which was significantly higher than all other treatments except FYM + vermicompost (VC) + soil + sand (2:2:2:1), (86.67±7.64 %), FYM + vermicompost (VC) + soil + sand (2:2:1:1), (83.33±5.77 %) and FYM + vermicompost (VC) + Soil + Sand (2:1:1:1) **(**80.00±8.66 %) with which it was found statistically *at par*. The lowest germination percentage was recorded in nursery soil alone (53.33±5.77 %) which was also significantly lower than other treatments.

Table 4.19: Germination study of *Clerodendrum colebrookianum* Walp by using different media

Treatments	Germination percentage	Days required for onset of germination	Days required for completion of germination	Days required for initiation of true leaves
Nursery soil alone	53.33±5.77	19.00±1.00	35.67±0.58	24.00±1.00
Sand alone	65.00±5.00	18.67±1.15	35.00±1.00	23.67±1.15
FYM+Vermicompost (VC)+ Soil + Sand (1:1:1:1)	78.33±2.89	15.00±1.00	32.00±1.00	19.67±0.58
FYM+Vermicompost (VC)+ Soil + Sand (1:2:1:1)	68.33±7.64	16.00±1.00	33.67±0.58	21.00±1.00
FYM+Vermicompost (VC)+ Soil + Sand (1:1:2:1)	66.67±5.77	14.33±0.58	31.33±2.08	19.33±0.58
FYM+Vermicompost (VC)+ Soil + Sand (1:1:1:2)	76.67±7.64	15.00±1.00	34.00±1.00	20.33±1.15
FYM+Vermicompost (VC)+ Soil + Sand (2:1:1:1)	80.00±8.66	15.67±1.15	30.33±0.58	21.00±1.00
FYM+Vermicompost (VC)+ Soil + Sand (2:2:1:1)	83.33±5.77	16.00±1.00	32.00±1.00	21.00±0.00
FYM+Vermicompost (VC)+ Soil + Sand (2:2:2:1)	86.67±7.64	15.00±1.00	34.00±1.15	20.00±1.00
FYM+Vermicompost (VC)+ Soil + Sand (2:2:1:2)	88.33±2.89	12.33±0.58	29.00±2.00	18.00±1.00
SEd (±)	5.10	0.78	1.01	0.77
$CD_{0.05}$	10.71	1.63	2.12	1.62

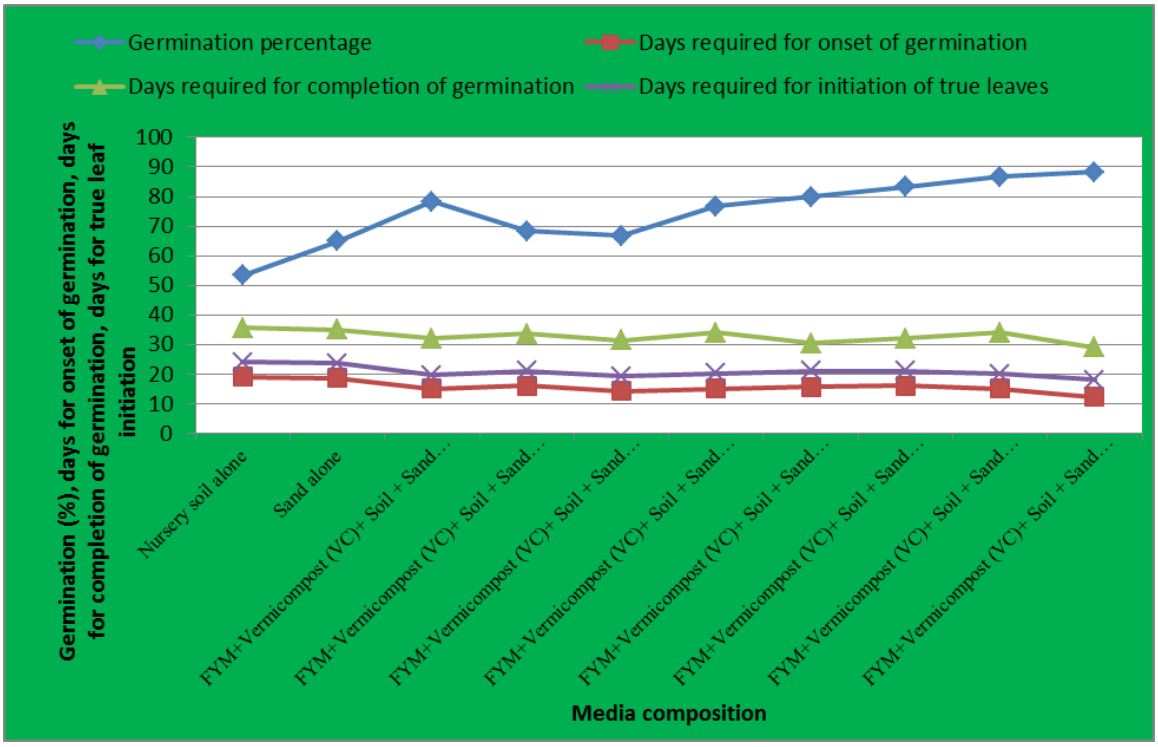


Fig. 4.8: Germination (%), days for onset of germination, days for completion of germination, days for true leaf initiation with different media composition

Significant differences were observed among the different media composition with respect to days required for onset of germination (Table 4.19). Among the different media, the maximum days required for onset of germination was observed in nursery soil alone (19.00±1.00) which was significantly higher than all other treatments except in sand alone (18.67±1.15) with which was found statistically *at par*. The minimum days for onset of germination was recorded in FYM + vermicompost (VC) + soil + sand (2:2:1:2) (12.33±0.58) which was significantly lower than rest of the treatments.

The effect of different media on days required for completion of germination was found to be significant (Table 4.19). The maximum days required for completion of germination was found in nursery soil alone (35.67±0.58), which was significantly higher than the rest of the treatments except sand alone (35.00±1.00), FYM + vermicompost (VC) + soil + sand **(**2:2:2:1**) (**34.00±1.15), FYM + vermicompost (VC) + soil + sand (1:1:1:2) (34.00±1.00) and FYM + vermicompost (VC) + soil + sand (1:2:1:1) (33.67±0.58) with which it was found statistically *at par*. While, the minimum days required for completion of germination (29.00±2.00) was recorded in FYM + vermicompost(VC) + soil + sand (2:2:1:2) which was significantly lower than all other treatments except FYM + vermicompost (VC) + soil + sand **(**2:1:1:1**) (**30.33±0.58) .

Nursery soil alone (24.00±1.00) required the significantly maximum days for initiation of true leaves except in sand alone (23.67±1.15). The minimum days required for initiation of true leaves was obtained in FYM + vermicompost(VC) + soil + sand (2:2:1:2) (18.00±1.00) which was significantly lower than all other treatments except in FYM + vermicompost(VC) + soil + sand (1:1:2:1) (19.33±0.58).

4.7 Acquisition of germinability

4.7.1 Dormancy

To determine the acquisition of germinability, seeds were harvested at three different stages i.e. immature seeds (green fruit), mature seeds and dehisced seeds. The experimental test showed very less germination from green immature fruits. The seeds collected at mature and fallen seeds gave good germination. So, there was no dormancy present in the seeds of harvested and fallen seeds. But the poor germination in green seeds might be due to under-developed embryo at the time of collection of the seeds (dormancy due to rudimentary embryo).

4.7.2 Germination Percentage

Seeds harvested at different stages indicated significant to highly significant difference in germination percentage (Table 4.20). The highest germination percentage was obtained in the harvested seeds (55.00±8.66 %) which was significantly higher than all other types of seeds. The lowest germination percentage was obtained in green seeds (8.33±2.89 %) which was significantly lower than rest of the harvested and fallen seeds.

4.7.3 Speed of germination

The data recorded on speed of germination was observed to have significantly varied among the seeds harvested at various stages (Table 4.20). The highest speed of germination was found in harvested seeds (0.30±0.07) which was significantly higher than the rest of the seeds followed by fallen seeds (0.19±0.02). The lowest speed of

germination was found in green seeds (0.08±0.03), which was also significantly lower than rest of the seeds.

4.7.4 Mean Germination Time

The variation in mean germination time due to the seeds harvested at various stages was found to be significant as shown in Table 4.20. The maximum mean germination time was obtained in harvested seeds (46.67±2.31) which was significantly higher than all other types of seed except fallen seeds (42.00±4.58) with which it was found statistically *at par*, while the significantly lowest mean germination time was obtained in green seeds (20.67±0.58).

4.7.5 Peak Value

Observations pertaining to peak value of the seeds were varied significantly as shown in Table 4.20. From the data presented in the Table, it is evident that the peak value of harvested seeds (0.09±0.04) was significantly higher than the rest of the seeds, which was followed by fallen (0.05±0.00), whereas the lowest peak value was obtained in green seeds (0.00±0.00).

4.7.6 Mean Daily Germination

Data presented in Table 4.20 indicated significant variation in mean daily germination of seeds due to seeds harvested at various stages. The maximum mean daily germination was obtained in harvested seeds (0.24±0.03) which was significantly higher

than all other types of seeds. The lowest mean daily germination was also found in green seeds (0.08±0.03) which was significantly lower than the rest of the seeds.

4.7.7 Germination Index

The difference among the seeds harvested at various stages was found significant (Table 4.20). Only the harvested seeds had the highest germination index (1.18±0.16), while, with respect to fallen and green seeds no germination index was recorded (0.00±0.00).

Table 4.20. Germination parameters of different seeds of *Clerodendrum colebrookianum* Walp

	Germination (%)	Speed of Germination	Mean Germination Time	Peak Value	Mean Daily Germination	Germination Index
Harvested	55.00±8.66	0.30±0.07	46.67±2.31	0.09±0.04	0.24±0.03	1.18±0.16
Fallen	31.67±2.89	0.19±0.02	42.00±4.58	0.05±0.00	0.15±0.02	0.00±0.00
Green	8.33±2.89	0.08±0.03	20.67±0.58	0.00±0.00	0.08±0.03	0.00±0.00
S. Ed(±)	3.33	0.04	2.86	0.03	0.02	0.08
$CD_{0.05}$	9.25	0.10	7.94	0.09	0.04	0.21

Table 4.21 : Percent survival of *Clerodendrum colebrookianum* Walp

Germplasm	Survival percent
Durtlang	50.00±3.00
Reiek	66.67±2.62
Luangmual	50.00±3.00
Lungdai	100.00±0.00
Serkhan	100.00±0.00
Zonuam	88.89±4.76
Chawlhhmun	100.00±0.00
Tanhril	55.56±5.32
Sakawrtuichhun	83.33±2.08
Lengpui	66.67±4.93
S. Ed (±)	2.76
$CD_{0.05}$	5.79

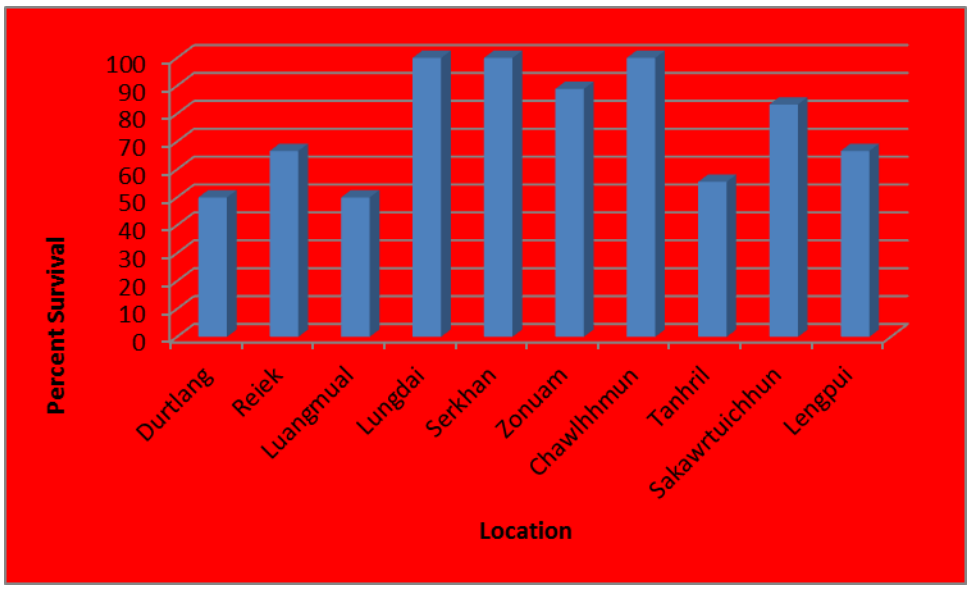


Fig. 4.9: Per cent survival of seedlings across different locations

4.8 Nursery parameters

4.8.1 Per cent survival

Data presented in Table 4.21 and Fig. 4.9 indicated that significant variation was observed among the seeds collected from different locations with respect to per cent survival. The maximum per cent survival was observed in Lungdai, Serkhan and Chawlhhmun (100.00±0.00), which was significantly higher than rest of the locations, while, the lowest per cent survival was obtained in Luangmual and Durtlang (50.00±3.00) which was significantly lower than rest of the locations.

4.9 Seedlings growth

4.9.1 Plant height

The data pertaining to plant height of the seedlings are presented in Table 4.22. Analysis of variance revealed significant difference in plant height among the germplasm collected from various locations.

At 30 days after planting the maximum plant height was observed in Lungdai(9.67±1.80 cm) which was significantly higher than most of the locations. It was followed by Reiek (8.33± 1.53 cm). Whereas, the significantly lowest plant height was obtained in Serkhan (3.77±1.95 cm).

The highest plant height at 60 DAP was recorded in Lungdai (30.37±3.81 cm), which was significantly higher than most of the germplasms. It was followed by Reiek (29.60±5.90cm). The lowest plant height at this stage was found in Serkhan (18.47±1.53 cm).

Luangmual recorded the highest plant at 90 DAP (56.03±5.18 cm), which was significantly higher than most of the other germplasms. It was followed by Sakawrtuichhun (53.67±12.19 cm), while, the lowest was obtained in Tanhril (34.57±8.26 cm).

Among the germplasm, Durtlang (95.83±4.65 cm) showed the highest plant height after 120 days, which was significantly higher than most of the germplasms. It was followed by Zonuam (94.00±2.65) and Reiek (93.03±1.72 cm). However, the lowest plant height was obtained in Tanhril (62.27±15.77 cm).

Table 4.22 Average height of the seedlings of *Clerodendreum colebrookianum* Walp.

Germplasm	30 days	60 days	90 days	120 days	150 days	180 days	210 days	240 days	270 days	300 days	330 days	360days
Durtlang	5.47±0.93	22.33±2.52	47.70±2.29	95.83±4.65	153.33±16.01	201.83±11.86	234.67±17.24	243.83±21.27	244.17±24.76	251.83±25.27	277.00±30.79	304.58±41.98
Reiek	8.33±1.53	29.60±5.90	50.33±1.60	93.03±1.72	146.00±2.00	178.67±12.79	197.17±19.41	210.00±22.52	227.67±27.02	255.33±29.02	277.67±34.53	311.61±38.13
Luangmual	7.87±2.10	26.60±4.62	56.03±5.18	89.37±6.92	135.33±3.06	164.33±21.73	170.67±29.54	183.33±32.72	193.50±36.01	210.33±6.43	238.00±47.89	255.00±24.58
Lungdai	9.67±1.80	30.37±3.81	53.47±6.26	89.67±10.21	132.27±21.11	158.00±37.72	170.33±48.95	184.00±52.12	189.00±55.67	204.17±53.58	224.67±65.13	233.97±71.97
Serkhan	3.77±1.95	18.47±1.53	35.50±13.14	69.17±23.00	116.00±10.58	142.67±6.43	155.33±16.97	172.00±24.25	180.67±24.01	191.00±24.27	206.00±31.18	220.86±43.46
Zonuam	6.77±3.01	23.87±3.90	51.67±7.23	94.00±2.65	148.33±20.55	183.33±0.58	192.00±9.17	197.67±12.66	200.67±15.95	209.00±14.18	224.33±32.32	242.82±29.60
Chawlhhmun	6.90±1.21	24.70±0.95	50.00±1.00	76.67±14.29	118.33±15.00	147.67±4.16	156.50±15.88	166.83±16.00	171.00±13.11	175.00±6.24	185.67±12.34	206.25±12.69
Tanhril	6.27±0.87	20.48±2.53	34.57±8.26	62.27±15.77	99.00±41.58	116.13±31.14	119.50±26.36	120.67±1.53	122.00±6.24	135.83±31.21	158.33±33.50	174.87±32.55
Sakawrtuichhun	6.83±0.15	27.45±4.68	53.67±12.19	71.67±14.05	118.67±24.13	120.00±7.00	128.17±3.25	137.13±32.20	143.00±35.17	157.00±40.51	178.60±5.97	197.10±20.60
Lengpui	7.33±0.25	24.23±1.20	47.93±7.24	67.93±10.17	116.67±12.01	164.27±8.60	189.83±1.61	209.27±4.41	216.33±7.02	219.77±10.87	250.33±10.50	279.63±14.29
S. Ed (±)	**1.36**	**2.92**	**5.02**	**9.14**	**14.82**	**15.17**	**16.80**	**18.19**	**19.82**	**23.04**	**24.39**	**27.64**
$CD_{0.05}$	**2.85**	**6.14**	**10.54**	**19.21**	**31.14**	**31.88**	**35.30**	**38.22**	**41.64**	**48.40**	**51.24**	**58.06**

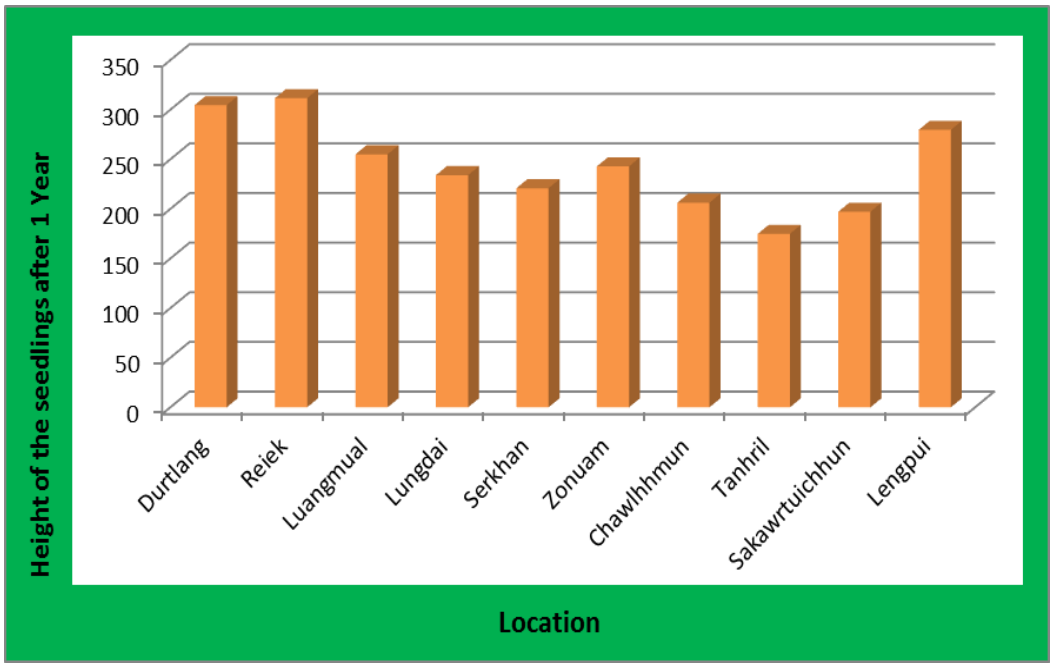


Fig. 4.10: Height of the seedlings across different locations after 1 year of growth

At 150 days after planting, the significantly highest plant height was obtained in Durtlang (153.33±16.01 cm). It was followed by Zonuam(148.33±20.55 cm), while, the lowest was found in Tanhril (99.00±41.58) which was significantly lower than most of the germplasm.

The significantly highest height after 180 days was obtained in Durtlang (201.83±11.86 cm), followed by Zonuam (183.33±0.58 cm), Reiek (178.67±12.79 cm) and Luangmual (164.33±21.73 cm) respectively. However, the lowest was observed in Tanhril (116.13±31.14 cm) which was significantly lower than other germplasm except Sakawrtuichhun (120.00±7.00 cm) and Serkhan (142.67±6.43 cm), with which was found found statistically *at par*.

At 210 days after planting, the maximum height was obtained in Durtlang (234.67±17.24 cm) which was significantly higher than rest of the germplasm. The significantly lowest plant height was obtained in Tanhril (119.50±26.36 cm).

The maximum height at 240 days after planting was obtained in Durtlang (243.83±21.27 cm) which was significantly higher than other germplasms except Reiek (210.00±22.52 cm). The lowest height was obtained in Tanhril (120.67±1.53 cm) which was significantly lower than most of the germplasm.

After 270 days, the highest plant height was also observed in Durtlang (244.17±24.76 cm) followed by Reiek (227.67±27.02 cm), while, the lowest was observed in Tanhril (122.00±6.24 cm).

The maximum plant height after 300 days was found in Reiek (255.33±29.02 cm) which was significantly high and found statistically *at par* with Durtlang (251.83±25.27

cm). However, the lowest was obtained in Tanhril (135.83±31.21 cm) which was significantly lower than most of the germplasm.

Among the germplasm, the maximum height after 330 days was obtained in Reiek (277.67±34.53 cm) followed by Durtlang (277.00±30.79 cm). The lowest height was obtained in Tanhril (158.33±33.50 cm), which was significantly lower than most of the germplasms.

At 360 days after planting, the maximum plant height (Fig. 4.7) was obtained in Reiek (311.61±38.13 cm) which was significantly higher than rest of the germplasms except Durtlang (304.58±41.98 cm), whereas, the significantly lowest plant height was obtained in Tanhril (174.87±32.55 cm).

4.9.2 Collar diameter

Data presented in Table 4.23 reveals that there was a significant difference in collar diameter among the different germplasms.

The highest collar diameter at 30 days after planting was obtained in Reiek (3.43±0.21 mm) followed by Sakawrtuichhun (3.17±0.15 mm), Luangmual (3.03±0.15 mm) and Lungdai (3.03±0.06 mm), whereas, the lowest was obtained in Serkhan (1.93±0.15 mm) which was significantly lower than all other germplasm.

The germplasm from Sakawrtuichhun (7.43±0.95 mm) recorded the highest in collar diameter at 60 days after planting which was significantly higher than rest of the germplasm except Chawlhhmun (6.68±0.32 mm), Luangmual (6.67±0.21 mm) and

Zonuam (6.57±1.09 mm). The significantly lowest collar diameter at this stage was found in Serkhan (4.93±0.85 mm).

The highest collar diameter at 90 days after planting was observed in Sakawrtuichhun (12.33±1.92 mm), which was significantly higher than other germplasms but statistically *at par* with Zonuam (11.33±1.53 mm), Luangmual (11.13±0.81 mm), Durtlang (11.00 ±1.00 mm) and Tanhril (10.67 ± 0.35 mm), respectively, while, the lowest was obtained in Serkhan (8.27 ± 1.27 mm).

Among the germplasm, Zonuam (13.67±1.53 mm) showed the highest collar diameter after 120 days, which was significantly higher than rest of the germplasms. It was followed by Reiek (13.20±0.72 mm), Durtlang (13.13±1.03 mm), Luangmual (12.83±0.29 mm) and Sakawrtuichhun (12.17±2.57 mm). However, the lowest collar diameter was obtained in Lengpuii (10.13±1.03 mm) which was significantly lower than most of the germplasms.

At 150 days after planting, the maximum collar diameter was obtained in Durtlang (19.47±0.50 mm) which was significantly higher than rest of the germplasm except Reiek (17.97±0.55 mm), but there was no statistical differene between these two. The significantly lowest collar diameter was found in Tanhril (14.10±0.44 mm) which was significantly lower than most of the germplasms.

The highest collar diameter after 180 days was obtained in Reiek (25.47±1.29 mm) followed by Durtlang (25.13±1.03 mm) and Luangmual (23.83±0.76 mm). However, the significantly lowest was observed in Tanhril (16.77±4.21 mm)

Table 4.23: Average collar diameter of the seedlings of *Clerodendrum colebrookianum* Walp.

Germplasm	30 days	60 days	90 days	120 days	150 days	180 days	210 days	240 days	270 days	300 days	330 days	360days
Durtlang	2.57±0.51	5.50±0.44	11.00±1.00	13.13±1.03	19.47±0.50	25.13±1.03	28.37±1.48	34.47±1.29	37.43±2.42	39.40±0.87	40.03±0.81	46.33±2.52
Reiek	3.43±0.21	6.00±0.50	9.23±0.75	13.20±0.72	17.97±0.55	25.47±1.29	28.70±0.52	34.00±0.80	38.23±1.00	41.17±1.35	44.03±2.79	46.33±3.21
Luangmual	3.03±0.15	6.67±0.21	11.13±0.81	12.83±0.29	16.23±0.68	23.83±0.76	27.53±1.50	29.70±0.96	32.93±2.73	35.53±1.42	38.03±2.51	41.43±2.71
Lungdai	3.03±0.06	6.13±0.48	8.33±0.58	11.03±1.00	15.00±0.90	18.67±1.24	19.00±1.73	22.67±1.53	22.77±1.20	25.53±1.22	26.63±3.37	37.30±3.42
Serkhan	1.93±0.15	4.93±0.85	8.27±1.27	10.33±0.58	15.33±1.53	18.33±0.58	20.97±2.15	25.73±2.19	27.00±2.65	27.70±3.68	29.37±1.17	36.51±3.59
Zonuam	2.77±0.50	6.57±1.09	11.33±1.53	13.67±1.53	17.00±1.73	21.33±0.58	22.30±1.23	26.67±1.53	29.00±2.00	29.90±3.35	31.57±0.60	35.67±3.21
Chawlhhmun	3.00±0.17	6.68±0.32	10.33±0.58	11.67±0.58	15.33±1.15	19.00±0.10	21.20±1.56	23.67±2.89	25.60±1.39	26.53±2.31	28.13±1.62	35.53±4.65
Tanhril	2.80±0.26	6.26±0.72	10.67±0.35	10.43±1.40	14.10±0.44	16.77±4.21	19.80±0.82	20.03±1.79	23.80±1.97	24.43±0.85	26.23±2.87	29.67±2.08
Sakawrtuichhun	3.17±0.15	7.43±0.95	12.33±1.92	12.17±2.57	17.40±1.30	17.00±1.91	20.00±1.00	23.33±2.66	25.10±2.01	25.77±3.68	27.20±3.30	35.67±4.16
Lengpui	2.90±0.10	6.52±0.10	9.20±0.72	10.13±1.03	15.10±1.01	20.17±1.04	24.07±1.01	28.77±0.40	34.80±1.91	35.90±1.49	39.73±0.55	40.73±2.00
S. Ed (±)	**0.22**	**0.54**	**0.82**	**0.93**	**0.87**	**1.39**	**1.04**	**1.49**	**1.59**	**1.88**	**1.89**	**2.73**
$CD_{0.05}$	**0.45**	**1.13**	**1.72**	**1.95**	**1.82**	**2.92**	**2.19**	**3.13**	**3.35**	**3.96**	**3.96**	**5.73**

At 210 days after planting, the highest collar diameter was obtained in Reiek (28.70±0.52 mm) which was significantly higher than rest of the germplasms except Durtlang (28.37±1.48 mm) and Luangmual (27.53±1.50 mm), whereas, the lowest was obtained in Lungdai (19.00±1.73 mm).

The maximum collar diameter at 240 days after planting, was found Durtlang (34.47±1.29 mm). Reiek (34.00±0.80 mm) followed it. The lowest collar diameter was obtained in Tanhril (20.03±1.79 mm) which was significantly lower than all other germplasms except Lungdai (22.67±1.53 mm).

After 270 days, the highest collar diameter was also observed in Reiek (38.23±1.00 mm) which was significantly higher than rest of the germplasm, except Durtlang (37.43±2.42 mm) with which it was found statistically *at par*. The significantly lowest collar diameter was observed in Lungdai (22.67±1.20 mm).

The maximum collar diameter after 300 days was found in Reiek (41.17±1.35 mm) which was significantly higher than rest of the germplasm, except Durtlang (39.40±0.87 mm) with which it was found statistically *at par*. However, the lowest was obtained in Tanhril (24.43±0.85 mm).

Similarly, among the germplasm, the maximum collar diameter after 330 days was obtained in Reiek (44.03±2.79 mm) which was significantly higher than all other germplasms. The lowest collar diameter was obtained in Tanhril (26.23±2.87 mm.

At 360 days after planting, the maximum collar diameter was obtained in Reiek (46.33±3.21 mm), which was significantly higher than rest of the germplasm. It was followed by Durtlang (46.33±2.52 mm), whereas, the lowest was obtained in Tanhril (29.67±2.08 mm).

Table 4.24: Number of leaves/plant of the seedlings *of Clerodendrum colebrookianum* Walp. collected from various locations

Germplasm	30 days	60 days	90 days	120 days	150 days	180 days	210 days	240 days	270 days	300 days	330 days	360days
Durtlang	7.67±0.58	11.33±1.15	19.33±3.06	24.67±3.06	54.00±5.20	76.33±8.50	81.67±6.66	90.37±0.32	93.67±6.35	96.0±1.00	104.33±0.58	111.67±2.89
Reiek	7.33±1.15	14.00±2.00	17.67±1.53	24.00±2.00	62.00±2.00	108.00±5.29	112.00±3.46	114.00±5.29	124.67±4.51	128.33±3.51	142.33±2.52	153.00±3.61
Luangmual	7.33±1.15	13.33±1.15	23.67±3.51	26.67±2.08	37.33±4.16	54.67±7.02	56.67±4.04	74.33±4.93	75.43±4.56	82.67±1.53	85.67±4.51	88.67±3.51
Lungdai	8.00±0.00	13.33±1.15	17.67±3.21	22.67±1.15	43.33±3.06	50.00±2.00	54.33±5.13	59.00±3.61	67.00±2.00	74. 67±2.52	86.33±2.52	91.67±2.52
Serkhan	6.67±2.31	10.67±1.15	15.33±3.06	21.33±2.31	50.00±4.58	54.00±8.54	60.67±2.52	68.33±3.51	74.67±3.06	85.67±1.53	91.00±6.24	99.67±3.79
Zonuam	7.33±1.15	13.33±2.31	20.67±3.79	22.67±1.15	42.00±5.29	50.67±4.16	54.00±3.61	65.11±3.47	74.33±2.52	86.00±1.00	93.63±1.70	99.33±1.53
Chawlhhmun	7.33±1.15	13.33±2.31	24.00±3.46	26.00±3.46	46.67±4.62	55.33±8.08	56.00±3.46	62.67±4.16	73.67±2.52	84.67±2.52	97.67±2.52	100.33±3.21
Tanhril	7.33±1.15	12.67±1.15	27.67±2.08	40.67±3.06	52.67±5.03	54.67±3.06	55.33±4.62	66.90±1.80	72.00±1.00	84.67±2.52	91.00±3.46	106.00±2.65
Sakawrtuichhun	8.00±0.00	14.00±0.00	28.67±2.89	32.00±1.73	58.67±3.06	62.67±5.03	64.33±5.03	68.35±4.04	75.22±1.34	80.67±1.53	91.11±0.84	97.33±2.08
Lengpui	7.33±1.15	12.67±1.15	17.33±3.06	24.00±2.00	45.33±4.16	63.67±1.15	64.33±3.21	66.17±1.04	68.67±6.51	74.67±2.52	86.33±3.51	94.33±3.51
S. Ed (±)	**-**	**1.18**	**2.54**	**1.86**	**3.52**	**5.05**	**3.24**	**3.05**	**3.36**	**1.36**	**2.65**	**2.57**
$CD_{0.05}$	**NS**	**2.49**	**5.34**	**3.91**	**7.40**	**10.62**	**6.80**	**6.41**	**7.06**	**2.86**	**5.56**	**5.41**

4.9.3 Number of leaves/plant

The data pertaining to number of leaves/plants are presented in Table 4.24. Analysis of variance revealed significant difference in number of leaves among different seedlings.

At 30 day after planting, there was no significant differences among the germplasms with respect to number of leaves. However, Lungdai and Sakawrtuichhun (8.00±0.00) and Serkhan (6.67±2.31) recorded the highest and lowest number of leaves respectively.

Among the germplasm, Reiek (14.00±2.00) and Sakawrtuichhun (14.00±0.00) showed the significantly highest number of leaves/plant at 60 days after planting,. The lowest number of leaves/plant at this stage was found in Serkhan (10.67±1.15) which was significantly lower than most of the germplasms

The highest number of leaves/plant at 90 days after planting was observed in Tanhril (27.67±2.08), which was significantly higher than other germplasm. while, the lowest was obtained in Serkhan (15.33±3.06).

Among the germplasms, Tanhril (40.67±3.06) showed the highest number of leaves/plant after 120 days, which was significantly higher than rest of the germplasms. However, the lowest number of leaves/plant was obtained in Serkhan (21.33±2.31) which was significantly lower than most of the germplasms.

At 150 days after planting, the highest number of leaves/plant was obtained in Reiek (62.00±2.00) which was significantly higher than rest of the germplasm. But, the

lowest number of leaves/plant was found in Luangmual (37.33±4.16) which was significantly lower than most of the germplasms except Zonuan (42.00±5.29) and Lungdai (43.33±3.06).

The highest number of leaves/plant after 180 days was obtained in Reiek (108.00±5.29) which was significantly higher than rest of the germplasms. It was followed by Durtlang (76.33±8.50). However, the significantly lowest number of leaves/plant was observed in Lungdai (50.00±2.00).

At 210 days after planting, the highest number of leaves/plant was obtained in Reiek (112.00±3.46) which was significantly higher than rest of the germplasm, whereas, the significantly lowest number of leaves/plant was obtained in Zonuam (54.00±3.61).

Reiek recorded the highest number of leaves/plant (114.00±5.29) at 240 days after planting, which was significantly higher than other germplasms. It was followed by Durtlang (90.37±0.32). The significantly lowest number of leaves/plant was obtained in Lungdai (59.00±3.61).

The highest number of leaves/plant at 270 days was also observed in Reiek (124.67±4.51) which was significantly higher than rest of the germplasm, while, Lungdai (67.00±2.00) recorded the significantly lowest number of leaves.

The highest number of leaves/plant after 300 days was found in Reiek (128.33±3.51) which was significantly higher than all other germplasms, but, found statistically *at par* with Zonuam(86.00±1.00) and Serkhan (85.67±1.53**)** lowest was obtained in Lungdai and Lengpui (74. 67±2.52).

Among the germplasm, Reiek recorded the highest number of leaves/plant at 330 days after planting (142.33±2.52) which was significantly higher than rest of the germplasms. The lowest number of leaves/ plant at this stage was obtained in Luangmual (85.67±4.51), which was significantly lower than most of the germplasms.

At 360 days after planting, the highest number of leaves/plant was obtained in Reiek (153.00±3.61) which was significantly higher than rest of the germplasm, whereas, the significantly lowest number of leaves/plant was obtained in Luangmual (88.67±3.51).

4.9.4. Morphological characteristics of the seedlings

Table 4.25 revealed that there was significant variation among the species with respect to biomass production, root length (cm), Root/shoot length ratio, Root fresh weight ratio (g) Shoot fresh weight (g), Leaf fresh weight (g), root dry weight, shoot dry weight and Root shoot dry weight ratio.

4.9.4.1 Biomass Production (g)

Among the germplasms, the maximum biomass production was recorded in Reiek (8074.01±419.04 g) which was significantly higher than rest of the germplasms except Lengpui (8042.77±778.68 g), Durtlang (8014.09±11.36 g) and Tanhril (7429.32±861.66 g) with which it was found statistically at par. Among all the germplasms, the lowest biomas production was recorded in Chawlhhmun (6301.93±113.67 g) which was significantly lower than rest of the germplasms except Zonuam (6311.19±50.68 g) and Lungdai (6412.05±254.76 g) with which it was found statistically *at par*.

4.9.4.2 Root Length (cm)

Observations pertaining to root length of the seedlings were found to be varied significantly as shown in Table 4.25. Among all the germplasms, the maximum root length was obtained in Reiek (89.47±5.25 cm) which was significantly higher than other germplasms except Chawlhhmun (79.24±8.06 cm) and Serkhan (79.15±6.13 cm). The lowest root length was found in Durtlang (65.57±6.10 cm) which was significantly lower than most of the germplasms.

4.9.4.3 Root /Shoot length ratio

The data on root /shoot length ratio of the plants are furnished in Table 4.25. Comparison of data on this parameter revealed that the root /shoot length ratio of the plants varied significantly with different germplasms. The highest root/shoot length ratio was recorded in Reiek (0.39±0.04) which was significantly higher than rest of the germplasms. It was followed by Chawlhhmun (0.32±0.02). Among all the germplasms, the lowest root /shoot length ratio was recorded in Durtlang (0.26±0.03).

4.9.4.4 Root fresh weight (g)

The difference among the different germplasms in root fresh weight was also found significant (Table 4.25). The maximum root fresh weight was obtained in Reiek (2686.67±132.88 g) which was significantly higher than most of the germplasms. It was followed by Lungdai (2678.33±112.76 g). The lowest root fresh weight was recorded in Tanhril (2285.00±90.97 g) which was significantly lower than other germplasms.

Table 4.25: Morphological characteristics of the seedlings *of Clerodendrum colebrookianum* Walp. collected from various locations

Germplasm	**Biomass Production (g)**	**Root length (cm)**	**Root/Shoot length ratio**	**Root fresh Weight (g)**	**Shoot fresh weight (g)**	**Leaf Fresh weight (g)**	**Root dry wt (g)**	**Shoot dry wt (g)**	**Root/Shoot dry weight ratio**
Durtlang	8014.09±11.36	65.57±6.10	0.26±0.03	2496.67±138.68	2861.76±61.63	2465.67±81.98	2090.07±62.75	2009.56±73.72	1.04±0.02
Reiek	8074.01±419.04	89.47±5.25	0.39±0.04	2686.67±132.88	3080.68±27.76	2496.67±80.31	2220.01±43.60	2228.48±83.42	1.00±0.03
Luangmual	7224.54±276.60	71.63±8.92	0.26±0.05	2568.33±161.97	2700.87±70.83	1955.33±38.03	2361.67±40.92	1848.67±89.15	1.28±0.08
Lungdai	6412.05±254.76	76.30±8.98	0.27±0.03	2678.33±112.76	1192.71±91.63	2541.00±80.17	2535.02±47.68	473.85±80.70	5.44±0.76
Serkhan	7299.72±357.56	79.15±6.13	0.30±0.03	2633.33±118.28	2023.72±112.49	2642.67±93.84	2526.67±28.10	1278.88±99.98	1.98±0.16
Zonuam	6311.19±50.68	71.98±4.88	0.30±0.02	2530.00±162.25	1495.53±113.82	2285.67±68.06	2260.67±83.51	776.36±79.51	2.93±0.22
Chawlhhmun	6301.93±113.67	79.24±8.06	0.32±0.02	2436.67±179.26	1013.27±59.46	2852.00±54.62	2086.67±80.83	790.00±62.89	2.65±0.15
Tanhril	7429.32±861.66	69.34±6.67	0.28±0.02	2285.00±90.97	2558.65±103.88	2585.67±47.17	2018.33±38.19	1706.45±31.77	1.18±0.04
Sakawrtuichhun	7156.39±621.37	71.65±7.50	0.30±0.05	2405.00±104.31	2394.05±132.35	2357.33±24.21	2185.03±30.96	1541.85±19.86	1.42±0.03
Lengpui	8042.77±778.68	66.75±4.56	0.31±0.02	2323.33±168.55	3201.11±129.86	2518.33±82.51	1740.01±65.01	2348.91±41.39	0.74±0.02
S. Ed (±)	**396.23**	**5.14**	**0.02**	**117.61**	**68.23**	**55.54**	**46.46**	**60.00**	**0.22**
$CD_{0.05}$	**832.49**	**10.81**	**0.05**	**247.11**	**143.36**	**116.69**	**97.60**	**126.07**	**0.47**

4.9.4.5 Shoot fresh weight (g)

A significant influence of the germplasms on shoot fresh weight of the plants was observed (Table 4.25). The germplasm obtained from Lengpui showed the highest shoot fresh weight (3201.11±129.86 g) which was significantly higher among all other germplasms except Reiek (3080.68±27.76 g) with which it was found statistically *at par*. The lowest shoot fresh weight was obtained in Chawlhhmun (1013.27±59.46 g) which was found significantly lower than all other germplasms.

4.9.4.6 Leaf fresh weight

The estimates for leaf fresh weight are presented in Table 4.25. The data presented in the Table revealed that maximum leaf fresh weight was obtained in Chawlhhmun (2852.00±54.62 g) which was significantly higher than all other germplasms, whereas, the significantly lowest leaf fresh weight was obtained in Luangmual (1955.33±38.03 g).

4.9.4.7 Root dry weight

The data presented in Table 4.25 reveals that there was significant difference in root dry weight among the different germplasms. Among all the germplasms, the maximum root dry weight was observed in Lungdai (2535.02±47.68 g) which was statistically *at par* with Serkhan (2526.67±28.10 g). While, the lowest root dry weight was observed in Lengpui (1740.01±65.01 g**)** which was significantly lower than the rest of the germplams.

4.9.4.8 Shoot dry weight

It is evident from the data presented in Table 4.25 that the shoot dry weight of the plants was influenced by different germplasms. Among all the germplasms, the highest shoot dry weight was obtained in Lengpuii (2348.91±41.39 g) which was significantly higher than all other germplasms except Reiek (2228.48±83.42 g). The lowest shoot dry weight was recorded in Lungdai (473.85±80.70 g) which was significantly lower than all other germplasms.

4.9.4.9 Root/shoot dry weight ratio

A highly significant difference in root shoot dry weight ratio was observed among the germplasms (Table 4.25). Lungdai recorded the maximum root shoot dry weight ratio (5.44±0.76) which was significantly higher than other germplasms. While, the lowest was obtained in Lengpui (0.74±0.02) which was significantly lower than all other germplasms except Reiek (1.00±0.03), Durtlang (1.04±0.02) and Tanhril (1.18±0.04).

Discussion

5.1 Phytosociology

The description and classification of the plant community in an ecosystem is known as phytosociology (Odum, 1971). Phytosociological analysis of a plant community is the first and foremost basis of the ecological study of any piece of vegetation and this study is important to understand the functioning of any community. The number of species reflects the gene pool and adaptation potential of the community (Odum, 1963). Quantitative analysis of vegetation helps in understanding the structure, composition and tropic organization of any community. Species composition and diversity vary from habitat to habitat within the communities exposing identical physiognomic characteristics (Nautiyal *et al*., 1999). Likewise, the life forms of species represent the adjustment of perennating organs and plant life history to environmental conditions (Nautiyal *et al*., 2001). It is an important characteristic in describing vegetation that offers a preliminary picture of the ecological character of the vegetation (Kershaw, 1973).

There was variation in percentage frequency in the range of 40 – 80 per cent among the different locations. The maximum frequency (80%) was obtained in Luangmual, which was followed by Durtlang and Chawlhhmun (70%) respectively whereas, the lowest was obtained in the germplasm of Lengpui (40%). For a particular species, higher frequency indicates its more frequent distribution at sites due to optimum soil and environmental conditions. Our study is in the line of the

findings of the Nautiyal *et al.* (2002) where they also obtained variation in per cent frequency among a number of individuals.

Similarly, among the different germplasms in our study, the highest abundance was obtained in Sakawrtuichhun (15.40), and the lowest was obtained in Lungdai (7.5). Similarly, the maximum density of *Clerodendrum colebrookianum* Walp. was obtained in the location Sakawrtuichhun (308.00 plants/ha) and the lowest was obtained in Serkhan village (152.00 plants/ha). Nautiyal *et al.* (2003b) also obtained variation in density among a number of individuals of alpine medicinal herbs.

There was variation with respect to average basal cover and Total Basal Cover (TBC) among the studied populations of *C. colebrookianum*. Among all the locations, the highest was reported in Luangmual (200.36 $cm^{2}m^{-2}$), while, the lowest was in Zonuam (47.88 $cm^{2}m^{-2}$). Nautiyal *et al.* (2002) and Nautiyal *et al.* (2003) also observed variation in TBC among a number of individuals in six alpine regions in Garhwal Himalaya.

The relative frequency, density and relative dominance of the germplasms varied among the different locations. Among the studied locations, the highest relative frequency was obtained in Luangmual (13.33%), while, the lowest was observed in Lengpui (6.66). Similarly, the maximum relative density was found in Sakawrtuichhun (13.48) and the lowest was in Serkhan (6.65). The highest relative dominance was obtained in Luangmual (20.44), and the lowest was observed in Zonuam (4.88).

Analysis of IVI provides information about the social status of a species and can be recognized as patterns of association of dominant species in a community (Parthasarathy and Karthikeyan 1997). Analysis of IVI in a management practices represented different combinations of species with different dominants and co-dominants. These includes *Achyranthes aspera L. Acmella oleraceae/Spilanthus acmella, Adhatoda vesica Mill, Adenostemma lavenia, Ageratum conizoids, Artemisia vulgaris, Bidens pilosa, Blumea lanceolaria, Cajanus cajans, Centela asiatica, Colocasia spp , Cuscuta reflexa Roxb, Eupatorium odoratum, Imperata cylindrical, Manihot esculenta, Mikania micrantha, Mimosa pudica L., Oroxylum indicum, Pteridium acquilinum, Solanum indicum L., Solanum torvum, Thysanolaina maxima, Trevesia palmate.* Supporting the findings of Mandal and Joshi (2014), the present findings in the current study suggest that vegetation experiencing stresses from biotic pressure are under serious threat. However, some of the species have managed to survive. This could be attributable to their broad ecological amplitude and greater adaptability against biotic influences.

Considering that the IVI provides an excellent marker for determining the status of distribution and availability across varying environmental and biotic conditions (Negi *et al*. 1992), value of IVI of *C. colebrookianum* were compared. Values varied from one population to other. The Importance Value Index (IVI) among the different geographical locations varied from 21.06 - 46.21. Among the studied locations, the highest IVI was observed in Luangmual (46.21), while, the lowest was observed in Lengpui (21.06*)*. This difference can be attributed to varying species number, topography, biotic and abiotic interferences in community (Nautiyal 1996). The higher value of IVI indicates that all the available resources are being utilised by

that species and left over are being trapped by another species as the competitors and associates. Lower importance value of species is an index of low grazing pressure by herbivores on the study sites, as vegetation is a reflex of interactions between the plants, animals, soils and climate. Moreover, each species of a community plays specific role and there is a definite quantitative relationship between abundant and rare species (Bhandari *et al*., 1999). The high IVI of a species indicated its dominance and ecological success, its good power of regeneration and greater ecological amplitude.

Abundance and frequency (A/F) ratio reveals that regular distribution of the species was totally absent and most of the species were contagiously distributed in all sites during all seasons. The highest A/F ratio was observed in Lengpui (0.31), while, the lowest was in Luangmual (0.11). Among all the locations, the distribution pattern of the species was found contagious. According to Odum (1971), contiguous distribution is the most common pattern in nature and is formed as a result of small but significant variations in the ambient environmental conditions. The current study also suggests that clumped distribution often occurs due to an uneven distribution of nutrients or other resources in the environment. Variations in the distribution pattern among sites and vegetation composition are associated with micro environmental and biotic factors (Singhal and Soni 1989). Patterns of distribution depend both on the physicochemical nature of the environment and the biological peculiarities of the organisms (Odum, 1971). The dominance of contagious distribution may also be due to the fact that the majority of herb species reproduce vegetatively in addition to their sexuality. However, observations indicated that contagious distribution in vegetation was due to multitude of factors and the vegetative reproduction may not be the only

reason (Kershaw, 1973; Saxena and Singh, 1982). The contagious distribution pattern was also reported by a number of ecosystems (Joshi and Tiwari, 1990; Bhandari *et al*.,1995; Pande *et al*., 1996; Bhandari *et al*., 1997; Kunhikannan *et al*., 1998).

5.2 Threatened categories

5.2.1 Threat identified

The following threats have been identified with respect to this highly medicinal species:

- **No wild, few plants cultivated**: There was no plant found in the wild state in the forests of Mizoram. Mostly the plants are found in the semi wild state as well as cultivated state.
- **Kitchen garden crop**: The plant is commonly grown as kitchen garden crop in the homested gardens and jhum land.
- **Habitat destruction:** Since last few years there was destruction of the habit of *Clerodendrum colebrookianum* Walp. in the natural populations.
- **Low seed viability and germination**: The seeds of *Clerodendrum colebrookianum* Walp. lost the viability very soon. If they are not sown within a specified time, they fail to germinate. The percentage of germination is also very poor.
- **Slow growth rate**: The initial growth rate of *Clerodendrum colebrookianum* Walp. is very slow.
- **Over exploitation:** The rural people harvest the leaves, seeds as well as stems to sell in the market without any measures for its conservation**.**

5.2.2 Threat status

According to the Red List Categories of IUCN (1993), extents of occurrence and population estimation are the major criteria used to assign threat categories besides population reduction and probability of extinction. On the basis of the extent of occurrence status of *C. colebrookianum*,, as per the IUCN, (1993) red list categories, it is critically endangered to endangered species on the basis of no of mature individuals, since in the present study the number of matured individual was observed between 152-308. In addition, on the basis of area of occurrence and occupancy the species have been identified as endangered to vulnerable. Consequently, quantitative data on population of these species would be helpful in the determination of their status by examining occurrence, population density of species, total basal area and their dominance (IVI) in these communities. These observations would also be helpful in determining the status of other species and can be applied for conservation strategies. In addition, immediate measures and policies are needed to initiate for conservation of this valuable medicinal plant and to prevent them from further extinction in Mizoram, north-east India.

5.3 Germplasm Variability

5.3.1 Morphological features

The north-east region harbors several medicinal plants and more importantly, they synthesize secondary metabolites of medicinal importance and therefore, offer greater possibilities of having novel bimolecules and even larger quantity of active components . In different climatic and ecological conditions, medicinal plants possess special morphological, physiological and adaptational features. These plants adopt

several adaptive strategies to cope with different soil and climatic conditions and it is considered that flowering (Kuniyal *et al*., 2003) and fruit characteristics are associated with different environmental conditions and genotype of a species (Singh *et al*., 1999). Furthermore, seed production of a species may indicate a compromise between ecological and physiological parameters (Siemens and Johnson, 1995; Kuniyal *et al*., 2003). As a result, variation in morphology, physiological and biochemical characters appear in the plants from different locations. Variations among the populations of a number of species have been reported (Kuniyal *et al*., 2003; Nautiyal *et al*., 2003; Vashistha *et al*., 2006) and therefore, efforts are desirable to explore these variations at morphological, and biochemical level to explore the level of genetic diversity and more importantly, to evaluate these natural populations for the selection of superior and elite germplasm for domestication and cultivation particularly of important medicinal herbs including *Clerodendrum colebrookianum*.

In the present investigation, significant variation was observed among the different populations with respect to morphological parameters, viz. plant height, collar diameter, number of leaves, root length, fresh weight of leaves, fresh weight of shoot, fresh weight of leaves, dry weight of root, dry weight of shoot, root/shoot dry weight ratio, and biomass production. In general, the variation in morphological characters among a number of accessions collected from different locations might be due to variation in genetic make-up of the plants. In the present investigation, the maximum morphological characters with respect to the plants of Sakawrtuichhun might be due to better microhabitats present in that particular location. Purohit *et al*. (1999) reported considerable variation in leaf morphology in different populations of *Podophyllum hexandrum* in Garhwal Himalaya. Variations in the length and width

of leaves in the plants are supposed to be sensitive to the varying environmental conditions (Lynn and Waldren, 2001). In addition, available soil nutrient level also plays important role in determining morphological variations in plants (Pigliucci *et al*., 1997; Kuniyal *et al*., 2002), climatic factors (Krishnan *et al*., 2000) and can be considered as indicator to alter assimilate investment pattern (Korner *et al*., 1989). Our study is in close conformity with the findings of Purohit *et al*. (2008) where they also obtained morphological variation among the natural populations of *Picrorhiza kurrooa*.

5.3.2 Flowering and fruiting characteristics

Phenology is the art of observing life cycle or activities of plants in their temporal occurance throughout the growing season. Phenological and phenomonological variations of the plants are the product of interactions between genotype and environment. However, these modifications in plants may be reversible when plants grown under diverse agro-climatic conditions (Bhatt and Purohit, 1984).

Flowering and fruiting are most critical stages of life of a plant species. Among important parameters, study of phonological behaviour of any wild plant species, which is being targeted for cultivation, is a pre-requisite exercise. It is helpful in developing and standardizing agro-techniques of targeted species. Knowledge of reproductive biology is essential for the conservation, management, and recovery of threatened species (Kuniyal *et al*., 2003; Murugan *et al.*, 2006) and to improve desired traits or to develop new varieties. A clear understanding of phenological behavior on time of anthesis, time and duration of stigma receptivity, fertilization, mode of pollination, seed development is necessary for breeding programmes to obtain better

traits (Rout *et al*., 2009). Assessment of phonological behavior of any wild plant species that is being targeted for cultivation is a pre-requisite step. It provides information about morphological and functional attributes that are useful to understand adaptation features (Nautiyal and Purohit, 2000) and determines the growth and developmental pattern of a plant. Phenological observations have been made for developing management and conservation of plants for valuable plant species (Sanz-Cortes *et al*., 2002; Hamann, 2004).

In the present study, the *C. colebrookianum* plants grown in different locations showed marked variation with respect to commencement and completion of different phenophases. Among the different locations, the maximum days required from bud initiation to bud differentiation was recorded in Zonuam (12.00±1.00*)* and minimum was recorded in Reiek (9.67±0.58). The germplasm at Durtlang required maximum days (43.00±1.00) from initiation of bud to flowering, whereas, the germplasm in Reiek required the lowest days (39.33±0.58) for flowering. Significant differences were observed among the different locations with respect to the number of flowers/inflorescence. The maximum number of flowers/inflorescence was observed in Lengpui (116.67±5.51) and the minimum number of flowers/inflorescence was observed in Tanhril (96.33±7.57). Among all the locations, the maximum length of inflorescence was observed in Reiek (24.10±1.82 cm) whereas, the lowest was in Lungdai (15.03±1.05 cm). Similarly, the highest fruit setting percentage was observed in Sakawrtuichhun (98.90±3.10 %), whereas, the lowest was in Chawlhhmun (87.03±3.23%). The highest number of fruits/inflorescence was found in Reiek (42.67±4.73) while, the lowest was in Tanhril (18.33±6.11). Among all the locations, Serkhan (49.67±0.58) required the maximum days for fruit maturation, while Reiek

recorded the minimum days (45.67±1.53) from setting of fruits to maturation. Among the different locations, the highest fruit retention percentage was observed in Reiek (45.67±1.53) and the lowest was in Tanhril (28.67±3.51).

Phenological behavior of plant species provides information about morphological and functional attributes, which are useful to understand adaptation features (Nautiyal *et al.,* 2001). In the present investigation, wide variation have been observed with respect to different phonological attributes *viz.* days required from bud initiation to bud differentiation, days from initiation of bud to flowering, number of flowers/inflorescence, length of inflorescence, fruit setting percentage, number of fruits/inflorescence, days for fruit maturation, fruit retention percentage. Such variation attributed due to genetic effect of the tree and varied environmental conditions (temperature, humidity, rainfall, light, etc.) among the different locations. Along altitudinal difference, the temperature is a major factor which is the main determinant of phenological plant development (Worral, 1993). Phenological and phenomenological variations of the plants are the product of interaction between genotype and environment. However, these modifications in plants may be reversible when plants are grown under diverse climatic conditions (Bhatt and Purohit, 1984). Similar variation in flowering and fruiting behaviour was also reported in different crops by different researchers (Natarajan and Srimathi, 2008, Bentos *et al*., 2008, Piechowski and Gottsberger, 2009, Adjaloo *et al*., 2012 and Kukade and Tidke, 2013).

5.3.3 Variability, Heritability and Genetic advance as percent of mean

Significant variations were observed among different accessions regarding various plant morphological characters. Variability was estimated in terms of coefficients of variability in relation to phenotype, genotype and environment. Genetic parameters were worked out with regard to estimates of heritability (broad sense), genetic advance and genetic gain. The genetic parameters are useful tools for predicting amount of gains to be expected from an improvement programme. The variation among the sources is commonly used as an estimate of total genetic variation and to calculate the degree of genetic control for a particular trait. Genetic variability such as heritability and genetic gain for particular properties are important tools for predicting genetic gains (Foster and Shaw, 1988). High magnitude of variability in a population provides opportunities for selection of promising individuals to evolve a variety with desirable characters. Burton (1952) suggested that the study of GCV together with heritability estimates could give appropriate estimates of genetic gain expected from a selection programme. Genetic diversity is the variability among different genotypes of species and it arises either due to geographical separation or due to genetic barriers to cross ability (Hegde and Varghese, 2008).

The estimates of phenotypic and genotypic coefficients of variability gave a clear picture of amount of variations present in the available germplasm. The estimates of phenotypic variance were higher than the corresponding estimates of genotypic variance for all the traits, indicating thereby, the influence of environment in the expression of these traits. Similar finding was also reported by Godbharle *et al*., (2010) in sorghum. Since these estimates solely do not provide means to assess the

nature of genetic variability, phenotypic and genotypic coefficient of variation were also estimated. Although estimates of PCV were higher than that of GCV, they were close to one another implying that the influence of environment on the expression of these traits were negligible hence selection based on phenotypic value is feasible**.** Coefficients of variability varied in magnitude from character to character (either low or moderate or high). Therefore, it indicated that there was a great diversity in the experimental material used.

Heritability has an important place in plant improvement programmes (Dorman, 1976) as it provides an index of the relative strength of heredity versus environments. It is also useful for ranking importance of each trait in cross-breeding programmes (Kumar *et al*., 2007). Johnson *et al*. (1955) reported that heritability estimated along with expected genetic gain was more useful and realistic than the heritability alone in predicting the resultant effect for the best genotypes.

Johnson *et al*. (1955) explained that provenance heritability didn't indicate the amount of genetic improvement that can be achieved through provenance selection. Therefore it has been suggested that high heritability coupled with high genetic gain was the true index for effective selection (Johnson *et al*., 1955 and Swarup and Chaugale, 1962). High provenance heritability was not associated with high genetic advance in most of the cases which was very much in agreement with the observations of Swarup and Chaugale (1962). The provenance heritability estimated for any character was useful when high selection gain in that character was also feasible (Kaul and Bahu, 1974). Similarly, narrow-sense heritabilities for growth traits and basic density appeared to decrease over generations.

Moderate heritability in conjunction with high genetic advance as percent of mean was observed for this trait which indicates the role of both additive and non-additive gene action governing the inheritance of this trait and offers the best possibility of improvement through progeny selection or any modified selection procedures aiming to exploit the additive gene effects. These results are in accordance with the findings of Rahman *et al*. (2002) in snake gourd.

In the present investigation, moderate heritability in conjunction with high genetic advance as percent of mean was obtained in plant height, no. of leaves, fresh weight of leaves, shoot fresh weight, shoot dry weight, root length, fresh weight of root, dry weight of root, indicates the preponderance of both additive and non-additive gene action governing the inheritance of this character.

Similarly, the high PCV with high GCV was obtained for number of leaves was in accordance with the findings of Bhagasara *et al*., (2017) in sorghum. Moderate heritability coupled with high genetic advance as percent of mean was observed for this trait which indicates the preponderance of additive gene action governing the inheritance of this character and offers the best possibility of improvement through simple selection procedures.

The high PCV and GCV for fresh weight of leaves and dry weight of leaves was in accordance with the experimental findings by Nandanwar *et al.* (2017) in *Desmodium gangeticum.* Moderate heritability coupled with high genetic advance as percent of mean was observed for these traits indicates the preponderance of additive gene action governing the inheritance of this character and offers the best possibility of improvement through simple selection procedures.

The PCV and GCV were high for fresh weight of roots indicating that variation among the genotypes was also high and better scope for the improvement of these characters through selection. Moderate heritability coupled with high genetic advance as per cent of mean was observed for this trait which indicates the preponderance of additive gene action governing the inheritance of this character and offers the best possibility of improvement through simple selection procedures. These results are in agreement with the findings of Srivastava *et al*. (2017) in Indian Ginseng.

The PCV and GCV were high for root dry weight indicating that variation among the genotypes was also high and better scope for the improvement of these characters through selection. Moderate heritability coupled with high genetic advance as percent of mean was observed for this trait. These findings are in agreement with Singh *et al.* (2017) in Ashwagandha.

The estimates of PCV and GCV were high for biomass production. Our finding is in close conformity with the findings of Sangwan *et al*., (2013) in ashwagandha. Similarly, high estimates of PCV and GCV recorded for fresh weight of shoot and dry weight of shoot indicates presence of high degree of genetic variability and thus a greater scope for selection. The present results were in accordance with the findings of Patil (2001) in fenugreek. High PCV and GCV recorded for root: shoot length ratio and root: shoot dry weight ratio also obtained in the present investigation. Our study is in close conformity with the findings of Teli (2016) in carrot and Kumari and Wani, (2017) in *Diospyros melanoxylon*. Moderate heritability coupled with high genetic advance as percent of mean was observed indicating the preponderance of additive gene action governing the inheritance of this

character and offers the best possibility of improvement through simple selection procedures.

In the present study, wide variability was recorded in number of leaves, fresh weight of leaves, fresh weight of root, dry weight of root, shoot dry weight, shoot fresh weight, biomass production, root length, root/shoot dry weight ratio and root shoot length ratio indicating the existence of more variability for these traits in the genotypes under study as they have high PCV and high GCV. Moderate variability was recorded for plant height and collar diameter.

High heritability coupled with high genetic advance as percent of mean indicates operation of additive gene action as in case of number of leaves, fresh weight of leaves, fresh weight of root, dry weight of root, shoot dry weight, shoot fresh weight, biomass production, root length, root/shoot dry weight ratio, root shoot length ratio. Hence, directional selection for these traits in genetically diverse material could be effective for desired genetic improvement. High genetic advance as percent of mean with moderate heritability indicates action of both additive and non-additive genes as in case of plant height, no. of leaves, fresh weight of leaves, shoot fresh weight, shoot dry weight, root length, fresh weight of root, dry weight of root, which offers the best possibility of improvement through mass selection and progeny selection.

Hence, the breeder should adopt suitable breeding methodology to utilize both additive and non-additive gene effects simultaneously, since varietal and hybrid development will go a long way in the breeding programmes especially in case of *C. colebrookianum*.

5.3.4 Correlation studies

The correlation is one of the most important tools which measures the degree and magnitude of association between various characteristics in tree improvement programme. A clear understanding of association among different traits is of great importance as it shows whether the choice of one character confirms the appearance or disappearance to the other thus it is useful indirect selection.

For plant breeders, knowledge of correlation is of paramount significance since all the biological attributes are the interplay of several genetic factors among themselves and their individual and combined interactions with the environmental factors. The knowledge of correlation, supplies information about how important is a particular character, which is not amenable to direct selection, can be made through indirect selection. It also provides information about the correlated response to directional selection to predict genetic advance and thus, can be used as selection indices for operating more efficient selection programmes. Correlation could be phenotypic, genotypic or environmental. Phenotypic correlation is between values directly measured on individual and includes genetic and non-genetic effects. Genotypic correlation is between breeding values and amounts for only genetic causes, which could be pleiotrophy, linkage or gene frequency disequilibrium. Environmental correlation is between non- genetic values and arises due to the fact that several observations are affected by the same amount of environment. Therefore, knowledge of the correlations is of great significance.

The genetic correlation coefficients between different characters in seed sources and progenies are generally similar in magnitude and nature, to corresponding

phenotypic correlation coefficient in the present investigation. However, in general, genotypic correlations were higher in magnitude than the corresponding phenotypic correlation coefficient which is similar to the finding of Jat (1993) and Bhagat and Jadeja (2003) reported in *C. borivilianum*.

In the present investigation, the correlation coefficients among the different characters were worked out at phenotypic and genotypic levels. The phenotypic and genotypic correlation coefficients among different characters showed that the plant height had significant positive association with number of leaves, fresh weight of leaves, collar diameter, root length, fresh weight of roots, dry weight of roots, shoot fresh weight, shoot dry weight, root: shoot length ratio, root: shoot dry weight ratio and biomass production.

The estimates of Pearson's correlation coefficients among the different traits indicated the plant height is significant and positively correlated with number of leaves, fresh weight of leaves, collar diameter, root length, fresh weight of root, dry weight of root, shoot fresh weight, shoot dry weight, and biomass production. Plant height is negatively correlated with root: shoot length ratio and root: shoot dry weight ratio.

In the present investigation, plant height had found significant and positive correlation with leaf number, fresh weight of leaves, collar diameter, root length, fresh weight of leaves, root dry weight, shoot fresh weight, shoot dry weight, root:shoot length ratio, root:shoot dry weight ratio and biomass production. Rameshkumar (2013) also reported positive significant association of plant height was observed with dry weight of root, root length, and collar diameter at both the genotypic and

phenotypic levels. Positive and significant association of plant height with root length, collar diameter and root fresh weight were obtained by Kandalkar *et al*., (1993). Positive and highly significant association of plant height with dry root yield was also reported by Kubsad *et al*., (2009).

Plant height was positive and significantly correlated with number of leaves and similar result was obtained by Ziblim *et al.* (2015). Plant height also had strong positive association with shoot dry weight. Our study is in close conformity with the findings of Shabnam and Iqbal, (2016) in wheat.

In the present investigation, strong and positive phenotypic correlation was obtained for plant height and biomass production. Charles *et al.* (2002) observed in an experiment that the stalk dry matter yield of kenaf increased with increase in plant height. Ejieji and Adeniran (2010) also observed that stem dry matter of grain Amaranth (*Amaranthus cruentus*) increased with the increase in plant height.

In our investigation, collar diameter was found positively and significantly correlated with plant height, number of leaves, fresh weight of leaves, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight and biomass production. Rameshkumar (2013) reported collar diameter was positively and significantly correlated with plant height, leaf length, leaf width and dry weight of root at both genotypic and phenotypic levels. Kandalkar *et al*., (1993) obtained positive correlation of collar diameter with plant height, root length and root weight at genotypic level only, while positive and significant correlation of same were obtained at phenotypic level. Kubsad *et al*., (2009) obtained positive correlation of collar

diameter with dry root weight, while positive and significant correlation with plant height. Dubey (2010) reported positive correlation of root diameter with plant height and dry root yield. Ramesh Kumar *et al*., (2011) obtained positive and significant correlation of root diameter with dry root weight per plant.

In our study, positive and significant phenotypic correlation of root length was observed with plant height, number of leaves, fresh weight of leaves, collar diameter, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight and biomass production. Rameshkumar (2013) also obtained positive and significant correlation of root length with plant height, and root dry weight of root at both genotypic and phenotypic levels Kandalkar *et al*., (1993) obtained positive correlation of root length with plant height, root diameter and fresh weight of root at genotypic level only, while positive and significant correlation of same were obtained at phenotypic level. Kubsad *et al*., (2009) obtained positive correlation of root length with plant height, root diameter and dry root weight. Dubey (2010) reported positive correlation of root length with plant height and root diameter. Rameshkumar *et al*., (2011) obtained positive and significant correlation of root length with dry root weight per plant. Sangwan *et al*., (2013) obtained positive and significant correlation of root length with collar diameter.

In our study, the root length was positively correlated with biomass production. This is in conformity with the findings of Nasir and Wani (2014)**.**

Positive and significant phenotypic correlation of dry weight of root was obtained with plant height, number of leaves, fresh weight of leaves, collar diameter, root length, root fresh weight, shoot fresh weight, shoot dry weight, root: shoot length

ratio, root: shoot dry weight ratio and biomass production. Rameshkumar (2013) also obtained positive, significant and strong correlation of this trait with plant height, leaf length, leaf width, days to flower initiation, days to maturity, root length and diameter of root at collar region at both the genotypic and phenotypic levels; while withanoloide content was negatively and significantly correlated with dry weight of root at phenotypic level only. Kandalkar *et al*., (1993) obtained positive correlation of root yield with plant height, root length and collar diameter at genotypic level only, while positive and significant correlation of same were obtained at phenotypic level. Kubsad *et al*., (2009) obtained positive and significant correlation of dry root yield with plant height, while positive correlation with root length and collar diameter. Dubey (2010) reported positive correlation of dry root yield with root length, collar diameter and plant height at both genotypic and phenotypic level. Rameshkumar *et al*., (2011) obtained positive and significant correlation of dry root yield with root length and collar diameter.

Positive and significant phenotypic correlation of shoot fresh weight was obtained with plant height, number of leaves, fresh weight of leaves, collar diameter, root length, root fresh weight, root dry weight, shoot dry weight and biomass production. Our study is in close conformity with the study of Riaz *et al.* (2013) in *Gossypium hirsutum* who also obtained positive and significant correlation of shoot fresh weight with plant height, root length, fresh weight of roots and shoots dry weight.

Traits under selection are often associated with each other in a very complex way. Correlations between characters have been studied to identify those which are easy to measurement for indirect selection for yield. Correlation coefficients, although

it is useful in quantifying the size and direction of trait associations, it can be misleading if the high correlation between two traits is a consequence of the indirect effect of the traits (Dewey & Lu, 1959). Thus, many breeders report to the path coefficient analyses to clarify interrelationships between yield and several other traits. Importance of the information obtained from the correlation coefficient can be enhanced by partitioning it into direct and indirect effects for a set of a prior cause - effect interrelationships (Kang *et al*., 1983).

5.3.5 Regression Coefficient

The value of regression coefficient of biomass with plant height (0.01784), no of leaves (0.01327), fresh weight of leaves (0.00345), Collar diameter (0.09796), fresh weight of root (0.00208), shoot fresh weight (0.00160) and shoot dry weight (0.00333) was highly significant whereas regression coefficient of independent variable biomass production with dependent variables root length (0.02304) and dry weight of root (0.00236) was significant. Regression coefficient of biomass production with shoot length (0.45952) and shoot dry weight (0.21684) was not significant. Similarly, the highest variability among different growth parameters of plant collected from different populations exist in number of leaves (73.4852 %) followed by fresh weight of leaves (69.7654 %), dry weight of root (65.2359), shoot dry weight (61.4629 %) biomass production (59.3984 %), shoot fresh weight (59.2603 %). The multiple regression equation models shows that maximum positive contribution was made by shoot fresh weight (59.25 %) followed by fresh weight of root 45.59 % and highest negative contribution made by dry weight of root (0.16 %). Our study is in close conformity with the findings of Shen *et al*. (2011) who studied the multiple stepwise regression analysis of *Gentiana rigescens*. Their results revealed

that length, width, and number of root, plant height, the first branch number, and the calyx number were the main factors that affected the biomass production. Kim *et al.* (2016) also obtained significant and positive regression coefficient among biomass production and collar diameter. The results of regression analysis of Meng *et al.* (2018) revealed that there was functional equilibrium present between the biomass production and leaf parameters and root-shoot ratio.

5.4 Microscopic Evaluation

Plant anatomy deals with the structure, contents and development of cells and tissues. It describes the physical form and external structure of plants. It is of primary importance for all aspects of research in plant sciences such as morphogenesis, physiology, ecology, taxonomy, evolution, genetics, reproduction etc. (Fahn, 1990). The systematic anatomy is mainly aimed towards relating structure particularly of vegetative organs to taxonomic classification of the plants in which the characters are exemplified. Application of systematic anatomy can also be extended to detection of adulterants and substitutes (Metacalfe and Chalk, 1979). It is now frequently investigated at the cellular level, and often involves the sectioning of tissues and microscopy. Studies on anatomy of plants have much significance in different sectors investigation. Anatomical studies can explain where, what, when and how level chemical compounds deposited, cellular changed, cellular abnormalities are occurred. The anatomical studied can be clarified the qualities of the wood properties. Anatomical studies can be a potential tool of taxonomic studies, mainly where there is no reproductive organ. The anatomical annotations are of importance in the

assessments and appraisals, use of the characters as an effective tool in interpreting phyletic evaluations and systematic delineations (Metcalfe and Chalk, 1950).

Plant anatomy provides a novel perspective on the microscopic structure of plants. Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. Quality control of crude drugs is a challenging task because of complex nature of chemical constituents. To ensure the quality of herbal products proper identification of the plant material is essential (Anon., 1996). Anatomical characters help for identification, when the morphological features are indistinct (Cutler *et al.,* 2008). Anatomical study of medicinal plants is significant in pharmacognosy and to prevent adulteration as well as evolve the specific parameters for authenticity and quality control of raw drugs (Bernerjee and Mukherjee, 2001; Gupta *et al*., 2001).

In recent years, natural compounds of plant based origin have received much attention as they are well tested for their efficacy and believed to be much safer than synthetic drugs. Therefore critical studies on modern approaches, *viz.* physiochemical characterization, biological evaluation, toxicity studies, molecular mechanism of action, is needed for management of various diseases. Anatomical characterization provides additional support towards authentication and elimination of adulteration of market sample of potent medicinal plants as *Clerodendrum colebrookianum.*

Despite of its usage in folk medicine due to its antimicrobial effect, the morphology of *Clerodendrum colebrookianum* is not known entirely. However, this

study might be the first study conducted to determine the anatomical and some microscopic characteristics of aerial parts of *Clerodendrum colebrookianum*.

Stems serve as a sink for several metabolites and as an important source of bioactive compounds. In the present investigation, the microscopic evaluation of transverse section shows prominent epidermal cells with dark inclusions, uniformly thick periderm and which encircle the vascular cylinder. There was a fine outgrowths on plants called as Trichomes. Just beneath the tracheids, a single layer epidermis, compactly arranged square cells are present and acts as a boundary between the plant and the external environment. This is followed by 5-6 layered sclerenchyma cells which are almost circular in shape. Immediately after sclerenchyma cells 3-4 layered parenchymatous cells are present. Below parenchymatous cells, ground tissues are distributed throughout and composed of circular cells.

The leaves *C. colebrookianum* are simple, opposite or rarely whorled. Leaf base is wedge- shaped to heart-shaped, margin entire to slightly wavy, tip pointed. The outer cell wall was covered by Trichomes. It was followed by multiple layers of palisade cells. The palisade cells are round in shape. Just beneath the palisade cell, collenchyma cells are present. The vascular bundles are scattered in the collenchymatous cells around the pith.

Leaves are important organs for photosynthesis and play an important role in survival and growth of a plant. Many previous studies have revealed that variations in leaf traits are the result of adaptations to growth habitats (Pandey and Nagar, 2002).

Quantification and visualization of morphological variations of leaves and other structures are essential for an overview of evolutionary and ecological processes of

phenotypic diversification and are the fundamental basis from which to develop more complex studies to achieve new perspectives on the interaction of phenotype, genotype and environment (Jensen, 2003).

Morphologically and anatomically, the leaf is the most variable plant organ and the difference such as trichome is occasionally specific for species, genera or even families (Bunawan *et al.*, 2011). Information on foliar micromorphology has been reported of capable of shedding more light on plants structural features and their possible functional attributes (Ashafa *et al*., 2008). Several studies have reported the significance of foliar microscopic features used to evaluate taxonomical delimitations of many plants (Yasmin *et al*., 2009). This in effect has helped in correct identification and authentication of many plants hence finding usefulness in standardization of herbal products obtainable from various medicinal plants indigenous to most communities. Anatomical characters of the leaf that have been used in some studies include: epidermal cell type, stomata, trichome and vascular bundle pattern and arrangement (Senthamari, *et al*., 2011). However, no such information is available on the anatomy of *Clerodendrum coleoobrokianum.* The study was therefore undertaken to obtain information on micromorphological features of these two important medicinal plants which would help in their identification and authentication.

Anatomic characters of leaf are used as taxonomic markers to assist in the correct identification of the plant species. Some particular groups of plants or taxa seem to be characterized by specific type of cross-sectional anatomy, epidermal features, which are the epidermis, stomata, gland and trichomes (Park, 1994).

Kereszty (1994) studied the leaves of various *Clerodendrum* species under the SEM and found significant differences, in especially the lower surface and the stomata, to separate species and infraspecific taxa. Metcalfe (1979) and Metcalfe and Chalk (1979) reported homogeneous mesophyll and dorsiventral leaves in the family verbenaceae. In *C. louwalbertsii* the leaves are dorsiventral, but in C. *triophyllum* the mesophyll is homogeneous. Several authors have described the peltate hairs found in many members of the family Verbenaceae (Cantino 1990; Kereszty 1994). The general morphology of the peltate hairs present in the two species studied was basically the same as that described by these authors, and no significant differences were observed between the peltate hairs of the two species: in both cases the hairs had an 8-celled head, a unicellular stalk and a base cell.

Among the Cuban *Cleroelendrum* taxa, Kereszty (1994) found only hypostomatic leaves and anomocytic, actinocytic, anisocytic and paracytic stomata. Inamdar (1969) also found only hypostomatic leaves but mostly diacytic stomata in the Indian *Clerodendrum* taxa studied by him. Cantino (1990) reported 3-celled diallelocytic stomata in al l the species of *Clerodendrum* subgenus Cyclonema he examined. *C. triphyllum* and C. *lomvalbertsii* both belong to the subgenus Cyclonema (Verdcourt 1992) but only diacytic, anisocytic, anornocytic and paracytic stomata were observed during the present study.

5.5 Seed Biology

5.5.1 Physical parameters of the seeds

The Analysis of variance related to physical parameters of the seeds presented in Table 4.13 revealed significant differences with respect to locations.

The maximum seed weight was obtained from the seeds collected from Reiek (0.40±0.02 g**)** followed by the seeds collected from Zonuam (0.38±0.03 g). Among all the locations the lowest seed weight was obtained in Chawlhhmun (0.21±0.01 g). Similarly, among the different locations, the significantly maximum seed length was obtained from the seeds collected from Reiek (7.38 ±0.46 mm) followed by Zonuam (7.02±0.28 mm). Among the different seeds collected from various locations, the significantly lowest length was obtained from the seeds collected from Durtlang (6.43±0.20 mm), followed by Sakawrtuichhun (6.46±0.24 mm). Similary, there was significant variation among the germplasms with respect to diameter of the seeds. The maximum diameter was obtained from the seeds collected from Reiek (5.86±0.48 mm) and the minimum was obtained from the seeds collected from Durtlang (5.02±0.30 mm). The variation in physical properties of the seeds might be due to different genetic make-up of the individual seeds.

5.5.2 Moisture Loss (%)

Seed moisture is a critical factor determining the viability and longevity of both recalcitrant and orthodox seeds. In our present investigation, the moisture loss was increased with the storage period right from 30 days after storage till 90 days. Among the seeds collected from various locations, the moisture loss was also varied significantly throughout the observation periods. It is clear from the data presented in Table 4.13, that at 30 days after storage, the maximum moisture loss was observed in found from the seeds collected from Tanhril (17.65±0.01 %), while the lowest was reported from the seeds collected from Serkhan (10.05±0.03 %). At 60 days after storage, the moisture loss followed the similar pattern as that of 30 days. The maximum was in Tanhril (18.26±0.02 %), and the lowest was in Serkhan (13.50±0.01

%). At 90 days after storage of the seeds, the maximum moisture loss was observed in found from the seeds collected from Durtlang (19.13±0.02%), while the lowest was from the seeds collected from Sakawrtuichhun (14.13±0.00%). The variation in moisture content of this plant might be due to different ripening stage of the fruits as well as different soil and climatic condition and inherent character associated with species.

5.5.3 Seed volume (cc)

The seeds varied significantly with respect to the volume of seeds also (Table 4.13). The highest seed volume was found in the seeds collected from Reiek (0.86±0.12 cc) and lowest was in Durtlang (0.55±0.07 cc). The variation in seed volume might be due to the size of the seed coat, in addition embryo of the seeds also increases the volume of the seed.

5.5.4 Imbibition (%)

The imbibition result presented in Table 4.13 showed that percentage imbibition was found to be the highest in Lungdai (43.91±1.98) and the lowest was observed in Reiek (30.70±2.67). Yaklich *et al*. (1984) claimed that porous seed coats are usually permeable and non-porous ones impermeable. The seeds of *Clerodendrum* have small opening or pore on the ventral side of the seeds. Water can pass through easily so that the imbibition speed can be enhanced by this opening. But there should be limitations for entering the water into the seeds. It also depends upon the size of the embryo and the hardness of the seed coat.

5.5.5 Seed germination

Storage of seeds is an essential step for the long-term conservation of plant genetic resources. Maintaining seed viability for longer period is very essential to preserve the genetic integrity in stored samples. Since very early days, simple techniques have been adopted to maintain the seed viability in both domesticated and wild sources (Onyekwelua and Fayose, 2007; Pradhan and Badola, 2008). Inappropriate storage medium such as room temperature storage often results in low seed germination, seed deterioration, and loss of viability, which are natural phenomenon during storage (Schmidt, 2002, Nasreen *et al*.,. 2000). Several factors, namely, temperature, nature of the seeds, seed moisture content, relative humidity, influence the seed longevity during storage (Onyekwelua and Fayose, 2007; Pradhan and Badola, 2008). Seed moisture content, temperature, and storage periods are among the main factors affecting above relationship (Roberts, 1988.). Long-term storage may lead to considerable reduction in germination or to eventual death of the seeds. Proper storage conditions, however, may effectively retain substantial viability in seeds over a considerable storage period (Butola and Badola 2004; Chen *et al*., 2007). Such approaches are especially crucial in case of endangered species, where judicial use of seeds as valuable genetic material through standardizing proper storage mechanism is a precondition to strengthen species conservation programme.

There are two types of seeds in nature. One is orthodox and another one is recalcitrant seeds. Orthodox seeds dry up-to low moisture content and can tolerate freezing temperature and can be stored for many years. But recalcitrant seeds are dessicated sensitive, loss viability in a short period and cannot be stored for prolonged. Attempted has been made on the storage of recalcitrant seeds, such as

'moist' storage, partially dry storage and cryostorage, but all these methods have their own limitations (Chin, 1989). The seeds *Clerodendrum colebrookianum* comes under the latter. The seeds were collected in the month of November and germination test conducted from December till it losses viability. The seeds we stored in ambient temperature (room temperature) losses viability after 6 months if stored in ambient conditions and by 8 months if stored in refrigerated conditions. The germination was initially low irrespective of the locations and storage conditions and it reaches the maximum by 3rd months and thereafter again decreases gradually and finally loss the viability by 6 months in ambient condition and 8 months in refrigerated condition. In the present investigation, in refrigerated storage, at 3rd month after storage, the maximum germination was obtained in the seeds collected from Reiek (96.67 ± 5.77) at 3 months after storage, while in ambient conditions, the maximum germination was obtained from the seeds collected from Reiek (90.00 ±10.00 %) at 2 months after storage. Germination test indicated that seeds of *C. colebrookianum* may not remain viable for extended periods at room temperature and even storage at low temperature (4°C-6°C) for long time. Loss of viability in stored seeds is a common phenomenon (Verma *et al*., 1996) and it increased with storage duration with storage condition is another factor (Dell, 1987). However, it was also observed that seed moisture content did not played key role as population with low seed moisture at the time of harvesting had high viability in *C. colebrookianum*. Further, seed moisture was slightly decreased after one year storage. Loss of viability as well as variation in seed viability among different natural populations may have the relation with growth and development of embryo which caused morpho-physiological dormancy as suggested by Walck and Hidayati, (2004) in another Apiaceae species *Osmorhiza depauperata*.

Germination of seeds of *C. colebrookianum* decreased with the increasing storage period, however, the magnitude of decrease was higher in seeds stored in polythene bags than the refrigerator. Possibly, the seeds stored at 4ºC could maintain an optimum moisture level thus remained viable for comparably longer period (Pandey *et al*., 2000), while those stored at room temperature, lost viability earlier (Nautiyal *et al.*, 1985). Our study is in close conformity with the study of Prakash *et al*., (2005) in some medicinal plants of Alpine region, where they also obtained maximum germination at refrigerated seeds as compared to normal storage and the germination was decreased with the storage period.

5.5.6 Seed viability

Clerodendrum colebrookianum seeds are recalcitrant so they loss the viability very soon. In the present investigation, observations reveals variation in seed weight, seed length, seed diameter and seed volume in addition to moisture content as well as imbibition rate of the seeds. Viability among the different populations irrespective of moisture content which may suggests morpho-physiological type of dormancy in this species. On the basis of present observations, it is further suggested that seed stored at refrigerated temperature remain viable for eight months. The slow loss of viability in seeds stored at low temperature (4ºC) was probably due to reduced rate of metabolic activities and inactivation of enzymes, thus helping to retain seeds viable (Some and Seetalakashmi, 1989). Harrington (1972) made generalization that orthodox seeds storage life is halved by each 5ºC increase in temperature or by each 1% increase in seed moisture content. In the present experimentation, seeds of *Clerodendrum colebrookianum* also follow the same a rule as the increase in moisture content in the seeds stored in polybags at ambient temperature was comparatively

higher as well as seeds stored in refrigerator. Further observations are needed to overcome morpho-physiological dormancy and bringing uniformity in germination behavior of *C. colebrookianum*

Viability tests conducted on seeds of *C. colebrookianum* indicated that germination percentage of significantly decreases over a 1-year period, even under low temperature storage conditions. This loss of seed viability may be a primary reason for low seed germination of *C. colebrookianum* in nature (Nadeem *et al*., 2000). This study demonstrated that loss of viability and moisture content of stored seeds increased with storage duration, a common phenomenon in stored seeds (Edwards and Mumford, 1985; Verma *et al*., 1996) associated with seed viability over time and with storage conditions (Dell, 1987). Prakash *et al.,* (2005) also observed decrease in viability with the storage period and more viability at refrigerated seeds as compared to normal storage in some medicinal plants of Alpine region.

5.6. Germination studies

5.6.1 Germination studies with different PGRs and chemicals

Data presented in Table 4.18 showed that there was significant difference among the seeds treated with different PGRs and Chemicals with respect to the germination parameters. This investigation clearly indicated that seed germination in *C. colebrookianum* differed among seed treatments and in nursery conditions. An earlier report (Baskin and Baskin, 1998) suggested that the mother plant environment (nutrient, light, and water and seed position on the mother plant) may influence the

seed germination among populations. Latitude and elevation could also play be important factors affecting seed germination among the different populations.

Our study revealed that KNO_3 could moderately improve germination of populations of *C. colebrookianum*. These observations are in agreement with a report of Qaderi and Cavers (2000) and Vashistha *et al.* (2009) showing that seed germination of *Onopordium acanthium* and *Angelica glauca* was significantly affected by KNO_3 concentration.

In our study, gibberellic acid treatments had negligible effects on seed germination of *C. colebrookianum*. Earlier studies (Chaudhary *et al*., 1996; Ojala, 1985; Vashistha *et al.* (2009) have reported similar observations for other species. The poor response to gibberellic acid may be due to the presence of naturally occurring germination inhibitors that could not be overcome by the application of gibberellic acid. Seed treatments with KNO_3 have improved germination in *Heracleum candicans* (Johsi and Dhar, 2003) and *Arnebia benthamii* (Manjkhola *et al*. 2003).

As compared with expensive plant growth regulators, the use of relatively inexpensive KNO_3 and $NaHClO_3$ have been suggested as beneficial tools for mass multiplication in cultivation (Butola and Badola 2004). Our study indicate that seed treatments with KNO_3 and $NaHClO_3$ are economically feasible for *C. colebrookianum* and can be easily applied by nursery workers and poor farmers developing mass planting stock. In total, our study indicates that the use of the seed treatments plus the use of seed storage at low temperatures would be useful in increasing seed germination and thus plantings of *C. colebrookianum*. An appropriate nursery-based

germination protocol and the non-uniformity in germination behavior in *C. colebrookianum* is also needed further research.

5.6.2 Germination studies with different media

The growing media plays a vital role in growth and development of any plant species as act as one of the growth influencing factors i.e., edaphic factor that act as precursor for initial stages of plant life. The supply of plant water and air to the growing plants can be greatly influenced by the physical composition of growing media (Beardsell and Nicholas, 1982) which may further effect the anchorage, nutrient and water holding capacity of the medium. These characteristics directly influence the seedling emergence and vigor and consequently to seedling quality (Baiyeri and Ndubizu, 1994).

In present study the substrate combinations suggested that the best media among different treatments for seed germination parameters is soil + sand + FYM + vermicompost as there were significant effects in all seed germination parameters. Joiner and Nell (1982) found similar results in peat + perlite mixture for *Aglaonema* and *Dieffenbachia*. Abirami *et al*. (2010) also revealed enhanced seed germination and seedling growth in *Myristica fragrans* Houtt by using different combinations of growing media. Manh *et al*. (2014) also reported that using substrate mixture of vermicompost with rice hulls ash and coconut husk following rate 1:1:1 respectively gave highest value of germination rate, plant height, leaf area, plant biomass. There is also ample evidence suggesting that growing media containing mixture of vermicompost increase the germination percent (Michelle and Bachman, 2000; Atiyeh *et al*., 2000 Arancon *et al.,* 2004) by influencing higher germination, increased

biomass, balanced composition of nutrients enhanced growth and development which may preserve soil humidity, increase nutrient content and improve soil structure which increase water absorption and maintains the cell turgidity, cell elongation and increase respiration at optimum level, leading to favourable seed sprouting. All these factors are favorable for seed germination and ultimate by increase seed germination percent, speed of emergence, seed vigour, germination index, germination value and reduce imbibition period (Bachman and Metzger, 2008; Zaller, 2007). Our present study is in close conformity with the findings of Dharambheer *et al.* (2016) in *Angelica glauca*.

5.7 Acquisition of germinability

The experimental test showed very less germination from green immature fruits. The seeds collected at freshly harvested and fallen seeds gave good germination. So, there was no dormancy present in the seeds of harvested and fallen seeds. But the poor germination in green seeds might be due to under-developed embryo at the time of collection of the seeds (dormancy due to rudimentary embryo).

In the present investigation, differences in germination were observed with respect to the type of the seeds. The highest germination percentage was obtained in the freshly harvested seeds (55.00±8.66) while, the lowest was obtained in green seeds (8.33±2.89). Our study is in close conformity with the findings of Newton *et al*., (2013) where they also obtained maximum germination from freshly harvested seeds. The decline in germination of green seeds may have been confounded by dormancy induction in a proportion of seeds. An alternative and more plausible explanation for this, however, may be the premature breaking of the connection of the seed to the

placenta, or perhaps selective abortion, before germinability and desiccation tolerance, respectively, were acquired. Ovule abortion can take place at any stage before seeds reach maturity (Lersten, 2004).

There was variation among the type of seeds with respect to speed of germination, mean germination time, peak value, mean daily germination and germination index. With respect to all these parameters, the highest value was obtained in freshly harvested seeds and the lowest in green seeds. Our study is in close conformity with the findings of Kitchen and Monsen (2001), where they also obtained maximum germination percentage and MGT from the freshly harvested seeds.

5.8 Nursery parameters

The ability to tolerate the surrounding environmental conditions is an essential element to the survival. Due to adverse environment, most of the plant species are habitat specific and flourish well only within a narrow range of environment. They have specialized adaptation to a specific set of environment and this means they are very susceptible to all sort of environmental changes (Körner, 1999). Therefore, identification of suitable location is prerequisite for the successful domestication and economically viable cultivation of these species.

Significant variation was observed among the seeds collected from different locations with respect to survival percentage. The maximum per cent survival was observed in Lungdai, Serkhan and Chawlhhmun (100.00±0.00), while, the lowest was obtained in Luangmual and Durtlang (50.00±3.00). Variation of seedling survival collected from different altitudes can be attributed due to variation in genetic make-up

of the seedlings. Survival depends on the continuous adaptation of the species to new environment, and a species that has gained high chance of survival in a particular habitat is termed as adapted species (Campbell and Sorensen, 1984). Similar studies on comparison of per cent survival among different seedlings have also been reported by many workers (Chauhan and Nautiyal, 2005; Chauhan and Nautiyal, 2006; Vashistha *et al*., 2007; Vashistha *et al*., 2008).

5.9 Seedling growth

Production of high quality seedlings in large-scale plantation program needs a readily available and suitable seedling growing medium (Hossain, 2004). Seedlings develop profuse root system immediately after germination under full sun. Development of seedling is better in full sunlight than under partial shade (Tewary, 1994). The seedlings exhibit faster growth on onset of monsoon and can attain a certain height at the end of growing season (Zabala, 1990). Growth is strongly dependent on soil conditions.

In the present investigation, significant variation was observed among the different populations with respect to seedling growth in terms of morphological parameters, *viz*. plant height, collar diameter, number of leaves, root length, fresh weight of leaves, fresh weight of shoot, fresh weight of leaves, dry weight of root, dry weight of shoot, root/shoot dry weight ratio, and biomass production. In general, the variation in morphological characters among different seedlings collected from different locations might be due to variation in genetic make-up of the plants. Our study is in close conformity with the findings of Matin and Khan, (2000) where they obtained significant variation in growth performance of seven species of *Albizia*

seedlings at nursery stage in Bangladesh. Seedling variation in relation to habitat has also been reported in *Celtis australis* (Singh *et al*., 2006) and *Magnolia officinalis* (Shu *et al*., 2012).

In our study, plant height of the seedlings varied significantly among the different locations. At 360 days after planting, the maximum plant height was obtained in Reiek (311.61±38.13 cm), while the lowest was obtained in Tanhril (174.87±32.55 cm). Dangasuk *et al*. (1997) in their provenance trials with *Faidherbia albida* seedlings reported that there was little variation among and within the Southern African and East African provenances in seedling height.

Similarly, at 360 days after planting, the maximum collar diameter was obtained in Reiek (46.33±3.21 mm), while, Tanhril recorded the lowest value (29.67±2.08 mm). Dangasuk *et al*. (2001) and Ibrahim (1996) also observed variation in seedling diameter for *Faidherbia albida* Provenance at the nursery stage after 3 months. Ibrahim *et al*. (1997) reported that the seedlings produced by seeds from southern African provenances had larger collar diameter than those from other regions and this is in conformity with our findings.

Highly significant variation in leaf number was observed at all ages of growth (Table 4.23). These present findings are in conformity with the observations of Dangasuk *et al*. (1997) who reported significant difference in leaf number of *Faidherbia albida* seedlings thirty days after germination. Singh *et al*. (2010) also observed significant variation in the number of leaves per plant in *Quercus glauca* and attributed it to wide range of distribution of the species.

It should be noted that all seedling characters should be considered during selection because selection done on the basis of one character alone may not give the desired level of superiority (Singh *et al*., 2010). Since the period of this study was short (1 year), further progeny tests in the field should be undertaken for a longer period so as to obtain definitive recommendations for early selection as "It is advisable that the age of early selection in any species should be sufficiently long to achieve a reasonable level of accuracy in selection" (Loha *et al*., 2006). Finally, selecting and analyzing additional locations in future studies could be considered in order to get a more suitable selection for breeding purposes and integration into agrarian systems.

Summary and Conclusion

The present investigation entitled "Resource Assessment and Seed Biology of *Clerodendrum colebrookianum* Walp. in Mizoram, India'' was carried out during 2013-2016 at Dept. of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl, Mizoram.

The salient findings emerged out from the present investigation are summarized below:

1. The maximum percentage frequency of *C. colebrookianum* Walp was obtained in Luangmual (80%), whereas; the lowest percentage frequency was obtained in the germplasm of Lengpui (40%). The highest abundance of the species was obtained in Sakawrtuichhun (15.40) and the lowest was obtained in Lungdai (7.5).
2. The maximum density of *C. colebrookianum* Walp was obtained in the location Sakawrtuichhun (308.00 plants/ha). However, among the studied locations, the lowest density was obtained in Serkhan village (152.00 plants/ha).
3. The maximum average basal cover was found in Luangmual (28.22 cm^2), whereas, the minimum was observed in Zonuam (9.39 cm^2*)* among all the studied geographical locations. Among all the locations, the highest Total Basal Cover was reported in Luangmual (200.36 cm^2m^{-2}*)*, while the lowest was observed in Zonuam (47.88 cm^2m^{-2}*)*.

4. A large number of species have been found associated with *C. colebrookianum* Walp in different locations includes *Achyranthes aspera L. Acmella oleraceae/Spilanthus acmella, Adhatoda vesica Mill, Adenostemma lavenia, Ageratum conizoids, Artemisia vulgaris, Bidens pilosa, Blumea lanceolaria, Cajanus cajans, Centela asiatica, Colocasia spp , Cuscuta reflexa Roxb, Eupatorium odoratum, Imperata cylindrical, Manihot esculenta, Mikania micrantha, Mimosa pudica L., Oroxylum indicum, Pteridium acquilinum, Solanum indicum L., Solanum torvum, Thysanolaina maxima , Trevesia palmate.*
5. The relative frequency, relative dominance, relative density varied from 6.66 - 13.33, 4.88 – 20.44, 6.65 – 13.48 respectively.
6. The Importance Value Index (IVI) was calculated in order to record the dominance and ecological success of selected species. Among the studied locations, the highest IVI was observed in Luangmual (46.21) and the lowest value with respect to IVI was observed in Lengpui (21.06).
7. Distribution pattern on the basis of A/F ratio indicates *C. colebrookianum* Walp. as contiguous.
8. The threat status identified for this species indicates *C. colebrookianum* Walp. as Critically endangered to endangered on the basis of no of mature individuals; vulnerable on the basis of number of locations, Endangered on the basis of area of occurrence and occupancy.
9. Morphological features of the plant of *C. colebrookianum* Walp among different germplasm indicated that the average height of the plant varied from 189.80 – 303.53 cm, collar diameter (27.89- 45.67), number of leaves ranged

(33.33 – 211.00), root length (36.84 – 94.11), root shoot length ratio (0.14 – 0.39), root fresh weight (266.67 – 1273.33), shoot fresh wt. (608.33 – 2086.67), leaf fresh weight (170.00 – 840.00g), root dry wt. (110.00 – 596.67 g), shoot dry wt. (220.00 – 886.67 g), root/shoot dry wt. ratio (0.44 – 1.13) and biomass production (0.88- 3.36).

10. Flowering and fruiting characteristics of *C. colebrookianum* Walp. among different Germplasms indicated that there was significant variation among the germplasm with respect to days required for flowering from initiation of bud (39.33 – 43.00), days required for bud differentiation from initiation of bud (9.67 – 12.00), number of flowers/inflorescence (96.33 – 116.67), length of inflorescence (15.03-24.10 cm), fruit setting per cent (87.03-98.90%), no. of fruits/inflorescence (18.33- 42.67), days required to maturation from setting of fruits (45.67 - 49.67), fruit retention percentage (28.67 – 45.67).
11. Estimation of phenotypic and genotypic coefficient of variation, heritability, expected genetic advance and genetic advance as percent of mean for different traits in *C. colebrookianum* Walp revealed that the genotypic variance ranged from 0.0039 – 156884.27, phenotypic variance 0.065 – 411584.16 respectively. The maximum value for genotypic coefficient of variation was in fresh weight of root(47.11) and lowest was in Plant height (15.76). Similarly, phenotypic coefficient of variation ranged between 21.85 - 72.83. The Broad sense of heritability ranged from 34.55 -59.18 in fresh wt. of leaves and root shoot length ratio respectively. The genetic advance varied from 0.10 – 530.75. Highest is in shoot fresh wt. and lowest in root shoot length ratio. The

genetic gain ranged from 20.16 – 67.73. Highest in shoot dry wt. and lowest in collar diameter.

12. Genotypic Correlation coefficients of *C. colebrookianum* Walp among different traits revealed that most of the morphological parameters were significantly to highly significantly correlated among each others. Biomass production was highly correlated with no of leaves, fresh weight of leaves, root length, fresh weight of root, and shoot fresh weight.
13. Phenotypic correlation coefficient was also worked out among different traits in *C. colebrookianum* Walp and it revealed that Biomass production was highly correlated with Plant height, no of leaves, fresh weight of leaves, collar diameter, fresh weight of root, and shoot fresh weight shoot dry weight and strongly with root length and positively with dry weight of root.
14. Pearson's Correlation coefficients was also estimated among the different traits in *C. colebrookianum* Walp. and the results revealed that the number of leaves was highly correlated with the plant height, biomass production was highly correlated with Plant height, no of leaves, fresh weight of leaves, collar diameter, and fresh weight of root, and shoot fresh weight, shoot dry weight and strongly with root length and positively with dry weight of root.
15. The value of regression coefficient of biomass with plant height (0.01784), no of leaves (0.01327), fresh weight of leaves (0.00345), Collar diameter (0.09796), fresh weight of root (0.00208), shoot fresh weight (0.00160) and shoot dry weight (0.00333) was highly significant whereas regression coefficient of independent variable biomass production with dependent variables root length (0.02304) and dry weight of root (0.00236) was

significant. Regression coefficient of biomass production with shoot length (0.45952) and shoot dry weight (0.21684) was not significant. The multiple regression equation models shows that maximum positive contribution was made by shoot fresh weight (59.25 %) followed by fresh weight of roots (45.59 %) and highest negative contribution made by dry weight of root (0.16 %).

16. The stem of *C. colebrookianum* Walp is surrounded by a fine outgrowths or appendages called as Trichomes or plant hairs. Just beneath the tracheids, a single layer epidermis, is present and acts as a boundary between the plant and the external environment This is followed by schlerenchyma cells which are almost circular in shape. Immediately after schlerenchyma cells 3-4 layered parenchymatous cells are present. Below parenchymatous cells, ground tissues are distributed throughout and composed of circular cells. Vascular bundles are present along with the pith. And lastly, pith was present in the middle of the stem and it is composed of soft, spongy parenchyma cells.
17. The leaves *C. colebrookianum* Walp are simple, opposite or rarely whorled. Leaves are 10-20 cm in length and 6-12 cm in breadth. Leaf base is wedge-shaped to heart-shaped, margin entire to slightly wavy, tip pointed. The outer cell wall of leaf was covered by Trichomes. It was followed by multiple layers of palisade cells. Just beneath the palisade cell, collenchyma cells are present. The vascular bundles are scattered in the collenchymatous cells around the pith.
18. The outermost layer of the root is called epidermis followed by cortex. Just beneath the cortex, a 5-6 layers of collenchymatous cells are compactly

arranged. Vascular bundles, xylem and phloem occupy the central region of the root. Xylem transports the water and minerals absorbed by the root up to the stems, leaves, and flowers. The phloem transports the sugars and other nutrients made by the leaves down to the root for immediate use or for storage during periods of dormancy.

19. The observation on seed biology indicates that the physical parameters of the seeds varied with respect to germplasm. The weight, length and diameter of the seeds ranged from 0.21 – 0.40 g, 6.43 – 7.38 mm and 5.02 -5.86 mm respectively. The moisture loss of the seeds was increased with the storage period. In 30 days the moisture loss per cent varied from 10.05 – 17.65 per cent, which was increased to 13.51 – 18.26 per cent by 60 days and 14.25 – 19.13 per cent by 90 days. The seed volume also varied with the germplasms. Highest volume was found in Reiek (0.86 cc) and lowest was in Durtlang (0.55 cc). Similarly, the imbibition, of the seeds varied from 31.09 – 43.91 per cent. Highest (43.91%) was in Lungdai and lowest was in Lengpui (31.09%).
20. Month wise viability test of the seeds of *C. colebrookianum* Walp at refrigerated conditions indicated that the seeds remain viable for a maximum period of 8 months. Similarly, under ambient condition, the seeds remain viable only for 6 months.
21. Similarly, with respect to germination, it has observed that in refrigerated storage, at 3 month after storage, the maximum germination was obtained in the seeds collected from Reiek (96.67 ± 5.77) at 3 months after storage, while in ambient conditions, the maximum germination was obtained from the seeds collected from Reiek (90.00 ±10.00 %) at 2 months after storage.

22. The germination test that conducted with different PGRs and chemicals revealed that maximum germination percentage (88.67±1.53%) was obtained in KNO_3 150 mM, the same treatment also required minimum days for onset of germination (6.33±0.58), for completion of germination (12.00±2.00) and for initiation of true leaves (6.67±0.58).
23. Similarly, germination study by using different media indicated that the highest germination per cent was observed in FYM+ vermicompost (VC) + Soil + Sand (2:2:1:2) (88.33±2.89%). The same treatment also required minimum days for onset of germination (12.33±0.58), for completion of germination (29.00±2.00) and for initiation of true leaves (18.00±1.00)
24. Acquisition of germinability test revealed that there was no dormancy present in the seeds of freshly harvested and fallen seeds. The seeds from green immature fruits do not give proper germination. The dormancy is due to rudimentary embryo. The germination per cent was highest in the harvested seeds (55.00%) followed by fallen seeds with (31.67 %) and the lowest in Green immature seeds (8.33%). The speed of germination was also highest in harvested seeds (0.30) and lowest in green seeds (0.08). Mean germination time ranged from 20.67 – 46.67. Highest in harvested and lowest in green seeds, peak value ranged from 0.00 – 0.09. Highest in harvested and lowest in green seeds. Maximum value of mean daily germination was in harvested seeds (0.24) and lowest in green seeds (0.08). Similarly, the germination index ranged from 0.00-1.18. Highest in harvested and lowest in green seeds.
25. Per cent survival of *C. colebrookianum* Walp ranged from 50.00 – 100.00. Highest was observed in Chawlhhmun and lowest was in Durtlang. Maximum

percent survival was observed in Lungdai, Serkhan and Chawlhhmun (100.00±0.00), and lowest percent survival was obtained in Luangmual and Durtlang (50.00±3.00).

26. The seeds collected from each germplasm were raised in the nursery beds and the performance of the seedlings were observed. The seedlings varied significantly with respect to plant height (174.87 - 311.61cm), collar diameter (29.67mm - 46.33mm), number of leaves (88.67- 153.00), biomass production (6301.93 g - 8074.01 g), root length (65.57 cm - 89.47 cm), root/shoot length ratio (0.26 – 0.39), root fresh weight (2285.00g - 2686.67 g), shoot fresh weight (1013.27 g – 3201.11 g). Leaf fresh weight (1955.33g – 2852.00 g), root dry weight (1740.01g – 2535.02g), shoot dry weight (473.85 -2348.91 g), root/shoot dry weight ratio varied from 0.74 – 5.44.

Conclusion

Based on the results, following conclusions have been drawn from the present investigation:

1. *Clerodendrum colebrookianum* Walp. is found in kitchen garden or as semi wild type grown mainly as vegetables crop. Due to increase in populations, construction of buildings and over exploitation, the habitat has been gradually destroyed. The threat status identified for this species indicates *C. colebrookianum* Walp. is critically endangered to endangered on the basis of number of mature individuals; vulnerable on the basis of number of locations and endangered on the basis of area of occurrence and occupancy.
2. There was great morphological variability within the species and differences was observed with respect to plant height, collar diameter, number of leaves,

root length, root/shoot length ratio, root fresh weight, shoot fresh weight, leaf fresh weight, root dry weight, shoot dry weight, root shoot dry weight ratio and biomass production.

3. The stem of *C. colebrookianum* Walp. is surrounded by trichomes, followed by epidermis, schlerenchyma cells, parenchymatous cells and ground tissues and vascular bundles are distributed. Similarly, leaves are simple, opposite. The outer cell wall of leaf was covered by trichomes, followed by palisade cells, collenchyma cells. Whereas, the outermost layer of the root is epidermis followed by cortex, collenchymatous cells and vascular bundles.
4. From the present study, it has observed that, the seeds of *C. colebrookianum* Walp. is recalcitrant seed. The seeds lost the viability very soon. If they are not sown within a specified time, they fail to germinate.
5. Viability test indicated that seeds of *C. colebrookianum* Walp. may not remain viable after 6 months of storage at room temperature and at refrigerated temperature after 8 months.
6. Similarly, with respect to germination, it has observed that in refrigerated storage, at 3rd month after storage, the maximum germination was obtained in the seeds, while in ambient conditions, the maximum germination was obtained at 2 months after storage.
7. The result of germination test of different stages of maturated seeds revealed that there was no dormancy present in the seeds of freshly harvested and fallen seeds. The seeds from green immature fruits do not give proper germination because of dormancy. The dormancy is due to rudimentary embryo.

Sakawrtuichhun

Chawlhhmun

Reiek

Lungdai

Durtlang

Serkhan

Plate 1: Germplasm variability of *Clerodendrum colebrookianum*

Zonuam

Tanhril

Lengpui

Luangmual

Plate 2: Germplasm variability of *Clerodendrum colebrookianum*

Initiation of Floral bud

Bud differentiation

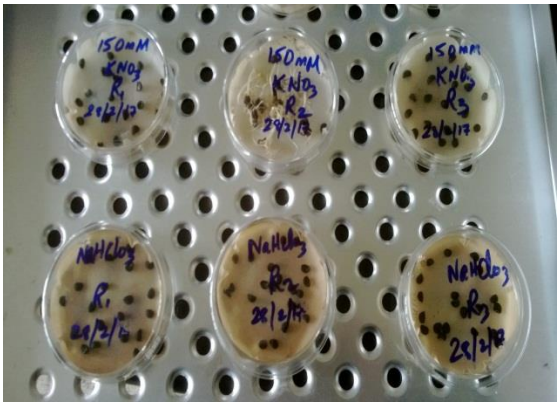
Pre flowering stage

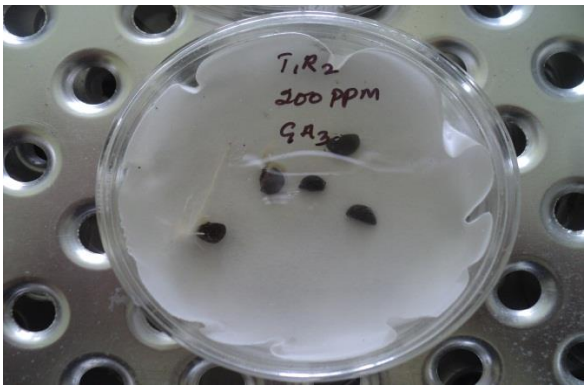
Full Bloom

Fruit setting stage

Fruit maturity

Plate 3: Diffferent Phenophases of *Clerodendrum colebrookianum*





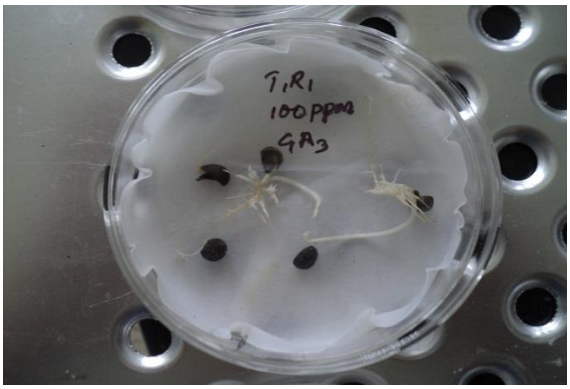


Plate 4: Germination with different PGRs

GREEN SEEDS

HARVESTED SEEDS

FALLEN SEEDS

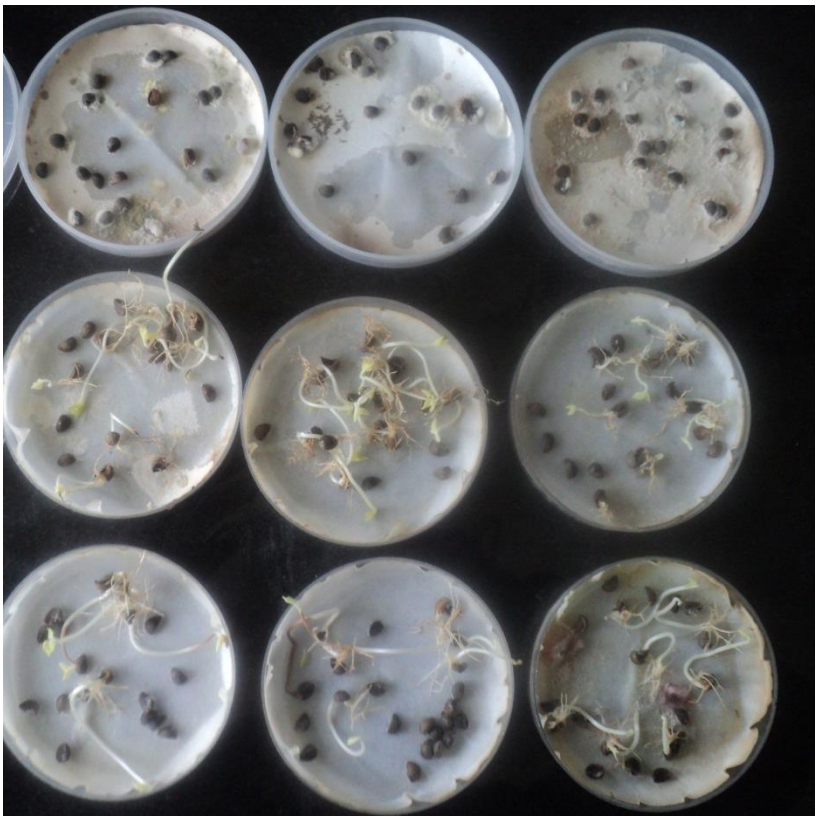


Plate 5: Germination of seeds harvested at different stages

Seed germination with VC and FYM

Nursery Soil

FYM:VC:Soil:Sand(:2:12)

Plate 6: Germination of seeds in Nursery






Plate 7: The growth of the seedlings from different locations

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CURRICULUM VITAE OF MR. R. ZOTHANKIMA

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Publications	1. Ralte, Zothankima., Hazarika, T. K. and Nautiyal, B. P. 2018. Seed Behavior of *Clerodendrum colebrokianum* Walp., a Vulnerable Medicinal Shrub of Mizoram, North-East India. *Environment & Ecology* **36** (1A): 214—220. 2. Hazarika, T. K., R, Zothankima. and Nautiyal, B.P. 2016. Economic dynamics of tissue cultured strawberry (*Fragaria* × *ananassa*) under the influence of integrated plant nutrients in humid sub-tropical hills of North Eastern India. *Indian Journal of Agricultural Sciences* **86** (2): 267–72. 3. Hazarika, T.K., Ralte, Zothankima, Nautiyal, B.P. and Shukla, A.C. 2015. Influence of bio-fertilizers and bio-regulators on growth, yield and quality of strawberry (Fragaria X ananassa) *Indian Journal of Agricultural Sciences* **85** (9): 1201–5.

Seminar/Symposium /Training attended

1. One month skill development Training Program in Biotechnology for Students of North-East India organized by Biotech Park Lucknow. 24th January – 23 February, 2018.
2. Participated in Training and Awareness Programme on protection of plant varieties and farmers rights on 28th & 29th March 2017 organised by Dept. of Forestry and Horticulture, Aromatic and Medicinal Plants.
3. Delivered oral presentation in National seminar on Biodiversity, conservation and utilization of Natural resources with reference to Northeast India (BCUNRNEI) on 30th -31st March 2017 organised by Dept. of Botany, Mizoram University.
4. Participated in Science communication Workshop (SciComm 101) held on 6th june, 2017 organised by *The Wellcome trust/DBT India Alliance.*
5. Poster presentation on International Conference on Natural Resources management for Sustainable development and rural Livelihoods on 26-28 October, 2017 organised by Dept. of Geography. Mizoram University, Aizawl.
6. Participated in workshop on Sustainable Management of Indigeneous knowledge on 01-03 march, 2016 organised by teri.
7. Participated in International Symposium on Sustainable Horticulture on 14th -16th March, 2016 organised by Dept. of Horticulture, Aromatic and Medicinal Plants.
8. Participated in Technical session/exhibition in Mizoram Science Congress on 13th-14th October, 2016 organised by Mizoram Science Congress 2016 organising committee.
9. Participated in Seminar on make in India: Science & Technology driven innovations organized by Mizo Academy of Sciences in collaboration with MISTIC on 4th November 2016.
10. Participated in National workshop on statistical analysis using excel software on 13th to 15th 2016, organized by Department of statistics, Pachhunga University College, Aizawl Mizoram.

Environment and Ecology 36 (1A) : 214—220, January—March 2018
Website: environmentandecology.com ISSN 0970-0420

Seed Behavior of *Clerodendrum colebrokianum* Walp., a Vulnerable Medicinal Shrub of Mizoram, North-East India

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Received 17 September 2017; Accepted 20 October 2017; Published on 10 November 2017

Abstract *Clerodendrum colebrokianum* Walp., East Indian Glory Bower locally known as Phuihnam in Mizoram, North east India is one of the most important medicinal plant. The present investigation was carried out during 2015-17 to study the germplasm variability with respect to the physical properties of the seeds of *Clerodendrum colebrokianum* collected from different locations. The study reveals that there was wide range of variability with respect to seed weight, seed length, seed diameter. There was significant variation among the seeds collected from different locations with respect to moisture loss, seed volume and water uptake (imbibition). The seeds losses viability after 6 months if stored in ambient conditions and by 8 months if stored in refrigerated conditions. The germination was initially low irrespective of the locations and storage conditions and it reaches the maximum by 3rd months and thereafter again decreases gradually. In refrigerated storage, at 3rd month after storage, the maximum germination was obtained in the seeds collected from Reiek (96.67 ± 5.77%) at 3 months after storage, while in ambient conditions, the maximum germination was obtained from the seeds collected from Reiek (90.00 ± 10.00%) at 2 months after storage.

Keywords *Clerodendrum colebrokianum* Walp., germination, North east India, Mizoram, Seeds.

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Introduction

The genus *Clerodendrum* is flowering plants belongs to the family Verbenaceae and is widely distributed in the tropical and warm temperat regions of the world, with most of the species occurring in tropical and Northern Africa, Asia, Egypt and Madagascar. *Clerodendrum* is a very large and diverse genus with about 580 identified species is distributed throughout the world [1]. It is the largest genus of the tribe Teucrieae. In India 23 species were recorded by [2], of

Table 1. Different locations and their geographical coordinates.

Sl. No.	Location	Latitude	Longitude	Elevation (m)
1	Durtlang	N 23°46´30.0´´	E092°44´00.3´´	1254
2	Reiek	N 23°41´45.7´´	E092°36´44.0´´	1148
3	Luangmual	N23°45´39.1´´	E092°43´19.4´´	1041
4	Lungdai	N 23°53´11.9´´	E092°44´34.5´´	1132
5	Serkhan	N 23°54´25.2´´	E092°44´27.6´´	1028
6	Zonuam	N 23°44´12.9´´	E092°42´11.1´´	1109
7	Chawlhh-mun	N 23°44´39.6´´	E092°41´32.1´´	942
8	Tanhril	N 23°44´21.1´´	E092°40´59.5´´	901
9.	Sakawrtui-chhun	N 23°76´11	E92°67´11´´5	440
10	Lengpui	n 23°84´05.99´´	E92°61´96.89´´	426.11

which 16 were recorded from Arunachal Pradesh by [3]. *Clerodendrum colebrookianum* Walp. commonly known as East Indian Glory Bower is a flowering shrub or small tree, characterized by a foetid smell. The species is found in tropical and subtropical regions of Asia including India, Myanmar, Bangladesh, Malaysia, Indonesia, Thailand, Bhutan and Nepal; and also in temperate China. It is erect reaches up to 1.5–3.5 m in height and is evergreen. Branchlets are usually 4-angled when young. Leaves are simple, opposite or rarely whorled. Leaf base is wedge-shaped to heart-shaped, margin entire to slightly wavy, tip long-pointed to pointed. Flowers are white and borne in 4-6-branched corymbose cymes, at the end of branches. Inflorescences loosely cymose orcapitate, in terminal or rarely axillary paniculatethyrses. Calyx is campanulate or cup-shaped, densely pubescent. Corolla with a slender tube; lobes 5, spreading. Fruit is a drupe with 4 1-seeded pyrenes, sometimes separating into 2 2-loculed or 4 1-locular mericarps. It flowers during post-monsoon, from August to December. East Indian Glory Bower is found in NE India, and parts of China and SE Asia. *C. colebrookianum*, has been reported to have antidiabetic, antihypertensive and sedative properties [4—7]. In Mizoram, it is considered as anti-cancer, used to increase breast milk [8]. Besides, it is one of the delicious vegetable recipes of local people.

The species *Clerodendrum colebrokianum* being important medicinal plants as well as vegetables is a native plant of Mizoram, North-east India. The plant is usually consumed for effective control of high blood pressure, hypertension and diabetes and found to possessed antimicrobial activity also. Local people consumed as vegetables instead of medicinal values. Unlike other vegetables, this plant is available throughout the year and also gives continuous income to the farmers. Once the plant has established, it can be last long as it is a perennial shrub. *Clerodendrum colebrokianum* is a shallow rooted crop and lack tap root, grows horizontally. From these roots a new plants arises and becomes difficult to maintain the proper spacing. Once we uproot the whole plant, a new plant has come up from the intact root which is left remaining. Leaf are the used part and is inhibited by ants and somewhat bitter in taste. The leaf boiled with water is proved to increase breast milk. The viability test showed that the seeds of *Clerodendron colebrokianum* are recalcitrant seeds and it cannot be stored for a long period.

Seed is a unit of life developing from fertilized ovule. They represent the mostcritical phase of a plant's life cycle and are responsible for the evolutionary continuum of plant species. Seed characteristics, germination preferences and seed dormancy patterns have been proposed as tools for understanding evolutionary patterns [9]. The time duration for which the seeds retain their viability varies with species and to some extent with the prevailing environmental conditions. There are seeds which remain viable for hundreds of years and those which lose their viability within a week or a month. What brings about the state of non-viability in a seed has been the subject of extensive investigation.

Materials and Methods

The investigation was carried out in the laboratory of Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl during 2015–2017.

Table 2. Physical parameters of the seeds from different locations.

Sl. No.	Location	Weight (g)	Length (mm)	Diameter (mm)	Moisture loss (%) 30 days	60 days	90 days	Seed volume (cc)	Imbibition (%)
1	Durtlang	0.22±0.02	6.43±0.20	5.02±0.30	17.01±0.02	17.92±0.02	19.13±0.02	0.55±0.07	34.82±3.79
2	Reiek	0.40±0.02	7.38±0.46	5.86±0.48	15.22±0.02	15.92±0.01	15.92±0.01	0.86±0.12	30.70±2.67
3	Luangmual	0.26±0.02	6.81±0.15	5.45±0.18	15.41±0.01	16.76±0.01	16.77±0.01	0.69±0.11	39.71±6.94
4	Lungdai	0.24±0.02	6.84±0.45	5.47±0.21	13.95±0.03	14.32±0.15	14.25±0.01	0.61±0.08	43.91±1.98
5	Serkhan	0.27±0.04	6.64±0.24	5.15±0.18	10.05±0.03	13.51±0.01	18.70±0.01	0.70±0.12	34.79±2.42
6	Zonuam	0.38±0.03	7.02±0.28	5.65±0.13	14.08±0.02	14.80±0.01	15.47±0.01	0.73±0.08	38.98±7.60
7	Chawlhhmun	0.21±0.01	6.92±0.41	5.50±0.19	13.06±0.01	14.31±0.02	16.04±0.01	0.68±0.08	36.29±9.82
8	Tanhril	0.25±0.01	6.71±0.38	5.22±0.20	17.65±0.01	18.26±0.02	18.28±0.01	0.64±0.11	33.83±5.11
9	Sakawrtui-chhun	0.24±0.03	6.46±0.24	5.10±0.35	12.63±0.02	14.12±0.01	14.13±0.00	0.76±0.08	32.48±1.48
10	Lengpui	0.23±0.02	6.66±0.21	5.37±0.24	14.17±0.01	15.08±0.02	15.85±0.01	0.74±0.12	31.09±2.10
SEd (±)		0.02	0.23	0.19	0.012	0.041	0.008	0.07	3.42
$CD_{0.05}$		0.04	0.49	0.40	0.026	0.087	0.019	0.15	7.19

Seeds of *Clerodendron colebrokianum* were harvested after ripening during the month of November, 2015 and 2016 from ten natural populations having altitudes between 426.11–1254 m asl. These populations include Durtlang, Reiek, Luangmual, Lungdai, Serkhan, Zonuam, Chawlhhmun, Tanhril, Sakawrtuichhunand Lengpui. The geographical co-ordinates of different locations are presented in Table 1. The seeds were collected to study the physical properties of the seeds in terms of seed weight, diameter, length, volume of the seeds, moisture content, imbibition, viability and germination percentage. After harvesting of the fruits, the seeds were air dried for one week and kept in a perforated polythene bags for further observations.

Immediately after collection of seeds, 20 seeds from each replication were randomly selected for recording the physical parameters of the seeds. The weight of the seeds was measured with the help of digital balance and expressed in gram (g). Similarly, the length and diameter of the seeds were measured with the help of verniercallipers and the result was expressed in mm.

The initial weight of the seeds was first measured by using digital weighing balance and recorded. The moisture loss of the seeds were measured at every 30 days interval till 90 days. After completion of this experiment, the moisture loss percentage at each level was calculated by using this formula:

$$\text{Moisture loss \%} = \frac{\text{Moisture loss}}{\text{Fresh weight}} \times 100$$

Seed volume was measured by water displacement method for which seeds were dipped in a known volume of water in a measuring cylinder and after immersing the seeds, the rise in water level was noted. Ten replicates with 10 seeds in each replicate was used for this purpose. Seed volume (V) was calculated using following formula:

$$V = V_2 - V_1$$

Where V_1 is initial water level and V_2 is final level after dipping the seeds. Mean seed volume with standard deviation was calculated.

To determine the water uptake by the seeds, five replicates of five seeds each was randomly taken and weighed individually for their initial weight using the electric balance. Seeds was then soaked in distilled water and kept at room temperature (25+2°C). Weight of these seeds was taken after every 24 h till the constant weight.

Water imbibition was estimated as percent increase in weight of seed using following formula:

$$\text{Imbibition rate} = \frac{\text{Imbibed weight - Initial weight}}{\text{Initial weight}} \times 100$$

Table 3. Monthwise germination per cent of seeds at refrigerated condition.

Sl. No.	Location	December	January	February	March	April
1	Durtlang	63.33 ± 5.77	76.67 ± 11.55	86.67 ± 5.77	73.33 ± 11.55	66.67 ± 5.77
2	Reiek	66.67 ± 5.77	66.67 ± 5.77	96.67 ±5.77	76.67 ± 15.28	56.67 ± 5.77
3	Luangmual	46.67 ± 5.77	93.33 ± 5.77	76.67 ± 5.77	66.67 ± 5.77	83.33 ± 15.28
4	Lungdai	40.00 ± 14.14	90.00 ± 10.00	73.33 ± 11.55	83.33 ± 15.28	76.67 ± 5.77
5	Serkhan	53.33 ± 5.77	83.33 ± 15.28	86.67 ± 5.77	63.33 ± 15.28	83.33 ± 11.55
6	Zonuam	43.33 ± 15.28	83.33 ± 5.77	76.67 ± 15.28	70.00 ± 20.00	70.00 ± 10.00
7	Chawlhhmun	50.0 ± 10.00	73.33 ± 11.55	63.33 ± 11.55	66.67 ± 5.77	70.00 ± 10.00
8	Tanhril	53.3 ± 15.28	76.67 ± 11.55	83.33 ± 15.28	80.00 ± 10.00	73.33 ± 15.28
9	Sakawrtuichhun	56.7 ±5.77	86.67 ± 5.77	86.67 ± 15.28	66.67 ± 15.28	63.33 ± 25.17
10	Lengpui	60.0 ± 20.00	80.00 ± 10.00	66.67 ± 5.77	66.67 ± 30.55	56.67 ± 5.77
	SEd (±)	7.22	6.83	6.50	5.74	7.95
	$CD_{0.05}$	15.16	14.35	13.65	12.06	16.71

Table 3. Continued.

Sl. No.	Location	May	June	July	August
1	Durtlang	43.33 ± 15.28	43.33 ± 5.77	3.33 ± 5.77	3.33 ± 5.77
2	Reiek	30.00 ± 10.00	40.00 ± 17.32	6.67 ± 5.77	0.00 ± 0.00
3	Luangmual	36.67 ± 15.28	26.67 ± 5.77	0.00 ± 0.00	0.00 ± 0.00
4	Lungdai	40.00 ± 17.32	30.00 ± 20.00	3.33 ± 5.77	0.00 ± 0.00
5	Serkhan	30.00 ± 10.00	30.00 ± 17.32	0.00 ± 0.00	3.33 ± 5.77
6	Zonuam	26.67 ± 5.77	40.00 ± 10.00	6.67 ± 11.55	0.00 ± 0.00
7	Chawlhhmun	33.33 ± 25.17	26.67 ± 5.77	3.33 ± 5.77	0.00 ± 0.00
8	Tanhril	40.00 ± 20.00	23.33 ± 5.77	3.35 ± 5.77	0.00 ± 0.00
9	Sakawrtuichhun	30.00 ± 20.00	26.67 ± 11.55	3.33 ± 5.77	3.33 ± 5.77
10	Lengpui	33.33 ± 20.82	20.00 ± 10.00	0.00 ± 0.00	0.00 ± 0.00
	SEd (±)	4.65	6.02	0.08	0.08
	$CD_{0.05}$	9.77	12.64	0.16	0.16

Germination test was conducted at monthly interval in refrigerated condition and ambient temperature. The seeds collected from different locations were kept in perforated polythene bags inside the refrigerator to study the effect of germination at monthly interval in refrigerated condition and ambient temperature.

Results and Discussion

The results of the present study as depicted in Table 2 revealed that among the various locations, significant to highly significant variation was observed among the seeds. The maximum seed wight was obtained from the seeds collected from Reiek (0.40±0.02 g) followed by the seeds collected from Zonuam (0.38±0.03 g). Among all the locations the lowest seed weight was obtained in Chawlhhmun (0.21±0.01 g). Similarly, among the different locations, the significantly maximum seed length was obtained from the seeds collected from Reiek (7.38±0.46 mm) followed by Zonuam (7.02±0.28 mm). Among the different seeds collected from various locations, the significantly lowest length was obtained from the seeds collected from Sakawrtuichhun (6.46±0.24 mm). Similarly, there was significant variation among the germplasms with respect to diameter of the seeds. The maximum was obtained from the seeds collected from Reiek (5.86±0.48 mm) and the minimum was obtained from the seeds collected from Durtlang (5.02±0.30 mm). The variation in physical properties of the seeds might be due to different genetic make-up of the individual seeds.

Seed moisture is a critical factor determining the

Table 4. Monthwise germination per cent of seeds at ambient condition.

Sl. No.	Location	December	January	February	March	April
1	Durtlang	50.00 ± 10.00	70.00 ± 10.00	86.67 ± 5.77	70.00 ± 26.46	66.67 ± 11.55
2	Reiek	50.00 ± 10.00	90.00 ± 10.00	80.00 ± 0.00	66.67 ± 15.28	60.00 ± 10.00
3	Luangmual	46.67 ± 15.28	66.67 ± 5.77	70.00 ± 10.00	63.33 ± 5.77	53.33 ± 5.77
4	Lungdai	43.33 ± 15.28	56.67 ± 5.77	63.33 ± 5.77	56.67 ± 5.77	50.00 ± 10.00
5	Serkhan	43.33 ± 5.77	76.67 ± 5.77	60.00 ± 10.00	86.67 ± 15.28	70.00 ± 10.00
6	Zonuam	33.33 ± 5.77	70.00 ± 10.00	70.00 ± 10.00	80.00 ± 20.00	50.00 ± 10.00
7	Chawlhhmun	46.67 ± 15.28	56.67 ± 15.28	86.67 ± 5.77	63.33 ± 15.28	43.33 ± 15.28
8	Tanhril	53.33 ± 15.28	76.67 ± 15.28	86.67 ± 23.09	70.00 ± 10.00	40.00 ± 20.00
9	Sakawrtuichhun	46.67 ± 5.77	53.33 ± 11.55	73.33 ± 5.77	70.00 ± 17.32	36.67 ± 11.55
10	Lengpui	36.67 ± 5.77	46.67 ± 15.28	73.33 ± 15.28	76.67 ± 5.77	40.00 ± 20.00
	SEd (±)	4.77	6.61	6.71	7.08	8.66
	$CD_{0.05}$	10.01	13.89	14.10	14.87	18.20

Table 4. Continued.

Sl. No.	Location	May	June	July	August
1	Durtlang	26.67 ± 5.77	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
2	Reiek	23.33 ± 15.28	3.33 ± 5.77	0.0 ± 0.0	0.0 ± 0.0
3	Luangmual	16.67 ± 5.77	6.67 ± 11.55	0.0 ± 0.0	0.0 ± 0.0
4	Lungdai	23.33 ± 20.82	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
5	Serkhan	23.33 ± 15.28	3.33 ± 5.77	0.0 ± 0.0	0.0 ± 0.0
6	Zonuam	13.33 ± 5.77	6.67 ± 11.55	0.0 ± 0.0	0.0 ± 0.0
7	Chawlhhmun	26.67 ± 23.09	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
8	Tanhril	13.33 ± 11.55	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
9	Sakawrtuichhun	26.67 ± 15.28	6.67 ± 11.55	0.0 ± 0.0	0.0 ± 0.0
10	Lengpui	30.00 ± 26.46	0.00 ± 0.00	0.0 ± 0.0	0.0 ± 0.0
	SEd (±)	4.86	2.58		
	$CD_{0.05}$	10.22	5.42		

viability and longevity of both recalcitrant and orthodox seeds. In our present investigation, the moisture loss was increased with the storage period right from 30 days after storage till 90 days. Among the seeds collected from various locations, the moisture loss was also varied significantly throughout the observation periods. It is clear from the data presented in Table 2, that at 30 days after storage, the maximum moisture loss was observed in found from the seeds collected from Tanhril (17.65 ± 0.01%), while the lowest was reported from the seeds collected from Serkhan (10.05 ± 0.03%). At 60 days after storage, the moisture loss followed the similar pattern as that of 30 days. The maximum was in Tanhril (18.26±0.02%), and the lowest was in Serkhan (13.50 ± 0.01%). At 90 days after storage of the seeds, the maximum moisture loss was observed in found from the seeds collected from Durtlang (19.13±0.02%), while the lowest was from the seeds collected from Sakawrtuichhun (14.13±0.00%). The variation in moisture content of this plant might be due to different ripening stage of the fruits as well as different soil and climatic condition and inherent character associated with species.

The seeds varied significantly with respect to the volume of seeds also (Table 2). The highest seed volume of was found in the seeds collected from Reiek (0.86±0.12 cc) and lowest was in Durtlang (0.55±0.07 cc). The variation in seed volume might be due to the size of the seed coat, in addition embryo of the seeds also increases the volume of the seed.

The imbibition result presented in Table 2 showed that percentage imbibition was found to be the high-

est in Lungdai (43.91±1.98) and the lowest was observed in Reiek (30.70±2.67). Claimed that porous seed coats are usually permeable and non-porous ones impermeable. The seeds of *Clerodendrum* have small opening or pore on the ventral side of the seeds. Water can pass through easily so that the imbibition speed can be enhanced by this opening. But there should be limitations for entering the water into the seeds. It also depends upon the size of the embryo and the hardness of the seed coat.

There are two types of seeds in nature. One is orthodox and another one is recalcitrant seeds. Orthodox seeds dry up-to low moisture content and can tolerate freezing temperature and can be stored for many years. But recalcitrant seeds are dessicated sensitive, loss viability in a short period and cannot be stored for prolonged. Attempted has been made on the storage of recalcitrant seeds, such as moist storage, partially dry storage and cryostorage, but all these methods have their own limitations. The seeds *Clerodendrum colebrokianum* comes under the latter. The seeds were collected in the month of November and germination test conducted from December till it losses viability. The seeds we stored in ambient temperature (room temperature) losses viability after 6 months of stored in ambient conditions and by 8 months if stored in refrigerated conditions. The germination was initially low irrespective of the locations and storage conditions and it reaches the maximum by 3rd months and thereafter again decreases gradually and finally loss the viability by 6 months in ambient condition and 8 months in refrigerated condition. In the present investigation, in refrigerated storage, at 3rd month after storage, the maximum germination was obtained in the seeds collected from Reiek (96.67 ± 5.77) at 3 months after storage, while in ambient conditions, the maximum germination was obtained from the seeds collected from Reiek (90.00 ± 10.00%) at 2 months after storage (Tables 3 and 4). Viability test indicated that seeds of *C. colebrokianum* may not remain viable for extended periods at room temperature and even storage at low temperature (4^{o}C – 6^{o}C) for long time. Loss of viability in stored seeds is a common phenomenon, and it increased with storage duration with storage condition as another factor. However, it was also observed that seed moisture content did not played key role as population with low seed moisture at the time of harvesting had high viability in *C. colebrokianum*, Further, seed moisture was slightly decreased after one year storage. Loss of viability as well as variation in seed viability among different natural populations may have the relation with growth and development of embryo which caused morpho-physiological dormancy as suggested earlier [10] in another Apiaceae species *Osmorhiza depauperata*.

Conclusion

Clerodendrum colebrokianum seeds are recalcitrant so they loss the viability very soon. In the present investigation, observations reveals variation in seed weight, seed length, seed diameter and seed volume in addition to moisture content as well as imbibition rate of the seeds. Viability among the different populations irrespective of moisture content which may suggests morpho-physiological type of dormancy in this species. On the basis of present observations, it is further suggested that seed storage at low temperature remain viable for eight months. Further observations are needed to overcome morpho-physiological dormancy and bringing uniformity in germination behavior of *C. colebrokianum*.

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