MORPHOLOGICAL AND BIOCHEMICAL VARIATION IN ADHATODA TYPES (*Adhatoda* spp.)

By
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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

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VELLANIKKARA, THRISSUR - 680 656
KERALA

2001

DECLARATION

I hereby declare that this thesis entitled **"Morphological and biochemical variations in adhatoda types (*Adhatoda* spp.)"** is a bonafide record of research work done by me during the course of research and that it has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Morphological and biochemical variations in adhatoda types (*Adhatoda* spp.)"** is a record of research work done independently by **Mrs. C. M . Manjusha**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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ACKNOWLEDGEMENT

Let me first thank the Almighty for enabling me to complete this task successfully.

I am extremely privileged to place my deep sense of gratitude to Dr. Alice Kurian, Associate Professor, Department of Plantation Crops & Spices and chairperson of my advisory committee for her kind concern, expert advice, utmost sense of patience and unparalleled support throughout the period of the investigation and preparation of the thesis. I am really grateful for the keen interest taken by her in the prompt correction of the manuscript, which has helped me a lot in the timely submission of the thesis.

My obligation to Dr. A.Augustin, Associate Professor (Biochemistry), AICRP on M & AP and member of my advisory committee cannot be expressed in words. I sincerely acknowledge the ever-willing help rendered in the biochemical analysis and constructive criticism in interpreting the results.

My heartfelt thanks are due to Dr. E.V.Nybe, Associate Professor and Head (i/c), Department of Plantation Crops & Spices for his invaluable help and critical suggestions during the course of preparation of the thesis.

I express my profound sense of gratitude to Dr. V.S.Sujatha for her scholarly suggestions and sincere help for the critical evaluation of the manuscript.

I owe thanks to Dr. P.A.Nazeem for providing the necessary facilities at CPBMB.

I sincerely acknowledge the help rendered in the statistical analysis of the data by Sri. S.Krishnan, Assistant Professor, Department of Agricultural Statistics.

I cannot forget the valuable suggestions and sustained interest taken by Dr. Z.Abraham, Officer-in-charge, NBPGR Regional Station, Vellanikkara, which has helped me a lot in interpretation of the results.

With immense pleasure, I thank each and every member of Department of Plantation Crops & Spices for their support and encouragement.

I am thankful to Mr. Sreekumar.P.K, (CCRP) for his photographic works and Dr.T.D.Babu and Dr. C.R.Achuthan, Research Associates, CPBMB for their help rendered during the conduct of the research work.

My profound sense of gratitude is also due to Chithra and Drowpadi Chechi for their invaluable support and help at the biochemistry lab.

Award of Junior fellowship by KAU is greatly acknowledged.

With immense delight, I acknowledge the cooperation and skilled assistance by all the labourers of College of Horticulture.

The timely help and loving encouragements extended by my dear friends Sanchu,Seena,Sonia,Viji,Reshmi,Shirish and Govind is highly cherished.

My soulful gratitude to Harikkuttan and Ajithettan for the prompt typing of the manuscript and timely help rendered.

I am forever beholden to my husband for his boundless affection and encouragement, which made me confident and optimistic during the course of my research work.

I am at loss of words to express my indebtedness to my in-laws, mother, sister and her family for their constant support and prayers.

The loving memories of my father who was very stable and affectionate gave me courage to face all the difficulties during my research work.

Dedicated to
my loving family

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

Introduction

INTRODUCTION

Plant based medical traditions make a very significant contribution to our country's health care. India is known for its plant resources from time immemorial and is one of the 12 biodiversity centres sheltering over 45,000 different plant species. Over 7500 species of plants are estimated to be used by 4635 ethnic communities for human and veterinary health care, across the various ecosystems from the transhimalayas to the southern tip of India and from the west coast to the far corners of the north east. The trade of medicinal plants in India is estimated to be to the tune of Rs. 550 crores per annum.

However, many of the medicinal plants are facing threat to their survival at varying degrees due to over exploitation and degradation, disturbance and outright loss of natural habitats. Germplasm conservation is one of the most important and urgent tasks of plant scientists today and this need is greatest in the tropics, particularly in tropical Asia where genetic diversity is very wide and existence of many species is threatened.

Changes in ecological and geographical conditions and plant reactions to them are directly or indirectly reflected in the rise of intraspecific taxum. Similarly, chemical differences in spontaneously occurring population which are genetically assimilated and controlled have led to the identification of chemical races in many commercial cultivated medicinal plant species. Earlier, physiological and morphological attributes had been the primary criteria for differentiating cultivars. But these characteristics alone have not always proven satisfactory. Natural compounds directly involved like proteins, secondary metabolites like alkaloids, phenolics etc. have been used as chemical markers to aid in positive cultivar identification.

Adhatoda sp. belonging to the family Acanthaceae, the family of the common cutflower crossandra is a medicinal plant well known as the source of the drug **vasaka** in the indigenous systems of medicine for bronchitis. Its leaves, flowers, fruits, and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis and asthma.The plant is of high demand in local markets and traditional preparations of vasa like *vasarishtam, vasakasavam, vasahareetaki* etc. are exported to the international markets. The crop is one among a few recommended for commercial cultivation in Kerala especially as an intercrop in the coconut gardens and also as a live fence .

It is an evergreen glabrous perennial shrub growing in moist deciduous regions of the country. *Adhatoda* derives its name from *'Adu'* and *'thoda'* which means the plant not eaten by goat. It is popularly known as malabar nut (English), atalodakam (Malayalam), adatodai (Tamil) arusha (Hindi) and vasa (Sanskrit). The classical texts of Ayurveda describe the properties of vasa as bitter (tikta), acrid, cooling (sita) and alleviating pitta. It is administered against dysponea (swasa), cough (kasa), fever (jwara), certain skin diseases (kusta) and wasting (kshaya).

Whole plant extract is used as sedative, antispasmodic, anthelmintic and leaf juice is stated to cure diarrhoea, dysentry and glandular tumour. The powder is reported to be used as poultice in rheumatic joints, as counter-irritant on inflammatory swelling of fresh wounds, urticartia and neuralgia. Vasicine which is the major alkaloid present is a promising cholagogue, uterotonic abortifacient and has proven useful for the control of postpartum haemorrhage. The leaves exhibit hypoglycaemic and selective anti-bacterial activity and used as insecticide and fungicide. The flowers are used to improve the circulation of blood and fresh ones are used in ophthalmia. The plant being a rich source of nitrogen is grown as green manure in rice fields where it also acts as an aquatic weedicide (CSIR, 1985).

Kerala physicians recognise two varieties of vasa, "atalodakam" considered as *Adhatoda zeylanica* which grows wild almost throughout India and "chittatalodakam" equated to *A.beddomei* which is confined to Travancore districts of Kerala and seen only under cultivation. The identity of chittatalodakam is questioned since *A.beddomei* is argued to be a type of *A.zeylanica* itself. Germplasm collection of *Adhatoda* types maintained at the Department of Plantation Crops and Spices, College of Horticulture,Vellanikkara showed wide variability in morphological features and exhibited characters intermediate to the two species. In view of this, the proposed study is aimed at characterizing the germplasm accessions and ascertaining the species identity of chittatalodakam based on morphological variation, which can be verified and supplemented through biochemical studies. The species identity of chittatalodakam if confirmed makes the study a step towards the conservation of *Adhatoda beddomei,* a rare and endangered species.

In this context the present study was proposed with the following objectives.

1) To characterize 52 accessions of *Adhatoda* available at the Department of Plantation Crops and Spices, College of Horticulture,Vellanikkara based on morphological, histological and biochemical variations and attempt grouping of accessions.

2) To analyse representative types of characteristic groups for variation in secondary metabolites like phenols, flavonoids, terpenoids and alkaloids and yield attributes.
3) To study the seasonal influence on morphological and yield attributes and distribution of secondary metabolites.

Review of literature

REVIEW OF LITERATURE

The genus *Adhatoda* had been mentioned earlier in the 17th century by Rheede (1678) and reports of its use in ayurvedic medicinal preparations trace back to centuries. Taxonomical and phytochemical studies of the plant had been attempted by many workers.Literature pertaining to morphological and biochemical variation in *Adhatoda* and other plants are presented below.

2.1. Taxonomic history of *Adhatoda*

Adhatoda Nees belonging to family Acanthaceae was earlier included in the genus *Justicia*. Linnaeus (1737) proposed the genus *Justicia* first based on the Asiatic species *Justicia adhatoda*. However, later in "Species Plantarum" he inducted ten more species into this genus, without altering the circumscription. At currently known *Justicia* includes about 300 species in it and is distributed along both the Old and the New world, exhibiting immense ranges of variations in the vegetative as well as floral features. So, Nees proposed the splitting of this genus into different genera like *Adhatoda, Gendarussa, Justicia, and Rostellularia*. Hooker (1885) reported two species of *Adhatoda*. *Adhatoda vasica* Nees and *A.beddomei* Clarke. Aiyar and Kolammal (1962) gave a detailed description of both the species with respect to morphological and histological variations. *Adhatoda vasica* Nees is also referred to as *A.zeylanica* Medic. This species had been mentioned in 'Flora of Calicut' by Manilal and Sivarajan (1982). Hussain *et al* (1992) described uses, biological activity and chemical constituents of *Adhatoda zeylanica* Medic (syn. *A vasica* Nees, *Justicia adhatoda* L.)

The chromosome number of *Adhatoda vasica* had been reported to be 34 by Mukherjee (1952), Hardas and Joshi (1954), Verma and Dhillon (1967), Mehra and Gill (1968) Datta and Maiti (1968) reported the chromosome number as 40 and 50 where as Grant (1956) reported it as 56. Krishnappa and Ramaiah(1982) detected

numerical variation in *Justicia*.They found chromosome number to be 2n=34 (*J.betonica*),2n=18 (*J.diffusa*), 2n 30 (*J gendarussa*), 2n=54 (*J.glabra*), 2n=36 (*J.glauca*) and 2n=18 (*J.latissica*). Saggoo and Bir(1986) studied different species and cultivars of *Justicia* and detected both diploid (n=9) and tetraploid (n=18) cytotypes .*J.procumbens* tetraploid individuals showed gigantism. Saggoo (1988) also studied chromosomal evolution in Indian *Justicia*. Pundarikakshudu and Bhavsar(1988) studied two types of *Adhatoda vasica* namely big leaf variety(BLV) and small leaf variety(SLV) and observed a diploid chromosome number of 34 in both the types.

Cross pollination with *Xylocopa* sp. as the major pollinating agent had been reported in *Adhatoda zeylanica* by Reddi *et al.*(1989).About 90% of fruit set was obtained through xenogamy as the mode of pollination where as only 50 and 75 % fruit set was obtained through autogamy and geitonogamy respectively

2.2 Phytochemistry of *Adhatoda*

Extensive phytochemical investigations in the crop have made possible identification of several alkaloids and their salts, essential oil, glycosides, triterpenoids, lignins, sugars, alcohol and hydroxyketones which are found to have several pharmacological activities in man.

Among alkaloids, the important and most common one is vasicine which is a quinazoline alkaloid with varying optical rotation. Vasicine is bitter and probably occurs in nature in its *l*-form and is racemized during the process of isolation It forms colourless crystals and is readily soluble in chloroform. The specific rotation of *l*-vasicine is strongly dependent on the nature of the solvent and concentration of solution. Photo-oxidation of vasicine yields a mixture of *l* and *d* forms of vasicinone, where as the naturally occurring vasicinone is laevorotatory.

Mithal and Schroff (1954) obtained vasicine as quaternary ammonium base on destructive distillation of vasaka leaves, Mehta (1960) isolated vasicine from the leaves of *A.vasica* by solvent extraction and crystals of vasicinone by keeping the solution overnight Bronchodilating action of vasicine and related compounds

including, vasicine and quinazol-4-one was studied by Cambridge *et al.* (1962). Vasicinone was obtained by aerial oxidation in sunlight of crude extracts of *Adhatoda vasica* or of pure vasicine. They suggested that this property was attributed to the quinazol-4-one ring system for they found it to be common to the parent substance and various simple derivatives.

Rangaswami and Seshadri (1971) examined flowers of *A.vasica* and reported the presence of vasicine, tritriacontane,β-sitosterol, luteolin,α-amyrin and β –sitosterol-D-glycoside. From the ether extract kaempferol and quercetin were isolated by column chromatography. Two crystalline flavonoid glycosides were obtained from ethyl acetate and n-butanol extracts.

Gupta *et al.* (1977) also isolated vasicine, vasicinone and vasicinol and made a comparative pharmacological study. Chowdhury (1979) could isolate *dl*-vasicinone after chromatographic resolution from a mixture of alkaloids obtained from ethanol extract of air-dried inflorescence. D'cruz *et al.* (1979) obtained an essential oil from the leaves of *A.vasica* which was shown to possess bronchodilatory activity. Atal (1980) conducted phytochemical and pharmacological investigations on the plant and reported the presence of the alkaloid vasicine which contributes 75 to 90% of the total alkaloids. Improved method of extraction and analysis of vasicine and vasicinone was given by Bhalla *et al.* (1982). Phytochemical investigation of the roots of *A.vasica* by Jain and Sharma (1982) could yield two novel alkaloids adhatodine and vasicol besides the already reported alkaloids vasicine, vasicinone and vasicinol. They also reported the presence of a new alkaloid vasicinolone which appeared to be an oxidative product of vasicinol. Isolation of vasicine hydrochloride by Atal *et al.* (1982) and vasicinone hydrochloride by Bhalla (1982) led to the use of more bronchodilatory compounds from *A. vasica.*

High performance liquid chromatographic (HPLC) determination of vasicine and vasicinone in *A.vasica* had been reported by Brain and Thapa (1983). Jain and Srivastava (1985) conducted preliminary phytochemical studies of *A.beddomei.* Ethanolic extracts of the whole plant yielded vasicine, vasicinone, deoxyvasicinone, betasitosterol and its glycoside. HPLC method of estimating vasicine in *A.vasica* had been standardised by Sharma *et al.* (1991). Singh *et al* (1991) characterized two new

aliphatic hydroxyketones from the aerial parts of *A.vasica*. From the petrol extract of aerial parts of *A. vasica* a new aliphatic alcohol had been isolated and characterized by Singh *et al.*(1992).

Agarwal *et al.* (1993) isolated lignins and Sarraf *et al.* (1994) isolated nine free sugars and two vitamins from leaves, root and bark of *A.vasica* Joshi *et al.* (1994) made an extensive investigation of quinazoline alkaloids of *A.vasica* and isolated the pyrrolo (2, 1b) quinazoline alkaloids l-vasicine, l-vasicinone, l-vasicol, anisotine, 3-hydroxy anisotine, and a new alkaloid vasnetine. Atta-ur-Rahman *et al.* (1997) isolated a new triterpenoid for the first time from the aerial parts of *A.vasica*.

2.3. Characterization based on morphological characters

2.3.1.Adhatoda

Kerala physicians recognise two varieties of *Adhatoda*, "atalodakam" which grows wild almost throughout India and "chittatalodakam" which is confined to the Travancore districts of Kerala and seen only under cultivation. Ayurvedic Materia Medica considers them as two different species, atalodakam equated with *Adhatoda zeylanica* Medicus and chittatalodakam with *Adhatoda beddomei* Clarke.

Hooker (1886) described the two species as follows.

Adhatoda vasica Nees : It is a dense shrub, 4-8 ft tall, sometimes arborescent upto 20ft, distributed from Punjab and Assam to Ceylon, Singapore, Malaysia and South East Asia. Leaves are elliptic 8 by 8 inch size, acute at both ends, entire, minutely pubescent and petiole 1 inch. Spikes 1-8inch, terminal often several together, dense and short bracts ¾ by ¼ inch, ovate or obovate elliptic ,bracteoles ½ by 1/8 inch falcate oblong. Calyx 1/3 by 1/8 inch, deeply 5 lobed ; lobes equal and lanceolate Corolla-tube 1/8-1/6 inch broad; lips white, palate transversely rose-barred. Stamens glabrous, anther cells accuminate at base, sometimes minutely white tailed. Ovary and style base minutely hairy. Capsule ¾ inch,clavate, pubescent, 4 seeded. Seeds ½ inch diameter, glabrous tubercular-verrucose.

A.beddomei Clarke:

A very large shrub distributed in South Travancore with leaves oblong, attenuate at both ends, entire, minutely pubescent 6 by 11/2 inch in size and petiole ½ inch. Peduncles axillary, opposite, stout heads 1 inch diameter, bracts ½ by 1/6 –1/4 inch, narrowed at base ; bracteoles ¼-1/2 inch, narrowly oblong. Calyx ¼ inch, sub-5-partite; segments narrowly oblong, acute, minutely pubescent especially within Corolla hairy; tube ½ inch, broadly cyllindric ; lips oblong upper emarginate, lower 8-lobed nearly to the base. Filaments stout, glabrous, except at base, anther-cells superposed, lower obscurely tailed. Ovary glabrous, 4 ovulate. style hairy, stigma minutely bifid. Capsule not known.

A mention about *A. vasica* had been made by CSIR (1948) as a small evergreen shrub occurring throughout the plains of India and in sub- Himalayan tracts ascending upto 4000 feet. Gamble (1957) also described *A.vasica* as a dense shrub distributed all over India, Ceylon and Singapore. It has long lanceolate leaves with acute ends, terminal spikes often several together, large flowers with long bracts. Ovary is pubescent, style hairy at base. *A. beddomei* is a smaller shrub distributed only in Kerala with small oblong lanceolate leaves with attenuate ends, shorter spikes occurring solitary. Ovary is glabrous, style terminal hairy and stigma minutely bifid

Aiyer and Kolammal (1962) after studying the pharmacognosy of Adhatoda gave a more detailed description of the two speices with reference to morphological features which agreed with Gamble (1957).

A.vasica Nees :

It is a large sized densely branched, bushy evergreen sub-herbaceous pubescent shrub growing to a height of two to two and a half meters or more. Leaves are large broadly elliptic or elliptic lanceolate, entire, tapering and acute at both ends, dark green above and paler beneath, upto 20 cm. long and 7 or 8 cm. wide with ten to twelve or even upto 15 pairs of rather close prominent secondary nerves.

The inflorescence is a long pedunculate short stout dense flowered prominently bracteate decussate often thyrsiform spike 5 to 10cm long. Flowers are subsessile in the axils of opposite bracts, large white or sulphur yellow. Bracts are sessile, 8 to 25 mm long and 6 mm. wide, ovate or elliptic, sub-acute and obscurely five to seven

ribbed. Bracteoles are 12mm by 3 mm size narrow, falcate oblong. Calyx is gamosepalous 8 to 12 mm long, glabrous or slightly pubescent and deeply five lobed with the lobes equal lanceolate or oblong lanceolate, acute and three nerved. Corolla is whitish, gamopetalous about 30 cm long. Its tube is short 3 to 8 mm by 12 to 8 mm broadly cylindrical to funnel shaped in the lower half, the upper half much laterally inflated ; the throat wide, coloured sulphur yellow and transversely marked with a few irregular rose red or yellow bands or streaks; the upper lip ovate oblong vaulted, obtuse or emarginate ; the lower-broad spreading and deeply three lobed with the lobes oblong rounded, the middle lobe being the broadest. Stamens – two attached near the top of the corolla tube; has long stout, curved filaments that are dilated and hairy at base and concealed under the vault of upper lip; Pistil-bicarpellary syncarpous, ovary-superior, minutely hairy or pubescent, two locular, with two ovules in each chamber, style-terminal filiform, hairy in its basal half with an obtuse entire or minutely bifid stigma at its tip.

A.beddomei Clarke:

The plant is distributed in hills of south Travancore from sea level to 900m elevation. It is a large sized glabrous shrub, similar to *A.vasica* in habit, but smaller, bearing simple, opposite, short petioled, oblong-lanceolate or elliptic-lanceolate, entire leaves 10 to 18 cm.long and 3 to 4 cm broad, attenuate at both ends with a prominent midrib and 8 to 10 pairs of distantly spaced minutely puberulous secondary nerves. Inflorescence;short peduncled condensed spikes or heads 1 to 2 or 2.5 cms in diameter, the peduncles mostly axillary, stout from 1.2 to 1.8cm.or occassionally upto 5 cm.long. Flowers: small, whitish, bisexual, zygomorphic. Bracts: ovate, subacute, puberulous, glabrate, obscurely 5 ribbed, narrowed at base, 1.3 cm long and 6 to 10 mm wide. Bracteoles: narrowly oblong about 6 to 10mm long. Calyx - about 6 mm long; sub-five partite, the segments linear oblong, acute and minutely pubescent especially on the inside. Corolla - about 2 to 2.5 cm long, creamy white, hairy with a short broadly cylindrical tube about 10mm long, the upper lips emarginate and lower three lobed or partite. Stamens – two, epipetalous, filaments stout and glabrous except at their base; Pistil-bicarpellary, syncarpous; ovary- superior, glabrous, bilocular with two ovules in each chamber; style –terminal hairy ending in a minutely bifid stigma.

Datta and Maiti (1968) collected *A.vasica* from different regions of the Eastern part of India and found six characteristic biotypes. They differed in structure and sometimes in number of chromosomes This suggested an evolution of cytotypes within this species brought about by translocation, fragmentation, deletion etc. as well as by polyploidy or aneuploidy. These chromosomal changes had probably selected out cytotypes best suited to their natural environments. Chromosomal differences may be related to the quantitative anatomical characters and to the quantity and quality of chemical substances available in this species. Datta and Samanta (1974) reported the existence of different cytotypes in this plant.

The two species *A.zeylanica* Medic. and *A.beddomei* Clarke had been described by CSIR (1985).The former is an evergreen, stiff perennial shrub 1.2 to 6.0m in height distributed throughout India upto an altitude of 1300m. Leaves are elliptic – lanceolate or ovate lanceolate, entire 5-30cm long, hairy, flowers are large, white with red or yellow-barred throats, in spikes with large bracts. *A.beddomei* is a medium sized shrub, 1-2 m in height, with broad oblong-lanceolate or elliptic-lanceolate 12.5 –18.0cm long leaves and white flowers in dense, bracteate spikes, commonly found in Kerala upto 900 m.

Henry *et al.* (1987) also described the two species *Justicia adhathoda* L. and *J. beddomei* Clarke. Former is a shrub with foetid smell, cultivated and often run wild; flowers white, throat barred with red or yellow. The latter is a large shrub with small flowers in short heads distributed in Coimbatore, South Arcot and Kanyakumari.

Pundarikakshudu and Bhavsar (1988) made detailed studies on the two types of *Adhatoda* . One type designated as small leaf variety (SLV) is shorter growing to a height of 3-5 feet. Stem is round and flattened only at the nodal region, branches are less in number and the plant appears like a small shrub. Internodes are 1 to 5 cm long, leaves small 10-15 cm long and 3-6 cm broad, dark green in colour with 8-10 pairs of veins. Petioles are comparatively shorter, 1-2 cm long. Inflorescence is a spike with short peduncle. Bracts and bracteoles are compactly arranged on the spikes. Peduncle 2-5 cm long and grooved, bracts are 1-2 cm long and 0.5 to 1 cm wide, ovate to lanceolate and densely arranged with 4 nerves.

In the other type designated as big leaf variety (BLV) plants grow upto a height of 6-8 feet. Stem is flattened rather than round and very much flattened at nodal region. Plants are more branched and dense giving a bushy appearance, internodes slightly longer and their length ranged from 3-6 cm. Leaves are 20-30 cm long and 4-8 cm wide, light green with 13-14 pairs of veins. Petioles are 1-3 cm long, spikes have long peduncles. Flowers, bracts and bracteoles are loosely arranged on the spikes Peduncle- 3-6 cm long and grooved. Bracts are very big, 2.5-3.1 cm in length, 1.2 to 1. 5 cm in breadth and generally showed 6 to7 nerves.

2.3.2. Other Crops

Characterisation and evaluation of sunflower (*Helianthus annuus* L.) germplasm was attempted by Mogali and Virupakshappa (1994). One ninety six germplasm accessions of sunflower were evaluated for quantitative and qualitative traits. They grouped the accessions on the basis of morphological traits like plant height, leaf shape, leaf colour, petiole length, branching pattern etc. While characterizing the germplasm, it was noticed that there was wide variability for all qualitative characters among the genotypes.

Vadukkoot (1996) evaluated morpho-anatomical variations in *Ocimum* spp. by selecting four species viz. *Ocimum tenuiflorum*, *O.gratissimum*, *O. basilicum* and *O.canum*. She compared various morphological observations of leaf, stem, inflorescence, floral and fruit characters between the species.

Cyto-morphological investigations in *Piper* spp.were done by Anand (1997) and prepared brief descriptions of eight *Piper* spp. based on the salient observations on morphology.Various morphological observations according to the descriptor included leaf colour, leaf shape, leaf tip, hairiness of leaf, stem characters like shape, colour, branching pattern etc. Based on the morphological comparisons, a key for identification of the eight species studied had been proposed.

Surveys were conducted to study the variability in Kodampuli *(Garcinia cambogia* Desr.) by Muthulakshmi (1998). Variability in vegetative, floral, fruiting and biochemical characters of Kodampuli was analysed. A wide variation was identified in tree characters like shape of the tree and branching pattern, leaf characters, floral characters and fruit characters.

2.4. Histological Studies

Datta and Mukerjee (1952) reported the anatomical features of *A. vasica*. Upper epidermis showed the presence of glandular as well as non-glandular hairs comprising of 2-3 cells. Stomata was found only on the lower epidermis along with epidermal hairs. Aiyer and Kolammal (1962) described the histological difference between the two species of *Adhatoda*. They observed that stomata were absent in the upper epidermis of *A. vasica* but were occasionally found in the upper epidermis of the leaf of *A. beddomei*. Thick walled one to three celled unbranched trichomes with broad bases were present in the epidermis of both the species. Each trichome arose from the centre of a circular cell, which was surrounded by elongate radiating epidermal cells.

Naik and Nirgude (1981) attempted classification of 16 taxa of *Chlorophytum* based on the variation in number and size of stomata on the adaxial and abaxial surfaces. Stomatal index on the abaxial side ranged between 12.1 and 42.3 and that on adaxial side ranged from 1.9 to 38.9 with a mean of 32.7 and 13.5 respectively among all the species. Although this was the case in most of the taxa the general figures were different for *C. laxum* and *C. tuberosum* where the stomatal index was higher on the adaxial side than the abaxial face.

Distribution of stomata in the leaves and stipules in 66 species representing 34 genera of 5 different families of order Malvales had been studied by Rao and Ramayya (1981). 86.36% of species were amphistomatic and a lesser number hypostomatic (13.64%). A orrelated study between stomatal frequency and distribution on one hand and plant habit on the other showed that most of the seasonals and 81.25% of the perennials were amphistomatic. Majority of the hypostomatics were trees.

Validity and significance of cuticular features in the taxonomy of 8 species of *Datura* had been discussed by Sharma (1992). The highest mean stomatal freequency (32.3) was noticed in adaxial leaf surface of *D. quercifolia*, while lowest (8.5) was found in *D. suaveolens*. These characters were found to be quite specific for each taxon. Foliar epidermal characters in 12 species of *Cinnamomum* were studied by

Baruah and Nath (1997). They found that epidermal cell wall nature, stomata, stomatal index and trichomes were found to be very useful in deciphering the individual species except in *C.tamala* and *C. impressinervium*. The leaves were hypostomatic in all the species investigated.

Vadukkoot (1996) compared various anatomical observations among four species of *Ocimum* viz. *O.tenuiflorum*, *O.gratissimum*, *O.basilicum* and *O.canum*. Stomatal count as well as its size, features of trichomes, mesophyll tissue and vascular architecture were recorded. From the morphological and anatomical observations made during the study it appeared that *O.gratissimum* and *O.tenuiflorum* were phylogenetically related and so also *O.basilicum* and *O.canum*. Menon (1999) found that *Plumbago zeylanica* had lower number of stomates per microscopic field than *P. rosea* on close examination of their lower leaf surface.

2.5. Isozyme pattern and enzyme activity

Isozymes are the multiple molecular forms of enzymes, separable by electrophoretic procedures, occurring within the same organism and having similar or identical catalytic activities. As enzymes are the primary products of a gene, variation in their structure will give reliable information about the variability in the genotype itself and less susceptible to environmental influence at the population level. Almost all studies of geographic variation within a species using isozyme markers had shown that most of the variation observed reside within rather than between population.

An analysis of peroxidase isoenzyme was made on 43 varieties of castor bean comprising three height groups viz. dwarf, medium tall and tall in order to visualise the relationship between isoenzyme pattern and heights by Athma *et al.* (1982). The dwarf plants were found to have more peroxidase activity than those of tall types. There was a great deal of variability for the number and intensity of peroxidase band in different height groups of castor bean. On studying the leaf peroxidase and esterase banding pattern in 10 sandal (*Santalum album* L.) types, Parthasarathi *et al.* (1985) found that the sandal plants with normal ovate wavy and normal ovate non-wavy leaves were found to be close genetically.

In order to characterise some species of the genus *Kaempferia*, Suvachittanont (1991) conducted isozyme electrophoresis of esterase. Two morphotypes of *K.galanga* viz. proh-hom and jangang were compared and esterase in proh-hom showed two bands where as in jangang only one band was found. These findings suggested that proh-hom and jangang should be regarded as different species since they exhibit different gene products. Peroxidase isoenzyme patterns in Vitaceae were studied by Bachmann(1994) and 313 *Vitis* cultivars were analysed for peroxidase in the phloem of dormant canes. Variability in neutral to basic peroxidase was useful for grouping *V.vinifera* and interspecific cultivars. The isozyme pattern of 26 varieties and 11 *Piper* spp. were studied by comparing peroxidase, esterase and glutamate oxaloacetate transaminase (GOT) by Sebastian *et al.*(1996). The eleven species were grouped into five based on isozyme variation at peroxidase and two based on the variation at esterase. Rossini *et al.* (1995) characterised 26 cultivars of soyabean (*Glycine max* L.) recommended for the southern region of Brazil based on their peroxidase activity. Characterization of soyabean cultivars by peroxidase test was also done by Ortega *et al.*(1995). High peroxidase activity was shown by 79% of cultivars and low activity by the rest. It appeared that the peroxidase test could be usefully incorporated in routine seed testing work for monitoring genetic purity of the seeds. Peroxidase activity was estimated quantitatively in 17 *Portulaca grandiflora* mutants by Reddy and Bhalla (1995). It was greatest in the short branched, light pink and big flower mutants and lowest in long branched, 5 petalled pink and small flower mutants. Peroxidase activity appeared to be correlated with growth and development.

Sen *et al.*(1998) compared esterase banding patterns in *Oryza sativa* L. and *O.rufipogon* Griff. There were two fast migrating bands in all *O.sativa* cultivars and only one in *O.rufipogon*. In addition the wild species had two slow migrating esterase bands which were not present in *O.sativa*.

Chaudhary *et al.* (1998) characterized rice cultivars using peroxidase isozyme and found that most of the cultivars exhibited its own unique isozyme pattern. The enzyme system studied provided simple pattern of bands along with intervarietal variation.

Eighteen species of genus *Curcuma* and 33 accessions of *C. longa* were analysed for isozyme variation in peroxidase, esterase and G.O.T. by Joseph (1999). Isoenzyme banding pattern for peroxidase in *C.longa* genotypes showed 17 variant isoenzymes and genotypes were grouped into eight. Genotypes were grouped into 17 based on esterase banding pattern and five groups based on G.O.T. banding pattern.

2.6. Secondary metabolites in chemotaxonomy

Very early in the development of chemistry of natural products, botanists and chemists recognized the possibilities for characterization and classification of plants on the basis of their chemical constituents. Knowledge of the structure and occurrence of natural products in plants and the potential uses of chemotaxonomy are becoming obvious. In recent times besides physiological and morphological attributes, chemical markers are also used as an adjunct to the classification methods.

2.6.1. Alkaloids.

Alkaloids generally include those basic substances, which contain one or more nitrogen atoms, usually in combination as part of a cyclic system. They are usually colourless, often optically active substances, which are very heterogeneous chemically. As a considerable number of alkaloids are specific to one family or to a few related plants or to a species, their study can be effectively utilized for chemo taxonomical works.

Pundarikakshudu and Bhavsar (1988) studied variation in alkaloid contents of two types of *A. vasica* designated as big leaf variety (BLV) and small leaf variety (SLV). The leaves and roots of BLV plants had in general higher alkaloid concentrations as compared to those of SLV plants. In September, the roots and leaves of BLV plants contained triple the amount of alkaloids present in the leaves and roots of SLV plants. The systematic classification of the genus *Atropa* was attempted by Heltmann (1979) by studying the alkaloid content of leaves and roots of *Atropa.belladonna, A. accuminata, A. baetica* and *A. pallidiflora.* European populations of *A. belladonna* were found to be morphologically similar, alkaloid

content in leaves and roots was 0.18-0.43% and 0.34-0.93% respectively. *A. baetica* from Spain contained a higher percentage of alkaloid (0.91%).

Fruits and leaves of 31 species of *Solanum* were assayed for total alkaloid and presence of solasodine by Maili *et al.* (1979). The analysis revealed that only 12 species contained solasodine and that *S.khasianum, S.elaeagnifolium, S.auriculatum* and *S.giganteum* contained sufficient quantity of the total alkaloid. Alkaloid profiles of 21 *Lupinus* species indigenous to North and South America had been determined by Kinghorn *et al.* (1980). Taxonomic classification of genus was related to the pattern of alkaloidal distribution.

Muzquiz *et al.* (1994) studied the variation of alkaloid components of lupin seeds in 49 genotypes of *Lupinus albus* from different countries and locations. Twenty samples were sweet, while 29 were bitter and the taste was positively correlated with the alkaloidal content. Vogel *et al.* (1997) analysed different populations of *Peumus boldus* for their alkaloid content. Plants were collected from different regions of Chile and it was seen that population of the South showed the lowest alkaloid concentrations while that of North and Central parts showed highest alkaloid concentrations. The alkaloid content of seeds and foliage of six *Erythrina* species was found to differ according to a study conducted by Mateos *et al.* (1998).

2.6.2 Phenolics

The potential of phenolic compounds in chemotaxonomic studies has become apparent as remarkable coincidence with phylogenetic classifications based on morphological characters and distribution of phenolic compounds has been observed by many workers.

2.6.2.1.Free phenols and phenolic acids

The distribution pattern of different phenolic acids in ten taxa of the tribe Amherstieae (Caesalpinioideae) was studied by Nageshwar *et al.* (1987). The quantified chromatographic data presented in terms of synthetic numerical indices indicated that there was a fair degree of relationship among the taxa and suggested that the tribe was homogeneous.

Twenty three members of Amaranthaceae were screened for leaf phenolics by Mangalan *et al.* (1988). Of the phenolic acids identified in this family, vanillic,

syringic, p-OH-benzoic, melilotic, gentisic and ferulic acids like resorcylic and o-coumaric were confined to the tribe Amarantheae. Taxonomic implications of phenolics in *Crepis* was studied by Manez *et al.*(1992). *Crepis vesicaria* yielded six types of phenolics from its aerial parts whereas *C.capillaris* yielded only three types. Qualitative differences in the phenolic composition of these species were substantial and could support morphology based taxonomic differences between *C.vesicaria* and *C.capillaris*. Umarani and Singh (1995) analysed leaf phenolics in *Herminium* (Orchidaceae) by two dimensional thin layer chromatography in conjunction with numerical taxonomy. The chromatographic spots could be considered as excellent markers specific to the species analysed. On basis of the calculations made, the 6 species studied belonged to two groups. Phenolic compounds in different olive varieties were determined by HPLC analysis by Esti *et al.*(1998). Demethyl oleuropecin was found in only two of the eight varieties studied so it could be used as a varietal marker.

2.6.2.2. Flavonoids

Flavonoids are mainly water-soluble compounds structurally derived from the parent substance flavone. As they are phenolic, they change colour when treated with base or with ammonia. They contain conjugated aromatic systems and thus show intense absorption bands in the UV and visible regions of the spectrum. The flavonoid data obtainable from two-dimensional chromatograms have been used directly by taxonomists for comparing the chemistry of different species within genera or for solving some particular problem of classification.

Rangaswami and Seshadri (1971) identified luteolin as the flavone in *Adhatoda vasica*.Chemotaxonomy of Acanthaceae-tribe Justicieae was attempted by Sarma (1998) and could obtain positive test for flavonoids. Lopes and Manaco (1979) studied the phylogenetic relationship in coffee through chromatographic analysic of flavonoid composition in ten species of coffee. They found *Coffea eugenioides* to be closely related to *C.arabica* and *C.salvatrix* of subsection Mozambicoffea. *C.stenophylla* seemed to be closer to subsection Mozambicoffea than to those of subsection Pachycoffea. They observed absence of similarity in flavonoid patterns of *C.arabica* and *C. congensis* while *C. liberica* and *C. dewevrei* were very similar in

their flavonoid composition. They could identify 75 flavonoids in total of which five were common to all the ten species. Out of the 30 individual spots, 12 were detected in the wild *Coffea salvatrix* and the pairing affinity values were higher for species of the same subsection.

Total flavanoid content of ten species of *Artemisia* subgenus *Dracunculus* of Kazhakstan flora were compared by Alyukina and Ryakhovskaya (1980). The content in aerial parts was found to vary from 36 to 57mg/g (of dry matter) in eight species while in the other two, this was only 18.0 and 5.7mg/g. Flavonoids of *Erythroxylum rufum* and *Erythroxylum ulei* were identified by Bohm *et al.* (1981). Different patterns of flavonoids were seen in different collection of *E.ulei* where as the four specimens of *E.rufum* examined were identical. There was also difference in pattern between the species. The flavonoids of two geographically distinct varieties of *Passiflora foetide* had been reported by Ulubelen *et al.* (1982). There was difference in the composition of flavonoids between *P.foetide var.hispida* and *P.foetide var. hibiscifolia*. The flavonoids were identified by standard spectral data.

Flavonoids in the leaves of 33 species and botanical varieties of *Nicotiana* were isolated and identified by the use of chromatographic, spectrometric and hydrolytic procedures by Jurzysta (1983). A few were unique to particular species, while others were common to all species. Mangalan *et al.*(1988) screened 23 members of Amaranthaceae for flavonoids. The distribution pattern of flavonoids clearly pointed to the existence of three distinct groups in the tribe Amaranthae characterised by the presence of flavonols, Gomphrenae by flavonols, flavones and glycoflavones and Celosieae by the absence of the entire flavonoid system. With a view to judge the taxonomic status, leaf flavonoids of three species of *Paspalum* viz., *P.paspaloides*, *P. serobiculatum* and *P.vaginatum* had been studied by Varma *et al.* (1991). Chemotaxonomical study of South Indian taxa of *Piper* was carried out by Ravindran and Babu (1994) to understand their inter relationships. Fourteen taxa were analysed for their flavonoids and based on presence or absence of these compounds, percentage similarity indices were calculated. The results in general supported the species delimitation and taxonomical relationships arrived at by conventional taxonomy using morphological characters. A chemical dichotomy was evident between the two

subgenera – Pipali (having erect spikes) and Maricha (having pendant spikes) thereby supporting the validity of the sectional classification.

2.6.3. Terpenoids

An enormous range of plant substances are covered by the word 'terpenoid' which are all based on the isoprene molecule CH2=C (CH3)-CH=CH2 and their carbon skeletons are build up from the union of two or more of these C5 units. Generally GLC (Gas Liquid Chromatography) and TLC (Thin Layer Chromatography) are used for separating these compounds and closely related pairs of isomers are satisfactorily separated and characterized by this procedure.

The most noted example of the use of terpenoids in plant taxonomy is the classical work of Mirov (1938) on the gum turpentines of pines. He detected many chemical varieties in *Pinus* spp. and found that *Pinus jeffreyi* was a relict Californian species with no terpenes in its turpentines but *P. ponderosa* turpentines contained α and β pinene and limonene. All the pentacylic and tetracyclic terpenoids of 22 *Quercus* spp. so far reported were identified and listed under different chemical groups by Fokina (1982). Occurrence of the individual groups of terpenoids in different species could be used as one of the chemotaxonomic characters. Triterpene chemotypes of some Polish populations of *Tanacetum vulgare* were analysed by Wilkomirski and Kucharska (1992). The major monotydrony triterpene alcohol and sterol from the leaves and flower heads of 7 population of *Tanacetum vulgare* were determined by TLC. It was shown that variation in contents of sterols and triterpenes in leaves was very small in different populations.

Holden and Mahlberg (1992) studied chemotaxonmy in *Euphorbia* spp. Qualitative and quantitative differences for components of the terpenoid profiles were employed to distinguish between accessions of *Euphorbia* spp. Triterpenoid profiles were diagnostic for a species and were similar for each of the accessions in a species collected from distant areas of Europe. By contrast, the 37 accessions of the *E.esula* complex were separated into 15 groups on qualitative and quantitative difference for components in the profiles. Steven *et al.* (1993) studied taxonomic and evolutionary significance of terpenoid variation within *Sedum* sectum *sedum*. Triterpenoids in epicuticular waxes of 37 plants of 25 species of *Sedum* sectum *sedum* were

investigated to determine their systematic and evolutionary significance in this taxon. The distribution of triterpenes in the species agreed with the intrageneric classification into series.

Meragelman *et al.* (1996) analysed terpenoid constituents of *Viguiera* spp. Two varieties collected from two different geographic regions of Argentina were shown to have very close chemical constituents.

2.6.4. Essential Oil

The genetic resources of medicinal and aromatic plants were being used to understand the biological activities of single and combinations of essential oil constituents and methods of separation and transformation of individual components. Chemotaxonomical works based on essential oil content and composition have been successfully used in many of the medicinal and aromatic plants.

D'cruz *et al.*(1979) obtained an essential oil from the leaves of *A.vasica* which was shown to possess bronchodilatory activity. CSIR (1985) reported the presence of a fragrant, golden yellow essential oil (0.075 % on dry basis) in leaves, roots and flowers of A. vasica on steam distillation. The oil contained limonene and the physico-chemical propterties and constituents of the oil are not yet fully known. The essential oil exhibits expectorant and rubefacient activity and has marked selective anti-bacterial activity against the strains of *Mycobacterium tuberculosis.*

Putievsky *et al.* (1992) studied variation in the essential oil of *Artemisia judaica* chemotypes. Differences were found in samples collected from various locations. Essential oil of *A.judaica* plants from the 2 regions where of two distinct chemotypes. These differences had been reported to reflect genetic differences between the two populations. Inter tree variation of essential oil composition of *Thuja occidentalis* was studied by Kamdem and Hanover (1993). Comparison of oil isolated from the foliage of individual trees revealed significant inter tree variation in oil composition. Total oil yield using steam distillation was almost identical among the ten trees analysed. Different geographic races of *Kaempferia galanga* were evaluated for rhizome and oil yield and found that there was significant variation in quantum yield of rhizome and oil between the races although oil quality remained the same (Presannakumari *et al.* 1994).

Diversity in Australian populations of *Murraya paniculata* with respect to the leaf volatile oil composition had been found by Brophy *et al.* (1994). The two types studied could be distinguished on the basis of habit, habitat, leaf morphology and pedicel length besides essential oil content and are thought to represent separate species.

The essential oil of 54 mature leaf samples of nine *Clerodendrum* taxa collected from various locations in Taiwan were analysed by gas chromatography by Hsiao and Lin (1995). The relationship among taxa was analyzed by cluster analysis of the gas chromatrogram and it indicated a congruence between morphological and chemical relationships at the inter specific level. All interspecific taxa were linked. Geographical variation in essential oil composition in *Pelargonium capitatum* was studied by Viljoen *et al.* (1995). A survey of the composition of volatile oil showed that the species displayed high levels of essential oil variation, which enabled the species to be divided into eight chemotypes correlated with geographical distribution. The essential oil of the seeds of 66 cultivars of *Anethum graveolens* were examined by Kruger and Hammer (1996). The oil content ranged from 1.91 to 7.25%. The three known chemotypes were detected based on the oil composition and some transition types were also observed. A comparative study of 13 fennel (*Foeniculum vulgare*) populations of different origins were carried out on the basis of morphological and chemical characters. Three distinct intraspecific chemical taxa could be separated based on the cluster analysis of the seed oil. It was also proved that the morphological characters could not be used to support any intraspecific chemical classification (Bernath *et al*, 1996). Maia *et al.* (1998) studied the essential oil variation in *Melampodium camphoratum*. Essential oil of aerial parts of two specimens of *M.camphoratum* from two different localities in the Amazon region were analysed by gas chromatography. The oil samples were remarkably different and may be characteristic of the chemotypes.

2.7. Seasonal variation in growth, yield characters and quality attributes

2.7.1. Growth and yield characters

The optimum stage of harvest of a crop is decided after a detailed study of growth and yield characters and content of active principle responsible for the pharmacological activity in the case of medicinal plants. The yield and quality of *Adhatoda* vary according to the locality, growth and age of the plant, conditions of soil and season of the year.

KAU (1999) while evaluating different medicinal plants suitable for a coconut based farming system observed the biometric and yield characters at different growth stages of *Adhatoda*. The root, stem and whole plant weight showed progressive increase with advance in age and the highest values were observed at the harvesting stage of two years.The leaf yield was maximum at harvest interval of 1 ½ years which showed a decreasing trend at second year probably due to reduction in leaf area at this stage.

Pareek *et al.* (1981) were of the opinion that in *Catharanthus roseus* harvesting at 200DAP gave better yields of leaves, stem and root. Granda *et al.*(1986) studied the foliage and root growth of *Rauvolfia tetraphylla* at bimonthly intervals from 8 to 36 MAP. The results indicated that foliage growth increased markedly during rainy season and decreased during dry season. Meera (1994) studied the effect of different stages of harvest on growth and yield of Jeevanti (*Holostemma annulare*). She found that the age of plant significantly influenced all biometric characters like vine length, branches/vine, diameter of vine etc. The number of leaves, total leaf area/plant, fresh and dry weight of stem and leaf showed an increasing trend upto 12 months and there after declined. The yield parameters were significantly influenced by different stages of harvest. It was found that an increase in crop duration brought about a progressive increase in all root characters and fresh and dry root yield per hectare. Shina (1998) studied the influence of stages of harvest on growth and yield of *Hemidesmus indicus*. The results indicated that plant height, number of branches, leaf area and leaf area index were significantly higher during the seventh month.

Menon (1999) reported that plant height, leaf area, root length and root girth increased with growth stages in *Plumbago rosea* and *P.zeylanica*. Leaf area increased

in the wet season and with the commencement of summer a marked decline was noted. Later, with the onset of southwest monsoon, there was a progressive increase in leaf area upto 16 MAP and declined thereafter. The number of roots and girth of roots tended to increase with age upto 16MAP and thereafter the values were on par. However, in respect of length of root, the observations showed that the roots continued to grow upto 18MAP. In respect of fresh weight of roots, the two species did not differ significantly. Fresh and dry weights of root increased progressively with age of the crop and maximum values were observed at 18 MAP. She found that the rate of growth in respect of economic characters showed a linear trend with age.

Paul (2000) observed that in *Andrographis paniculata* (family Acanthaceae) plants harvested at 3 and 4 MAP yielded maximum fresh weight than when harvested at earlier dates. Though maximum dry weight of whole plant, leaf, stem and root was recorded at 4 MAP the difference was not significant. Hence optimum stage of harvest was fixed as 3 months after transplanting.

2.7.2. Quality attributes

The enormous variation of secondary metabolism in plants has long been appreciated and variation in yield and chemical composition depend on pedoclimatic conditions and on the ontogenic stage of the plant.

Reproductive and vegetative phases are the two stages in the life cycle of a plant which show variation in their metabolic activity. When the initiation and development of leaves is suddenly switched to the initiation of floral primordia and subsequently floral parts, a drastic change in the physiology of the plant is reflected (Noggle and Fritz, 1976).

The work done by Pandita *et al.* (1983) revealed that the percentage of the various alkaloids in *Adhatoda* varied with the season; that the plant was rich in its alkaloidal content in the months of August – October. At that time of the year the total alkaloidal content was about 2% of which vasicine was about 95%. Among the minor alkaloids were deoxyvasicine, about 3% and vasicinone in traces. The total alkaloid content decreased after October and reached a minimum level in March, when the total percentage was about 0.4 to 0.6%. It was also noted that the vasicine percentage decreased to 45% after October and that of vasicinone increased. The vasicinone

content was maximum in January-February at about 25% of total alkaloids. The alkaloid percentage again increased after April when new foliage was formed and reached a maximum in August- September when the plant was about to flower. Arambewala *et al.*(1988) reported that the higher amount of vasicine was found in the inflorescences and in the month of July-September.

Pundarikakshudu and Bhavsar (1988) studied variation in alkaloid contents of two types of *A. vasica* designated as big leaf variety (BLV) and small leaf variety (SLV). In the leaves and roots of BLV, there was gradual increase in alkaloid concentration from September to January. In January, leaves and roots contained the highest amount of alkaloids. In the subsequent collections till June, a gradual drop in the alkaloidal contents was noticed. Plants collected in July showed a rising trend in the concentration of alkaloids. Unlike in SLV plants there was a biphasic change of alkaloid concentrations in BLV plants. The amount of alkaloids reached the optimal level first in September-October and again in January. There was a sharp fall in November. After January alkaloid concentrations started decreasing in a way similar to that seen in SLV plants. BLV plants had in general higher alkaloid concentrations as compared to those of SLV plants. In September the roots and leaves of BLV plants contained triple the amount of alkaloids present in the leaves and roots of SLV plants.

Seasonal variation of alkaloid in *A.vasica* was also studied by Rajani and Pundarikakshudu (1996). The concentration of total alkaloids in leaves of nine to ten year old plants of big leaf variety of *A.vasica* was determined throughout the year. Some variation was observed but compared with young plant all leaves of nine and ten year old plants contained high concentrations of alkaloids.

Paul and Joseph (1997) made a comparative study on the accumulation of primary and secondary metabolites in reproductive and vegetative phase of *Adhatoda vasica*. The abundance of economically important alkaloids in root, stem and leaves were estimated in the two growth phases which was correlated with the accumulation of primary metabolites like carbohydrate, lipids, protein and starch. Leaf, stem and root samples were collected during the month of April (reproductive phase) and September (vegetative phase). The leaf contained maximum amount of alkaloids when compared to root and stem that also in its vegetative phase. It was about 5.81% and

4.84% in vegetative phase and reproductive phase respectively. Root and stem though lower in their alkaloid content differ with respect to the two phases. In the stem and root, the alkaloid content was higher in the reproductive phase. The stem as a whole showed poor alkaloid percentage. It was concluded that leaves of the plant at its active vegetative growth phase was suitable for collection and extraction of vasicine.

The alkaloid content of *Chelidonium majus* was lowest at blossoming (about 0.5% in herb, 0.8% in root and rhizome) and highest in summer (1.7 and 2.2.% respectively) and again decreased during autumn according to Kustrak *et al.*(1982). A period extending from early August to late October was recommended for harvesting the plant. Alkaloid content in plants from different sites at any time were slightly different.

Amador *et al.* (1996) analysed the seasonal influence on the biosynthesis of ergoline alkaloid in the genus *Cuscuta* of family Convolvulaceae from the samples of four species (*C.americana, C.campesteris, C.corymbosa and C. tinctoria*) collected in the rainy season as well as in the dry season and the chromatographic profiles of alkaloids were determined. Alkaloids were found only during the rainy season in all samples.

Seasonal changes in the concentrations of four taxoids in *Taxus baccata* during the autumn - spring period were investigated by Glowniak *et al.* (1999). From November to March the total levels of taxoids differed between the needles and stems. Total levels in fresh needles were stable from December to March. The highest concentration in the stems were found throughout the whole year and the same was true for the fresh needle except for samples collected in November and December. These results confirmed that epigenetic factors, such as date of collection and thus phylogenesis and plant tissue determined taxoid levels during the late autumn period in *T.baccata.* Also owing to the thermo liability of taxoids, the influence of low temperatures in December and January could explain the highest observed concentrations of taxoids in the fresh stems and needles.

Letchamo (1996) recorded developmental and seasonal variations in flavonoids of diploid and tetraploid camomile (*Chamomilla reculita*) ligulate florets. The highest flavonoid content was recorded in the second and third flower harvests.

With the aging of the plants and or repeated harvest of the flowers from the same plant, flavonoid content sharply decreased reaching the minimum level at the fourth harvest. There were marked differences in flavonoid accumulation in relation to harvest frequencies and the sowing seasons. Flowers of spring sown diploids had more flavonoid content than autumn sown cultivars while autumn sown tetraploids had more flavonoid content compared with the cultivars sown during spring.

Materials and methods

MATERIALS AND METHODS

The study on "morphological and biochemical variations in adhatoda types (*Adhatoda* spp.)" was conducted during 1998-2000 at the College of Horticulture, Vellanikkara. The 52 *Adhatoda* accessions maintained at the Department of Plantation Crops and Spices served as the experimental material.

3.1 MATERIALS

The 52 *Adhatoda* accessions were multiplied through rooted cuttings. Terminal cuttings were raised in polybags filled with potting mixture in December and transplanted to main field in June. The 52 accessions were planted on mounds at a spacing of 45cm x 45cm and three plants were maintained for each entry. They were initially observed to record the morphological and histological characters. Among the 52 types of *Adhatoda*; 13 types which were assumed to belong to the two different species viz. *A. zeylanica* and *A. beddomei* were selected and planted separately on ridges with two replication and ten plants for each accession. They were harvested in two growth stages 6MAP and 12MAP during first week of January (dry season) and first week of July (wet season). Later, based on the flower characters and cluster analysis, five more types which consisted of types typical to the two species and types intermediate between the two species were also studied for their biometric and biochemical characters during wet season.

3.2 METHODS

3.2.1 Variability in morphological characters

The 52 *Adhatoda* accessions were critically observed at different growth stages to note the following morphological characters:

Table1. *Adhatoda* types utilized for the study

Sl.no.	Type	Place of collection	Sl..no.	Type	Place of collection
1	Acc.1	Elathur	27	Acc.32	Thodupuzha
2	Acc.2	Amminikad	28	Acc.33	Parur
3	Acc.3	Pilicode	29	Acc.34	Mannuthy
4	Acc.4	Pappinissery	30	Acc.35	Kottapuram
5	Acc.5	Malappuram	31	Acc.36	Othukungal
6	Acc.6	Kottakkal	32	Acc.37	Vellanikkara 1
7	Acc.8	Pandalam	33	Acc.38	Changanassery
8	Acc.9	Kozhikode	34	Acc.39	Edakkoli
9	Acc.10	Panniyur	35	Acc.40	Nattika
10	Acc.11	Poojapura	36	Acc.41	Thrissur
11	Acc.12	Puthukad	37	Acc.42	Poonoly
12	Acc.13	Madakkathara	38	Acc43	Chittoor
13	Acc.14	Mannarkad	39	Acc.44	Ranni
14	Acc.15	Odakkali	40	Acc.45	Kothamangalam
15	Acc.18	Thiruvalla	41	Acc.47	Ninnukuzhi
16	Acc.19	Perinthelmanna	42	Acc.49	Pazhuvellam
17	Acc.20	Mukkom	43	Acc.50	Moovattupuzha
18	Acc.21	Nileswar	44	Acc.51	Palluruthy
19	Acc.22	Kasargode	45	Acc.52	Ponmudi
20	Acc.23	Kudlu	46	Acc.53	Kodungalloor
21	Acc.25	Nedupuzha	47	Acc.54	Alakode
22	Acc.27	Pariyaram	48	Acc.55	Aluva
23	Acc.28	Guruvayur 1	49	Acc.56	Palode
24	Acc.29	Atholi	50	Acc.57	*Adhatoda zeylanica* (germplasm collection)
25	Acc.30	Pandiparamba	51	Acc.58	*A.beddomei*(„)
26	Acc.31	Guruvayur 2	52	Acc.59	Vellanikkara 2

3.2.1.1. Vegetative characters

The vegetative characters noted were plant height, number of branches, pubescence of branches and shape of stem. Leaf characters like colour (colour of upper and lower surface), shape (narrowly elliptic-lanceolate/broadly elliptic-lanceolate), leaf tip (acute/attenuate), number of secondary nerves, leaf length and leaf breadth were noted. Leaf area was computed as follows

Leaf area = $-17.69 + 3.02l + 1.97b$ where l and b are length and breadth of a leaf respectively.

All the leaves from a twig were observed for recording leaf characters.

3.2.1.2. Inflorescence and floral characters (in flowered types)

Inflorescence was observed for the following characters.

a. Length of peduncle – average length of five peduncles per spike.

b. Nature of spike - compact or spreading.

The floral characters noted were shape, size and number of ribs of bract, shape and size of bracteole, length, pubescence and number of lobes of calyx, colour and presence of streaks on corolla, corolla tube length, attachment and pubescence of stamens, filament length, pubescence and size of ovary, pubescence of style and number of lobes in stigma.

3.2.2. Variability in histological characters

The following histological observations, which differed between the two species of *Adhatoda*, were recorded in 52 accessions.

3.2.2.1. Stomatal count

Epidermal peelings were taken from upper and lower surfaces of the mature leaves using quickfix (adhesive), stained with safranin and mounted on slides. For each type, stomatal counts were taken from ten different fields. Stomatal index was worked out as number of stomates per unit leaf area.

3.2.2.2. Trichomes

Number of trichomes as well as average number of cells in them was counted from the slide prepared for taking stomatal count. Counts were taken from ten different fields and expressed per unit leaf area.

3.2.3. Stages of harvest

The selected 13 accessions were harvested in two seasons-dry (first week of January) and wet (first week of July). In addition to the vegetative characters recorded for the characterization of 52 accessions, the following yield attributes were also recorded at two stages of harvest-root length, root girth, fresh weight and dry weight of whole plant, stem, leaves and root. To record the dry weight, plant samples were oven dried at 60-80 °C and weight was recorded when it reached a constant value.

3.2.4. Estimation of vasicine

The procedure suggested by Kannan *et al.* (1959) was followed to estimate the vasicine content. Twenty grams of oven dried (60-80°c) and finely powdered whole plant samples were exhaustively extracted with methanol in a water bath using a Soxhlet extractor. Extraction was carried out until the solvent became colourless. The extract was concentrated to dryness by keeping in an exhaust chamber and the weight of crude extract was noted. To the residue, 20 ml of 5% aqueous lead acetate solution was added to remove the phenols present. The flask was shaken well and filtered to remove the lead phenolate and the residue in the funnel was washed with 20 ml of 5N H_2SO_4 to remove the suphates and the filtrate was collected. Again added 10 ml of 5N H_2SO_4 along the sides of the filter paper and washed thoroughly with distilled water. Precipitated lead sulphate was removed by filtration. The filter paper and flask were washed well with water until free of alkaloid. The combined washings and filtrate were taken in a separating funnel and added 50 ml of chloroform for extracting alkaloid. Chloroform extract was separated as bottom layer and was collected. This was repeated thrice and the chloroform extracts were pooled. This was transferred to

the separating funnel along with 50 ml of 0.1 N H_2SO_4. Collected the bottom layer. To the top aqueous solution added 50ml of 0.1N H_2SO_4, 25ml of 25% ammonia (to make the solution distinctly alkaline) and 50 ml chloroform. The two bottom layers were mixed in a beaker. Reduced the volume to dryness. Dissolved the residue in 10 ml of 0.1N HCl and excess of acid was titrated against 0.1N NaOH using bromothymol blue as indicator taking the appearance of greenish blue colour as the end point.

Calculation:

1 ml of 0.1 N HCl = 0.188g of the alkaloid (estimated as vasicine)

$$\% \text{ of vasicine} = (\text{Titre value} - \text{blank value}) \times \frac{0.188}{20} \times 100$$

3.2.5. Qualitative tests for secondary metabolites

Selected *Adhatoda* types were subjected to qualitative analysis of secondary metabolites like phenols, flavonoids, terpenoids and alkaloids. Thin layer chromatography (TLC) was conducted for this purpose.

3.2.5.1 Preparation of crude extract

Five gram of oven dried powdered sample was extracted with methanol until the solvent became colourless. The extract was dried in an exhaust chamber and equal volume (approximately 10 ml) of methanol was added to all samples, which was used for spotting on TLC plates.

3.2.5.2. Preparation of TLC plates

The plates were prepared by coating homogeneous slurry of silica gel G on glass plates of 20cm x 20cm size. To prepare about six plates, 30 grams of silical gel G dissolved in 60 ml of distilled water was sufficient. Silica gel and water were shaken vigorously to get a homogeneous mixture and poured into the TLC plate gel applicator of GCME type. The plates were passed one by one through the slit quickly to avoid setting of the silica within the applicator itself. The plates were allowed to dry for about 10 minutes, after which they were placed in aluminium racks and kept in chromatographic oven at 110°C for 30 min. These desiccated plates were used for spotting.

3.2.5.3. Spotting on TLC plates

Capillary tubes which were calibrated to 5,10,15 and 20 µl using micropipette were used for spotting the sample on TLC plates. Maximum resolution of the spots was obtained with 10 µl of the sample. Spotting was done 2 cm above the lower edge of the plate maintaining a distance of 1.5cm between two consecutive spots. Inorder to avoid the excessive spreading of sample, intermittent application of small doses of the sample was done. After application of each small dose, the solvent was evaporated from the point of application by spraying hot air from a hair dryer. This was repeated till all the 10 µl volume of the sample was completely applied.

3.2.5.4. Running solvent systems

Various running solvent systems were tried for phenol, flavonoid and triterpenoid and the following systems which gave better elution of spots were selected. For alkaloids the solvent system suggested by Pandita *et al.* (1983) was followed.

Compound	Running solvent system	Ratio
Phenol	Hexane: Ethyl acetate	3:1
Flavonoid	Hexane: Ethyl acetate	3:1
Triterpenoid	Hexane: Ethyl acetate	9:1
Alkaloid	Chloroform: Methanol	9:1

The solvent system was poured in the tank and lid was placed tightly. To saturate with the vapours of the volatile components of the solvent system, filter paper sheets were placed adjacent to the walls of the tank inside. After 30 minutes, the spotted plates were placed in the tank such that the spots were above the surface of solvent system. In about 45 to 60 minutes elution of spots achieved 2/3rd length of the plate when the plates were removed and placed under an exhaust flow of air to evaporate the solvents from the silica gel coat on the plate.

3.2.5.5. Preparation of spray reagent

Spray reagents used to detect the presence of phenols, terpenoids and alkaloids were as follows

Phenols – Folin & Ciocalteu's Phenol Reagent

Triterpenoids – 50% sulphuric acid

Alkaloid – Dragendorff reagent

Uniform spraying was done with fine droplets of spray reagent in an exhaust chamber. The sprayed plates were kept at 110 °C in a chromatographic oven to develop coloured spots.

In the case of phenols, spots turned blue in colour where as in the case of terpenoids spots first turned light brown and on vigorous heating turned dark brown. For alkaloids pink and orange spots were observed.

To detect the presence of flavonoids the eluted oven dried plates were viewed under CAMAG UV betrachter and yellowish green fluorescent spots indicated the presence of flavonoids.

Photographs were taken on the same day before the plates got cooled as the spots spread on cooling.

3.2.6 Isozyme analysis

Polyacrylamide gel electrophoresis (PAGE) was carried out using Hoefer Mighty Small TM II gel system for separating multiple forms of two enzymes – esterase and peroxidase. Acrylamide monomers ($CH=CHCONH_2$) were co-polymerized with N-N methylene bis acrylamide [CH2 ($NHCONH=CH_2$) 2bis] to obtain the gel. Freshly prepared ammonium persulphate acted as catalyst and N, N, N, N – tetramethyl ethylene diamine (TEMED) as chain initiator.

Polyacrylamide gel was preferred because of its chemical inertness, high resolution, easiness in handling and preparation.

3.2.6.1. Gel Preparation

The following stock solutions were prepared

1. Monomer stock solution

Acrylamide – 30.0g

Bisacrylamide – 0.8 g

Volume made upto 100ml with distilled water

2. 4x Resolving gel buffer (1.5 M TrisCl, pH 8.8)

Tris base – 18.5 g

Adjusted the pH to 8.8 with 1N HCl

Volume made upto 100 ml with distilled water

3. 4x stacking gel buffer (0.5 M Tris Cl, pH 6.8)

Tris base- 0.6g

Adjusted the pH to 6.8 with 1N HCl

Volume made upto 100 ml with distilled water

4. Chain initiator (10% APS)

Ammonium per sulphate – 0.1g

Volume made upto 1 ml with distilled water (prepared fresh)

5. Destaining solution (40% Methanol, 7% Acetic acid)

Acetic acid – 70ml

Methanol – 400 ml

Volume made upto 1000ml with distilled water

Running gel solution was prepared by mixing the stock solution in the proportion given in the following gel recipe

Gel recipe used for standardization

	7.5%		8.5%		10%	
	10 ml	20ml	10ml	20ml	10ml	20ml
Monomer	2.49	4.98	2.83	5.66	3.33	6.66
Resolving Buffer	2.50	5.00	2.50	5.00	2.50	5.00
Distilled water	4.94	9.88	4.60	9.20	4.10	8.20
10% APS	50µl	100µl	50µl	100µl	50µl	100µl
TEMED	5µl	10µl	5µl	10µl	5µl	10µl

In order to get better molecular sieving and good resolution of bands 10 percent strength gel was selected and a stacking gel solution of following proportion was used.

Stacking gel solution

Monomer	– 0.8ml
Stacking gel buffer	– 1.25ml
Distilled water	– 3 ml
APS	– 50 µl
TEMED	– 5 µl

Working solution (running gel + stacking gel) was gently poured in between the plates kept in gel casting unit using a gel caster. Polymerisation was achieved within 20 to 30 min. For both the isozymes analyzed, stacking gel to a width of 1-1.5cm was used for better resolution of bands. Combs were pushed in between the caster plates for making wells for loading samples. Gel should be devoid of gas bubbles.

3.2.6.2. Preparation of sample

Plant parts like root, stem, tender leaves and mature leaves were used for analysis. As maximum resolution was obtained with mature leaves they were selected as the ideal part. Leaf sample collected was washed thoroughly and wiped with filter paper to remove moisture.

Extraction buffer (Tris Cl pH 7.0)

Tris Cl	- 21.1995 g
Citric acid	- 2.6275g
L-Ascorbic acid	- 0.5284 g
Cystein HCl	- 0.5269 g

The volume was made upto 500ml and stored in amber coloured bottle at 4 °C

500mg of plant sample was taken in a pre-cooled mortar. To this, 1 ml extraction buffer containing 17% sucrose and 40 µl of 1% insoluble PVP (Polyvinyl pyrrholidone) were added. PVP was added to prevent the interference of phenols during extraction. Samples were ground at 5 °C and centrifuged at 16,000 rpm for 15

min in a Kubota high speed centrifuge. Supernatant was mixed with treatment buffer in 1:1 proportion

2x Treatment Buffer (0.125 M Tris Cl, pH 6.8, 20% glycerol)

4x Tris Cl, pH 6.8 – 2.5 ml

Glycerol – 2.0ml

Bromophenol blue – 0.2mg (100 µl of 1% solution)

Volume was made upto 10ml with distilled water and 1 ml aliquots were transferred to eppendorf tubes and stored at –4 °C.

After polymerization, the gels were transferred to electrophoretic apparatus. The upper and lower tanks were filled with pre-chilled electrode buffer of pH 8.3

Electrode buffer (0.025 M Tris, pH 8.3, 0.192 M glycine)

Tris base – 1.5125g

Glycine – 7.2g

Volume made upto 500ml with distilled water

10 µl of sample mixed with equal volume of treatment buffer was loaded to the well after removing the combs. Upper tank was connected to cathode and lower one to anode. The electrophoretic run was carried at 4 °C. A constant current of 10mA per plate was maintained for esterase and 5mA per plate for peroxidase. It took 2 ½-3 hours for completion of the run.

Staining solution for peroxidase :

100 ml of stain contained

0.2M acetate buffer, pH 5.6- 100ml

Benzidine - 0.1g

H_2O_2 (3%) - 0.4 ml

Destaining solution (40% Methanol, 7% Acetic acid):

Acetic acid - 70 ml

Methanol - 400 ml

Volume made upto 1000ml with distilled water.

Fresh stain was prepared each time. Acetate buffer and benzidine were mixed, heated to boil, cooled and filtered. Hydrogen peroxide was added at the time of staining. The gels were immersed in the staining solution with continuous shaking

until clear brown bands appeared. The gels were then destained in destaining solution. Photographs were taken on the same day and zymograms were drawn on graph paper.

Staining solution for esterase (Sadasivam and Manickam, 1992):

200 ml of staining solution contained

Sodium dihydrogen phosphate	– 2.8 g
Disodium hydrogen phosphate	– 1.1 g
Fast blue RR salt	– 0.2 g
Naphthyl acetate	– 0.03 g

Volume made upto 200 ml with distilled water.

Fresh stain was prepared each time. Gels were immersed in the staining solution with continuous shaking until clear bands appeared. Destained the gels with 7% acetic acid. Took photographs on the same day and zymograms were drawn.

3.2.6.3. Nomenclature of the isozymes

The enzymes were designated by the following abbreviations.

1.Peroxidase – PRX

2. Esterase – EST

3.2.6.4. Numbering of isozymes

For numbering, all the isozymes of an enzyme studied were pooled. The fastest moving anodal band was numbered 1 (eg. PRX-1) and the slower ones were given subsequent numbers. Relative mobilities (Rm) of bands were calculated as per the formula

$$Rm = \frac{\text{Distance moved by band}}{\text{Distance moved by the dye}}$$

3.2.7. Enzyme activity of peroxidase

One gram of mature leaf was extracted in 3 ml of 0.1M phosphate buffer (pH 7) by grinding with a pre-cooled mortar and pestle. 50µl of 0.1% insoluble PVP was added at the time of extraction to prevent the interference of phenols. Centrifuged the homogenate at 18000g at 5 ^{0}c for 15 min. The supernatant was used as the enzyme

source. Pipetted out 3 ml buffer solution, 0.05 ml 20mM guaiacol solution (dissolved 0.214ml guaicol in water and made upto 100ml), 0.1 ml enzyme extract and 0.03ml freshly prepared H_2O_2 solution (diluted 0.14ml of 30% H_2O_2 to 100ml with water such that the extinction of the solution was 0.485 at 240 nm) in a cuvette. The buffer was brought to 25 °C before assay. Mixed well the contents and placed the cuvette in the spectrophotometer. Enzyme activity was assayed at 436nm and allowed the absorbance to increase by 0.05. A stopwatch was started and time required in minutes (Δt) to increase the absorbance by 0.1 was noted.

Calculation

Since the extinction coefficient of guaiacol dehydrogenation product at 436nm under the conditions specified was 6.39 per micromole, enzyme activity per litre of extract was calculated as below.

$$\text{Enzyme activity units / l} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1}$$

$$= 500/\Delta t$$

3.2.8 Essential oil content (%)

The essential oil was extracted by water distillation in Clevenger apparatus. Fifty grams of oven dried and finely powdered leaf sample was taken in the round-bottomed flask and distilled with 200 ml of distilled water. The duration of distillation was fixed as 5h as no further increase in the oil content was observed beyond this period. The steam volatile oil being lighter than water, condensed and collected on the top of the Clevenger trap. The volume of oil was noted. The percentage of essential oil in the sample was worked out.

3.2.9. Statistical analysis

The data collected were subjected to statistical analysis using MSTATC package. Analysis of variance was performed for the 52 types following the procedure

by Panse and Sukhatme (1978). Phenotypic, genotypic and environmental variances were estimated using the formula suggested by Burton (1952). Phenotypic and genotypic coefficient of variation and heritability were calculated by the formula suggested by Burton and Devane (1953). The genetic advance expected for the genotype at five per cent selection pressure was calculated using the formula by Lush (1949) and Johnson et al. (1955) with value of the constant K as 2.06 as given by Allard (1960). Genetic gain (genetic advance as percentage of mean) was estimated using the genetic advance calculated by the above method. The selected types of *Adhatoda* which were observed for yield attributes were subjected to pooled analysis of variance.

3.2.9.1. Cluster analysis

Non hierarchical Euclidean cluster analysis was followed to study the divergence among 52 accessions of *Adhatoda* according to Spark (1973). The characters used were plant height, number of branches, leaf area and petiole length.

Result

RESULTS

The observations related to the investigations on 'morphological and biochemical variations in adhatoda types (*Adhatoda* spp.)' and the results obtained are presented below under the following headings:-

1. Characterization of 52 accessions of *Adhatoda*

a) Morphological variation

b) Histological variation

c) Isozyme pattern and enzyme activity

d) Variability analysis

2. Variability in representative accessions

a) Morphological variation

b) Biochemical variation

3. Seasonal variation in morphological and biochemical characters.

4.1 Characterization of 52 accessions of *Adhatoda*

4.1.1. Morphological variation

4.1.1.1. Vegetative characters

The data on vegetative characters like plant height, number of branches, leaf length, leaf breadth, leaf area, leaf colour, leaf shape, number of secondary nerves and petiole length presented in Table 2 revealed significant variation between the accessions.

The mean height of plants among *Adhatoda* types under study ranged from 26.0cm in Acc.3 to 77.67 cm in Acc.56 (Plate 1a). Number of branches ranged between 3.0 and 7.7 the maximum value being in Acc.11. Leaf length showed wide variation ranging from 11.07cm in Acc.58 to 24.80cm in Acc.59 . Leaf breadth ranged from 3.53cm to 7.23 cm in Acc.58 and Acc.59 respectively. Leaf area calculated as a function of leaf length and leaf breadth was widely varying among the accessions ranging from 26.68 cm^2 in Acc.58 to 77.73 cm^2 in Acc.59. Petiole length was seen to vary from 0.57cm in Acc.58 to 3.17cm in Acc.59.

Plate 1. Variability in morphological characters of *Adhatoda*

1a. Plant habit

1. *A. zeylanica*
2. Intermediate
3. *A. beddomei*

1b. Leaf colour

1. Dark green
2. Light green

1c. Leaf shape

1. Broadly elliptic lanceolate (*A. zeylanica*)
2. Broadly elliptic lanceolate (Intermediate)
3. Narrowly elliptic lanceolate (Intermediate)
4. Narrowly elliptic lanceolate (*A. beddomei*)

Plate 1a

Plate 1b

Plate 1c

Plate 1d. Inflorescence

1. *A. beddomei*
2. *A. zeylanica*

1e. Corolla

1. *A. zeylanica*
2. *A. beddomei*

1f. Ovary pubescence

1. *A. zeylanica*
2. *A. beddomei*

Plate 2. Distribution of stomata

2a. Stomata absent on upper epidermis (*A. zeylanica)*

2b. Stomata present on upper epidermis (*A. beddomei*)

2c. Lower epidermis – similar for both the species

Plate 1d

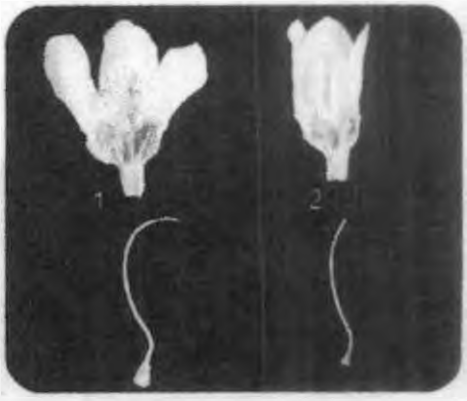

Plate 1e

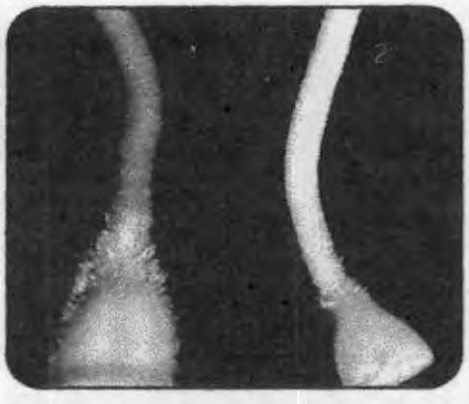

Plate 1f

Plate 2a

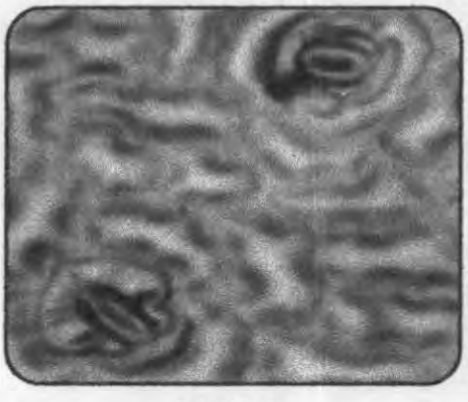

Plate 2b

Plate 2c

4.1.1.1.a. Pubescence of branches and leaves : All the types possessed pubescent branches and leaves.

4.1.1.1.b. Shape of stem : Stem was cylindrical at the internodes and flattened at the nodes in all the types.

4.1.1.1.c. Leaf colour : The colour on upper and lower side of the leaf was recorded. Leaves of six types had light green colour on the upper side and others had dark green colour on the upper side. The lower side of leaves of all the types were light green in colour(Plate 1b).

4.1.1.1.d. Leaf shape : The leaves were in general elliptic-lanceolate in shape. Twenty four types had broader leaves than the others. The leaves whose breadth was less than 5cm were considered as narrowly elliptic lanceolate and those with more than 5 cm as broadly elliptic-lanceolate(Plate 1c).

4.1.1.1.e. Leaf tip: All the types had leaves with attenuate ends.

4.1.1.1.f. Number of secondary nerves: The number of secondary nerves ranged from 8-10 in Acc. 10 to 13-15 in Acc.51. More number of secondary nerves was generally seen in broadly elliptic lanceolate leaves.

4.1.1.2. Inflorescence characters

Among the 52 types of *Adhatoda* studied, only nine types flowered during the course of study. Flowering season extended from October to April. Inflorescence was a prominent bracteate spike with axillary peduncle. Flowers were subsessile in the axils of opposite bracts, white, bisexual, strongly zygomorphic and devoid of any scent. The two stamens were attached near the top of the corolla tube. Pistil-bicarpellary, syncarpous with bilocular ovary. Two types of flowers could be observed - one type was larger, white with purplish streaks on the inner side of the corolla and the other type was smaller, white without purplish streaks. The inflorescence and floral characters are presented in Table 3.

4.1.1.2.a. Peduncle length and nature of spike: The length of peduncle ranged from 3.3cm to 5.6cm in Acc.14 and 7.5 to 8.5cm in Acc.53. Two types of inflorescences were seen- one was more compact and smaller and the other type was spreading and longer (Plate 1d). Among the nine types flowered, five types had smaller compact inflorescence and four types had larger spreading ones.

Table 2. Variability in vegetative characters of *Adhatoda* types

Type	Plant height (cm)	No. of branches	Leaf length(cm)	Leaf breadth(cm)	Leaf area(cm2)	Petiole length(cm)	Leaf colour	Leaf shape	No.of secondary nerves
Acc.1	37.57	5.0	12.03	3.93	41.13	1.80	darker	narrowly EL	9 to11
Acc.2	66.20	6.3	18.30	5.53	51.29	1.60	lighter	broadly EL	11to12
Acc.3	66.67	5.7	16.17	4.20	46.33	1.73	darker	narrowly EL	9 to11
Acc.4	53.33	5.3	17.40	4.20	41.81	1.67	darker	narrowly EL	10 to11
Acc.5	69.40	6.3	15.87	4.30	47.94	1.67	darker	narrowly EL	10to11
Acc.6	66.50	3.3	16.70	4.80	45.04	1.53	lighter	narrowly EL	10 to13
Acc.8	58.33	4.3	16.90	3.57	34.00	1.30	darker	narrowly EL	11 to12
Acc.9	55.00	4.0	17.70	4.30	36.60	1.25	darker	narrowly EL	11 to12
Acc.10	54.97	5.3	13.70	5.50	34.36	1.67	darker	broadly EL	8 to10
Acc.11	65.47	7.7	16.70	4.33	44.13	1.30	darker	narrowly EL	10 to11
Acc.12	50.00	3.0	15.20	4.23	35.00	1.23	darker	narrowly EL	10to11
Acc.13	47.57	4.7	16.57	4.83	39.95	1.23	darker	narrowly EL	10 to11
Acc.14	67.17	3.0	14.87	4.17	44.55	1.00	darker	narrowly EL	10to11
Acc.15	71.20	6.7	20.40	5.77	51.76	1.67	darker	broadly EL	11to12
Acc.18	70.33	2.3	21.90	6.87	64.09	2.60	darker	broadly EL	11to12
Acc.19	57.30	4.3	18.43	5.17	43.90	1.20	darker	broadly EL	11 to13
Acc.20	26.37	3.3	15.20	4.87	45.65	1.77	darker	narrowly EL	11
Acc.21	61.83	5.0	15.97	3.87	36.67	1.40	darker	narrowly EL	10to12
Acc.22	53.07	5.3	17.07	4.73	46.22	1.53	darker	narrowly EL	10 to11

Table 2. (contd.)

Acc.23	50.03	4.7	13.67	3.80	28.34	1.27	darker	narrowly EL	8 to9
Acc.25	63.07	4.0	17.67	6..47	30.12	1.97	darker	broadly EL	10to12
Acc.27	76.73	6.3	17.40	6.03	57.42	1.83	darker	broadly EL	10 to11
Acc.28	26.00	4.0	11.20	4.37	36.43	1.50	darker	narrowly EL	9 to11
Acc.29	28.17	4.3	14.63	5.10	44.43	1.47	darker	broadly EL	11 to13
Acc.30	71.17	5.3	17.20	4.90	39.61	1.60	darker	narrowly EL	11
Acc.31	33.50	6.0	16.77	5.23	39.10	1.70	lighter	broadly EL	10 to11
Acc.32	48.23	2.0	14.87	4.40	52.43	0.90	darker	narrowly EL	11
Acc.33	61.07	5.3	17.40	5.13	41.79	1.47	darker	broadly EL	10 to11
Acc.34	26.07	4.3	14.00	4..43	37.30	1.27	darker	narrowly EL	11
Acc.35	77.07	7.7	22.70	6.60	68.09	3.13	darker	broadly EL	13 to 15
Acc.36	38.73	2.0	15.60	5..57	42.15	1.90	lighter	broadly EL	9 to10
Acc.37	62.20	4.3	18.00	4.70	43.75	1.60	darker	narrowly EL	9 to11
Acc.38	27.03	3.0	15.50	3.60	34.06	1.53	darker	narrowly EL	10 to11
Acc.39	46.01	3.7	13.37	7.00	40.42	1.63	darker	broadly EL	10 to13
Acc.40	54.90	4.0	11.60	5.67	39.25	1.20	darker	broadly EL	10to12
Acc.41	58.17	5.0	15.53	4.27	41.21	1.50	darker	narrowly EL	11 to12
Acc.42	50.67	3.7	14.20	4.37	38.37	1.10	lighter	narrowly EL	10to12
Acc.43	61.33	5.3	19.57	6.23	47.62	1.70	darker	broadly EL	10to12
Acc.44	53.37	4.0	15.70	4.40	37.77	1.67	darker	narrowly EL	10 to11
Acc.45	38.67	3.0	12.50	3.67	41.74	1.07	darker	narrowly EL	9 to10
Acc.47	39.13	5.3	15.67	4.07	36.97	1.08	darker	narrowly EL	10 to11

Table 2 (Cont`d)

Acc.49	72.03	6.7	17.03	4.57	50.33	1.37	darker	narrowly EL	10to12
Acc.50	76.00	6.7	17.90	5.10	46.63	1.57	darker	broadly EL	11 to13
Acc.51	73.50	6.3	18.37	5.07	54.90	1.90	darker	broadly EL	12 to13
Acc.52	60.17	7.3	22.60	7.00	75.20	2.40	darker	broadly EL	11 to13
Acc.53	67.03	5.7	21.97	6.63	85.05	2.37	darker	broadly EL	11 to13
Acc.54	62.10	5.3	17.73	5.10	36.93	1.37	darker	broadly EL	9 to12
Acc.55	50.67	6.0	21.70	6.53	51.03	2.07	lighter	broadly EL	11 to 15
Acc.56	77.67	5.7	18.43	6.23	64.73	2.30	darker	broadly EL	10 to11
Acc.57	74.03	4.0	19.47	5.43	65.68	1.80	darker	broadly EL	13 to15
Acc.58	42.30	3.7	11.07	3.53	26.68	0.57	darker	narrowly EL	10 to11
Acc.59	66.63	3.7	24.80	7.23	77.73	3.17	darker	broadly EL	12 to13
CD 5%	21.58	2.9			9.98	0.67			
1%	28.60	3.9			13.20	0.88			

EL-Elliptic lanceolate

Table 3. Inforescence characters of Adhatoda types

Type	Length of peduncle (cm)	Nature of spike	Flower		Bract			
			Colour	Purplish streaks	Shape	No. of ribs	Length (cm)	Width (cm)
Acc.22	5.0-5.5	compact	white	absent	ovate	5	2.0-2.5	0.8-1.2
Acc.23	5.0-5.8	spreading	white	present	ovate	8	2.0-2.5	1.4-1.5
Acc.25	4.5-7.1	compact	white	absent	ovate	8	2.4-3.0	1.2-1.4
Acc.27	3.5-4.5	compact	white	absent	ovate	5	2.0-2.2	0.9-1.1
Acc.28	4.5-7.9	compact	white	absent	ovate	5	2.9-3.1	1.1-1.9
Acc.34	3.3-5.6	compact	white	absent	ovate	5	1.8-3.2	1.2-1.4
Acc.51	4.9-7.0	spreading	white	present	ovate	7	2.8-3.1	1.5-1.7
Acc.53	7.5-8.5	spreading	white	present	ovate	9	3.0-3.6	1.6-2.8
Acc.55	7.0-7.4	spreading	white	present	ovate	8	2.8-3.6	1.2-1.7

Table 3 (contd.)

Bracteole		Calyx			Corolla tub	Filament	Ovary	Style
Shape	No. of ribs	Pubescence	No. of lobes	Length (mm)	length (mm)	length (cm)	pubescent	Pubescence
lanceolate	3	pubescent	5	11	4.0-5.0	1.8-2.0	glabrous	hairy at base only
lanceolate	3	pubescent	5	10	4.0-5.0	2.2-2.3	pubescent	,,
lanceolate	3	pubescent	5	8	3.0-4.0	2.2-2.5	glabrous	,,
lanceolate	3	pubescent	5	7	2.0-4.0	2.0-2.5	glabrous	,,
lanceolate	3	pubescent	5	9	2.0-3.0	2.3-2.8	glabrous	,,
lanceolate	3	pubescent	5	9	2.0-4.0	2.0-2.2	glabrous	,,
lanceolate	3	pubescent	5	11	2.0-5.0	2.2-2.6	pubescent	,,
lanceolate	3	pubescent	5	9	3.0-4.0	2.2-2.3	pubescent	,,
lanceolate	3	pubescent	5	10	2.0-4.0	2.2-2.4	pubescent	,,

4.1.1.2.b. Bract and bracteole characters: All the flowered types had ovate bracts. Number of ribs ranged from seven to nine in longer spreading spikes and five in smaller compact spikes except Acc.25 which had eight ribs. There was much variation in length and breadth of bracts which ranged from 1.8 to 3.6 cm and 0.8 to 2.8 cm respectively. All the types had narrow lanceolate bracteole with three ribs. There was not much variation in the size of bracteole.

4.1.1.2.c. Calyx : All the types had five lobed pubescent calyx. Length of calyx ranged from 7mm in Acc.27 to 11mm in Acc.22and 51.

4.1.1.2.d. Corolla : The corolla colour was white in all the nine types. The flower types which were large in size borne on longer and spreading spikes were seen to possess purplish streaks on the inner side of corolla. The purplish streaks were absent in the other type of inflorescence. There was not much variation between types in the corolla tube length and shape. Corolla tube was cylindrical and length ranged from 2.0 to 5.0mm. (Plate 1e).

4.1.1.2.e. Stamen - attachment, pubescence and filament length

There were two stamens in a flower attached near the top of corolla tube each on opposite sides. Stamens were hairy at the base and glabrous towards the tip in all the types. There was not much variation in the filament length which ranged from 1.8 to 2.0cm in Acc.22 and 2.3 to 2.8cm in Acc.28.

4.1.1.2.f. Ovary and style : The larger flowers with purplish streaks had large sized highly pubescent ovary where as smaller flowers without purplish streaks had small sized glabrous ovary(Plate 1f). In all the flowered types style was hairy only at the base and glabrous towards the tip and the stigma was bifid.

4.1.2. Histological variation

Leaf epidermal peelings were observed for stomata and trichome characters and the observations are presented in Table 4.

4.1.2.1. Stomata

Presence of stomata on both the leaf surfaces as well as stomatal count were noted. Among 52 types observed, 28 types had amphistomatic leaves (stomates are

Table 4. Stomata and trichome characters of *Adhatoda* types

Type	Stomata				Trichome			
	Upper surface	stomatal index (per mm^2)	Lower surface	stomatal index(per mm^2)	Upper surface		Lower surface	
					No.of cells	Trichome index(per mm^2)	No.of cells	Trichome index(per mm^2)
Acc.1	present	24	present	282	3	21	3	21
Acc.2	absent	-	"	310	2	20	3	3
Acc.3	present	27	"	352	2	19	2	3
Acc.4	absent	-	"	183	3	20	3	3
Acc.5	present	-	"	268	2	42	2	38
Acc.6	present	23	"	268	3	34	2	40
Acc.8	absent	-	"	254	2	18	2	20
Acc.9	present	12	"	308	3	12	2	16
Acc.10	present	19	"	335	2	18	2	22
Acc.11	absent	-	"	254	3	26	2	32
Acc.12	present	8	"	268	2	19	3	30
Acc.13	present	11	"	296	2	11	2	13
Acc.14	present	11	"	310	2	24	2	25
Acc.15	absent	-	"	254	2	15	2	16
Acc.18	absent	-	"	183	2	18	2	20
Acc.19	absent	-	"	374	2	42	2	49
Acc.20	absent	-	"	296	2	6	3	14
Acc.21	present	11	"	211	2	6	3	7
Acc.22	absent	-	"	197	2	21	2	30
Acc.23	absent	-	"	268	2	15	2	18
Acc.25	present	11	"	225	3	30	3	26
Acc.27	present	21	"	260	2	19	2	20
Acc.28	present	10	"	272	2	16	2	18
Acc.29	absent	-	"	220	20	11	2	15
Acc.30	present	18	"	242	3	10	3	12

Table 4 (Contd.)

Acc.31	present	21	"	282	2	62	2	34
Acc.32	present	8	"	254	3	23	2	20
Acc.33	present	20	"	352	3	8	3	10
Acc.34	present	49	"	310	3	15	2	14
Acc.35	absent	-	"	239	2	15	3	18
Acc.36	absent	-	"	239	3	15	3	16
Acc.37	absent	-	"	239	3	16	3	18
Acc.38	absent	-	"	410	2	31	33	36
Acc.39	present	8	"	269	2	15	2	20
Acc.40	absent	-	"	242	2	16	2	20
Acc.41	present	15	"	366	3	58	3	45
Acc.42	present	11	"	309	3	22	3	20
Acc.43	absent	-	"	211	2	27	2	34
Acc.44	present	8	"	333	3	20	2	24
Acc.45	present	12	"	211	3	19	3	23
Acc.47	present	32	"	268	2	34	2	34
Acc.49	absent	-	"	338	2	27	2	38
Acc.50	absent	-	"	252	3	21	3	25
Acc.51	absent	-	"	282	2	27	2	26
Acc.52	absent	-	"	268	3	42	3	27
Acc.53	absent	-	"	352	3	7	3	49
Acc.54	absent	-	"	282	2	16	2	18
Acc.55	absent	-	"	268	2	19	2	22
Acc.56	absent	-	"	239	3	23	2	18
Acc.57	absent	-	"	352	3	34	2	36
Acc.58	absent	-	"	366	3	18	3	22
Acc.59	present	10	"	268	2	24	5	26

present on both surfaces of leaf) and 24 types had hypostomatic leaves (stomates only on lower side). There was variation in the stomatal index among the types and it ranged from 183 to 410. (Plates 2a and 2b).

4.1.2.2. Trichomes

There was no significant difference in the number of trichomes as well as number of cells in the trichomes between the different types of *Adhatoda*. Trichomes were present on both the surfaces of leaf more abundant in the region of veins. The number of cells in the trichome varied from 1 to 4 and trichome index ranged from 6 to 62 in the upper surface and 7 to 49 in the lower surface.

4.1.3. Isozymes

4.1.3.1.Peroxidase

Mature leaves were used for analyzing the banding pattern of peroxidase. (Fig.1, Plates 3a to 3h). Three bands such as PRX-1 (Rm=0.659), PRX-2 (Rm=0.585) and PRX-3 (Rm=0.549) were expressed in the present running system. The bands PRX-2 and PRX-3 were recorded in all types whereas only the types other than Acc.13, 19,25,34 and 51 expressed PRX-1 (Rm=0.659). Based on the banding pattern of peroxidase the 52 accessions were grouped into two (Table 5).

4.1.3.2.Esterase

Variation in the banding pattern of esterase isozyme was observed in the accessions. A total of seven bands of esterase were observed in the 52 types (Fig.2, Plate 4a to 4h). The bands EST-1 (Rm=0.422) and EST-7 (Rm=0.089) were seen in all the accessions. These types could be grouped into twelve based on the esterase banding pattern and no band was found typical to an accession (Table 6).

4.1.4. Peroxidase activity

The peroxidase activity of mature leaves ranged from 170 to 500 units per litre. Based on the activity all the 52 types were grouped as high (390-500 units per litre) medium (280-390 units per litre) and low (170-280 units per litre) Table 7.

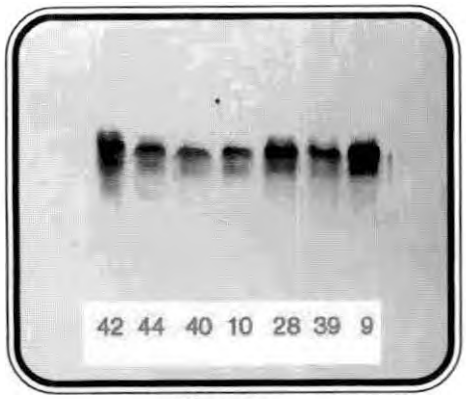

Plate 3a

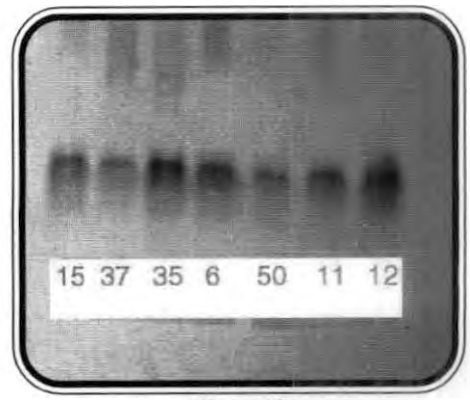

Plate 3b

Plate 3c

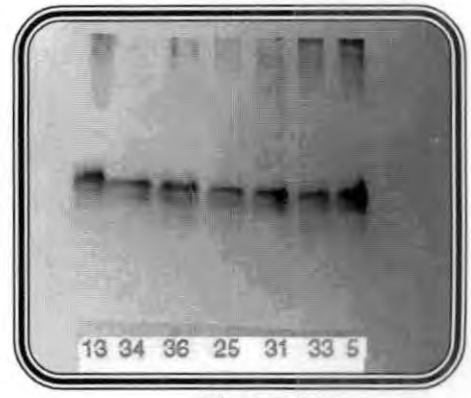

Plate 3d

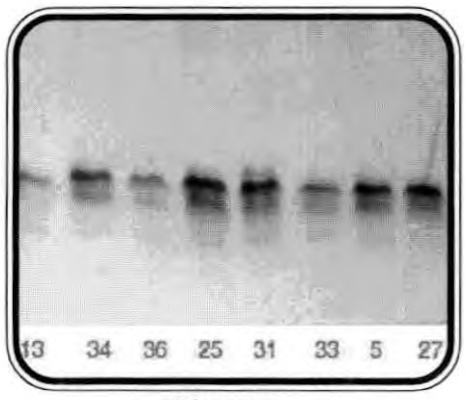

Plate 3e

Plate 3f

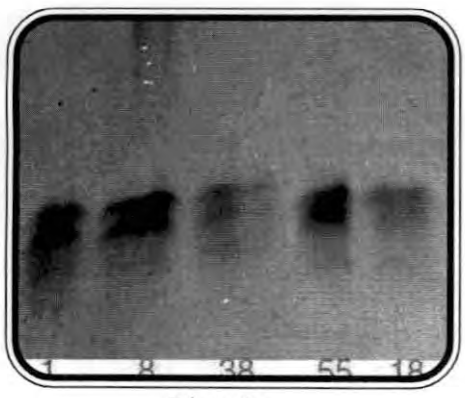

Plate 3g

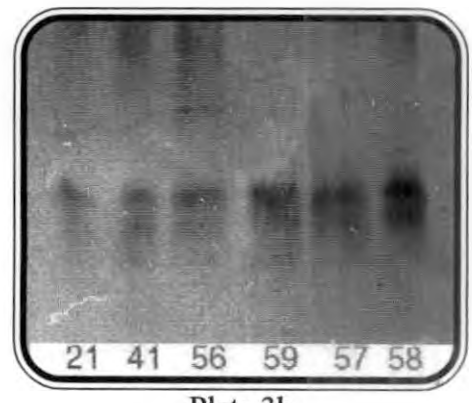

Plate 3h

Banding pattern of peroxidase in *Adhatoda*

Fig.1 Zymogram of peroxidase isozyme in *Adhatoda*

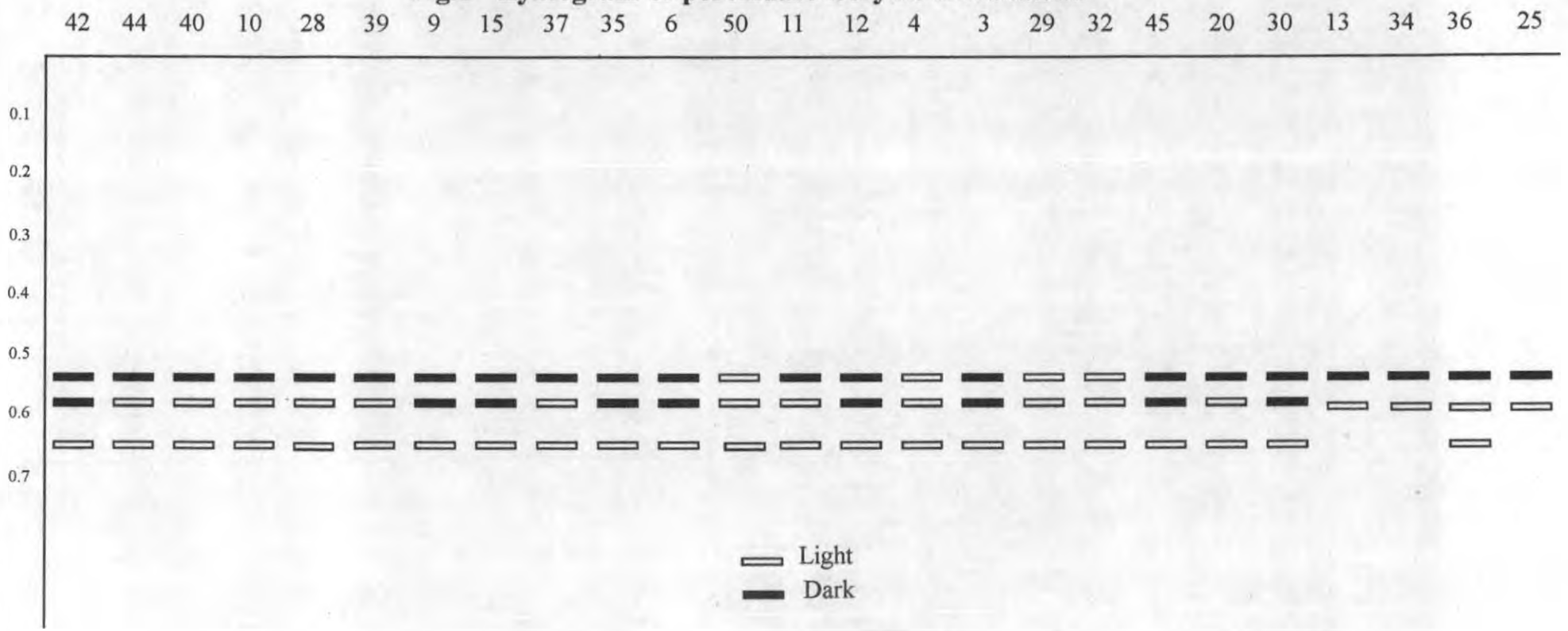

Fig.1(contd.)

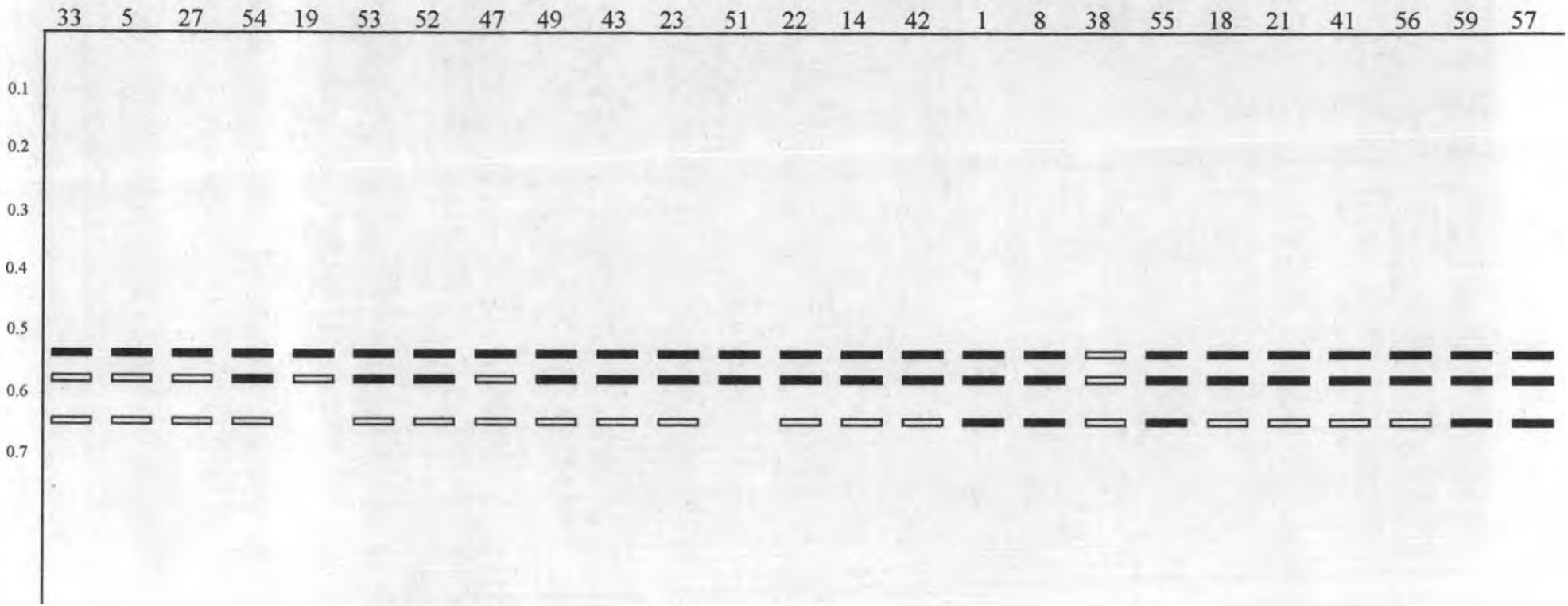
33 5 27 54 19 53 52 47 49 43 23 51 22 14 42 1 8 38 55 18 21 41 56 59 57
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Table 5. Grouping of *Adhatoda* types based on banding pattern of peroxidase

Group 1 PRX 1,2 and 3	Group 2 PRX 2 and3
Acc.1,2,3,4,5,6,8,9,10,11,12,14, 15,18,19,20,21,22,23,24,27,28, 29,30,31,32,33,35,36,37,38,39,40,41,42, 43,44,45,47,49,50,52,53,54, 55,56,57,58,59	Acc.13,19,25,34,51

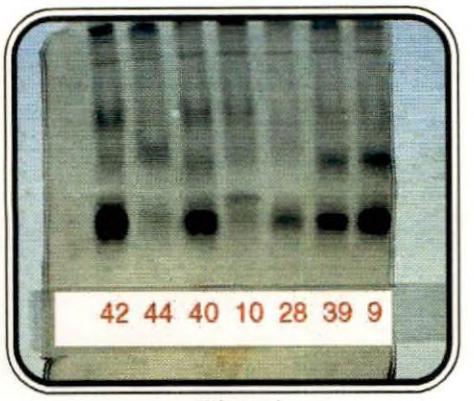

Plate 4a

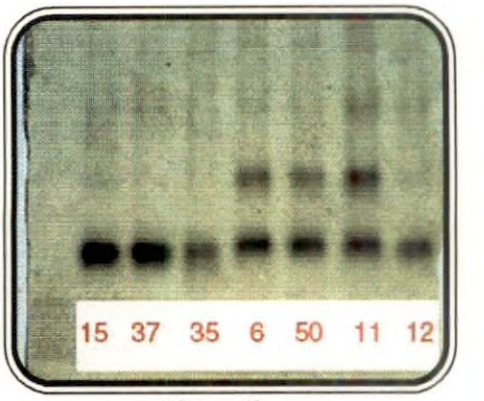

Plate 4b

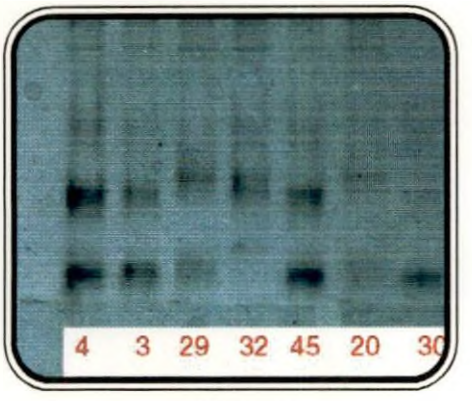

Plate 4c

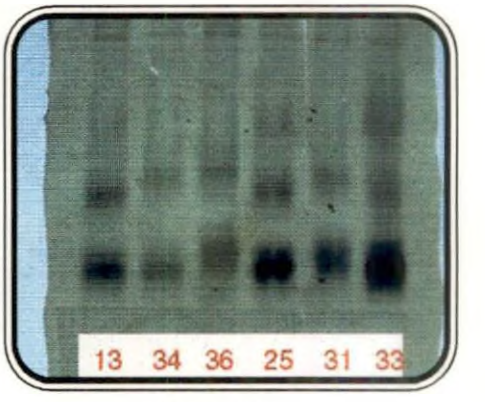

Plate 4d

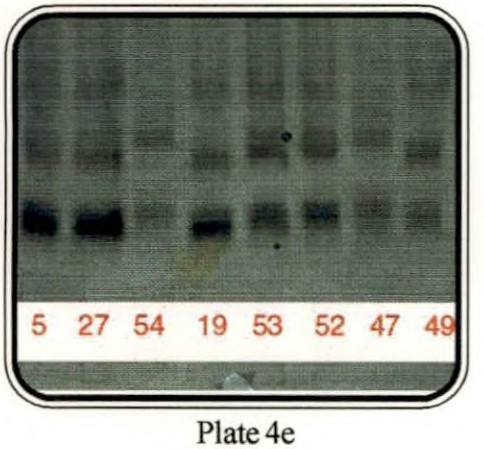

Plate 4e

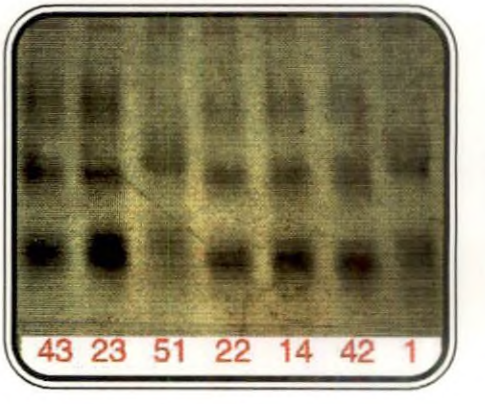

Plate 4f

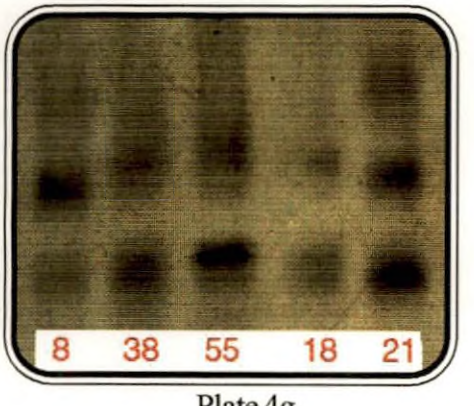

Plate 4g

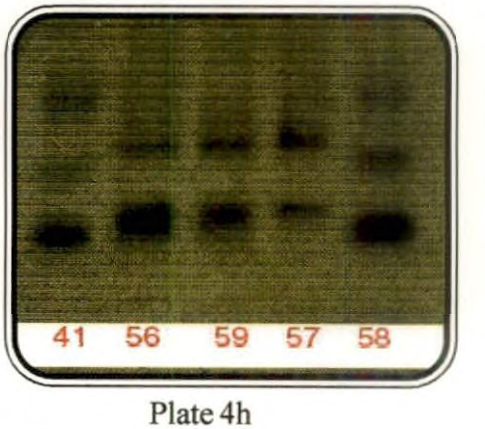

Plate 4h

Banding pattern of esterase in *Adhatoda*

Fig 2. Zymogram of esterase isozyme in *Adhatoda*

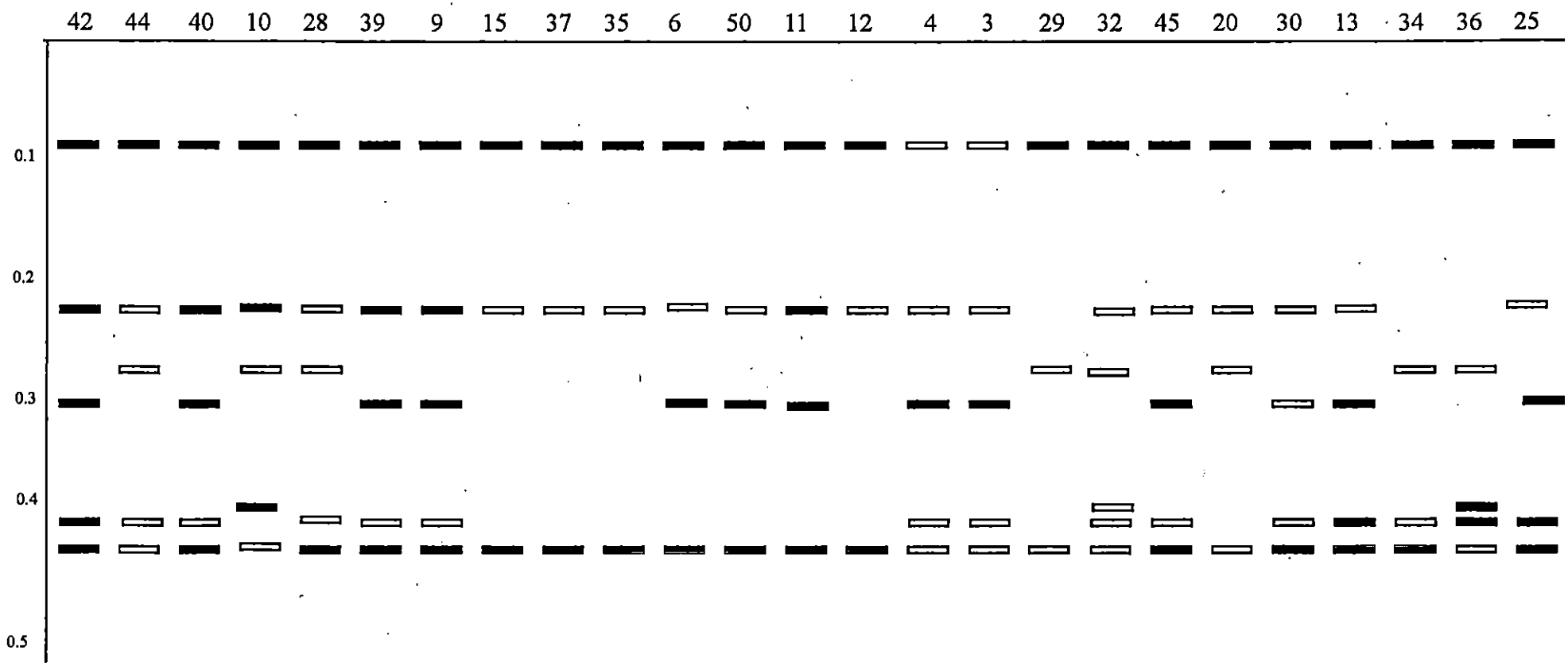

Fig.2(contd.)

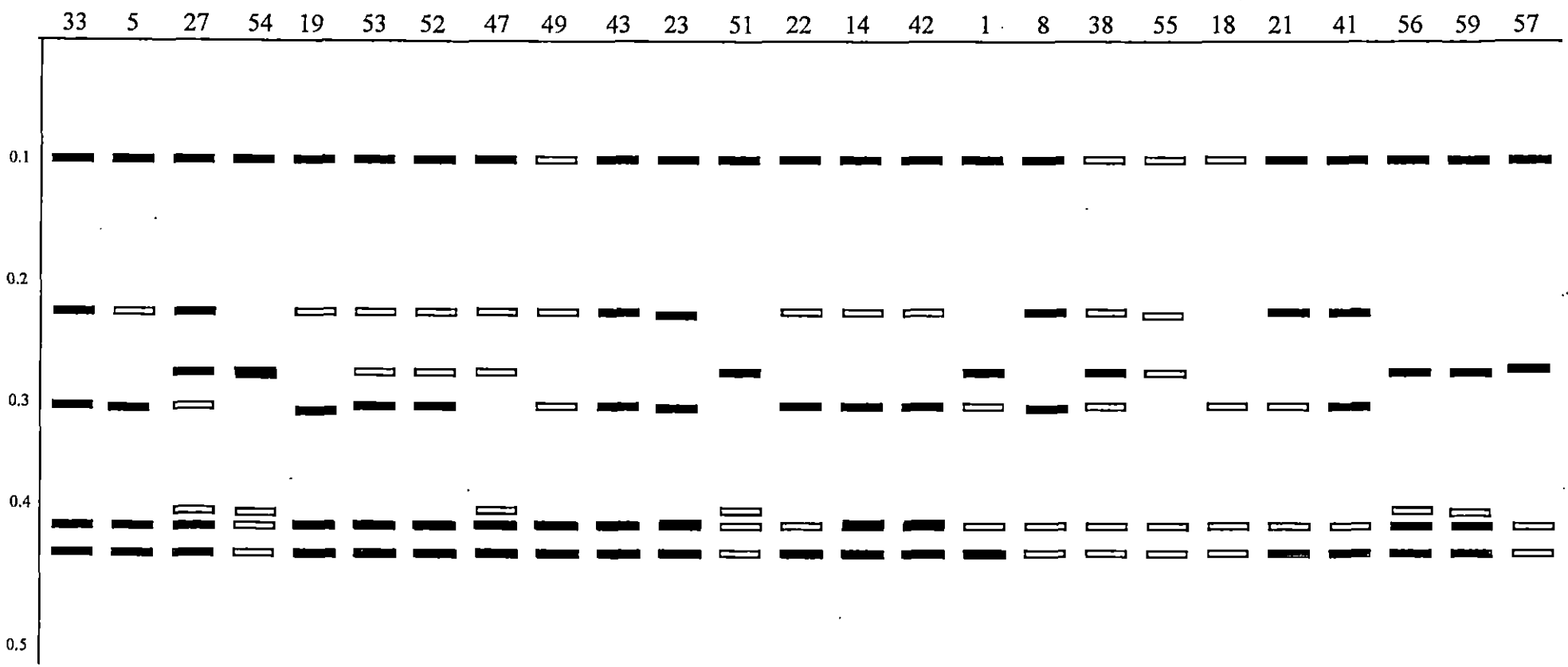
33 5 27 54 19 53 52 47 49 43 23 51 22 14 42 1 8 38 55 18 21 41 56 59 57
0.1
0.2
0.3
0.4
0.5

Table 6. Grouping of *Adhatoda* types based on banding pattern of esterase

Group	Esterase bands present	Types
1	EST-1,2,4,6 and 7	Acc.2,3,4,5,8,9,13,14,19,-21,22,23,25,27,30,33,39,-40,41,42,43,45,49,58
2	EST-1,2,5,6 and 7	Acc. 28,44,55
3	EST-1,3,5,6 and 7	Acc. 10
4	EST-1,5 and 7	Acc.29
5	EST -1,2,3,5,6 and 7	Acc. 32,47
6	EST-1.5.6 and 7	Acc. 20
7	EST-1,2,5 and 7	Acc.34
8	EST-1,2,3,5 and 7	Acc.31,36,51,54,56,59
9	EST-1,2,4,5,6 and 7	Acc.38,52,53
10	EST-1,2,4,5 and 7	Acc.1
11	EST-1,2,4 and 7	Acc.6,11,18,50,57
12	EST-1,2,and 7	Acc.12,15,35,37

Table 7. Grouping of *Adhatoda* types based on peroxidase activity

High (390 -500units/l)	Medium (280 -390units/l)	Low (170-280 units/l)
Acc.8,9,23,35,42,53	Acc.1,5,10,14,22,28, 30,39,40,43, 44,45,49,51,52, 54,55,56,58,59	Acc.2,3,4,6,11,12,13,15, 18,19,20,21,25,27,29,31, 32,33,34,36,37,38,41,47,50,57

Table 8. Genetic variability in *Adhatoda*

	Plant height	No.of branches	Leaf area	Petiole length
Mean	55.55	4.74	45.65	1.62
SD	10.53	1.51	5.02	0.33
Gcv	0.23	0.19	0.25	0.27
Pcv	0.34	0.34	0.29	0.26
E cv	0.24	0.38	0.14	0.26
Heritability	0.48	0.32	0.75	1.08
Genetic advance	0.18	1.07	20.56	1.32
Genetic gain	0.33	0.23	0.45	0.82

4.1.5. Variability analysis of morphological characters

The data pertaining to population mean, genotypic, phenotypic and environmental coefficient of variation, heritability, genetic advance and genetic gain derived based on biometric characters are presented in Table 8.

For plant height, number of branches and leaf area, phenotypic coefficient of variation (pcv) was greater than genotypic coefficient variation (gcv). But the reverse was the case for petiole length where gcv was more than pcv. Environmental coefficient of variation was 38.29% for number of branches, 25.60% for petiole length, 24.32% for plant height and only 13.65% for leaf area. Heritability was maximum for leaf area (74.7) followed by petiole length (51.92), plant height (47.7) and number of branches (31.7). Genetic advance was the highest for leaf area (1.55)and lowest for number of branches (0.65). Genetic gain was very high for petiole length (66%) and the lowest value (1.7%) was recorded for plant height.

4.1.6. Cluster analysis

Based on the vegetative characters viz. plant height, number of branches, leaf area and petiole length, the 52 accessions were grouped into 3 clusters (Table 9). Cluster I had the maximum number of accessions (25) followed by cluster III (20) and II (7). Cluster II comprised of tall types with larger leaves and the flowered types showed white flowers with purplish streaks on the inner side of corolla. Cluster III included shorter types with small leaves and small white flowers without purplish streaks inside. Cluster I showed, vegetative characters intermediate to cluster II and III and included both the floral types. The mean plant height of cluster I (63.87cm) was intermediate to cluster II (70.42cm) and cluster III (39.96cm). Number of branches (5.4), leaf area (70.08) and petiole length (2.5cm) was the highest for cluster II compared to cluster I and III (Table 10,Fig.3). The distance between cluster centroids illustrated in Fig.4 showed that cluster I was closer to cluster III (1.93) than cluster II(3.02).

Table 9. Accessions in three clusters

Cluster 1	Cluster 2	Cluster 3
Acc.2,3,5,6,11,12,15,18,19, 20,22,23,25,27,29,32,36,37, 40,41,43,49,50,52,54	Acc.35,51,53, 55,56,57,59	Acc. 1,4,8,9,10,13,14,21, 28,30,31,,33,34,38,39, 42,44,45,47,58

Table 10. Means of variables for the clusters in *Adhatoda*

Variable	Cluster 1	Cluster 2	Cluster 3
Plant height(cm)	63.87	70.42	39.96
No.of branches	5.35	5.05	3.88
Leaf area(sq. cm)	43.71	70.08	39.52
Petiole length (cm)	1.54	2.54	1.4

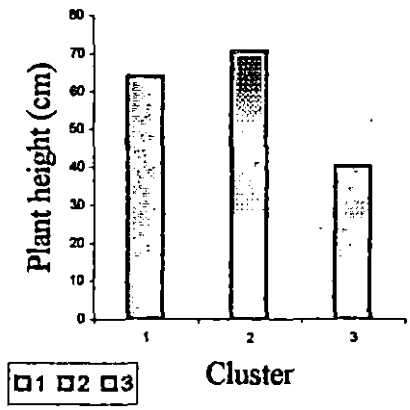

Fig 3a.Plant height

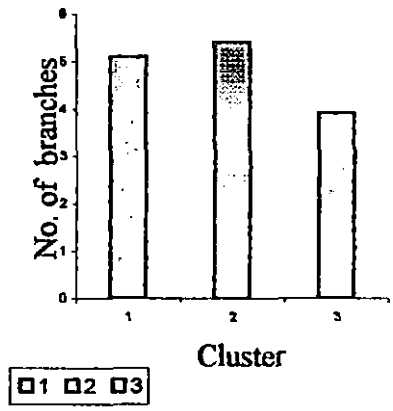

Fig 3b. No. of branches

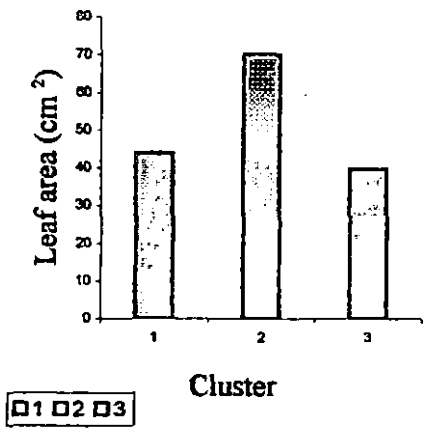

Fig 3c. Leaf area

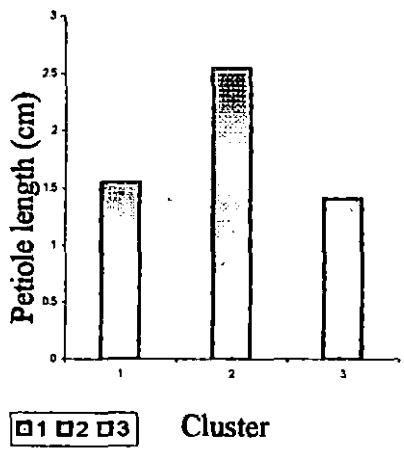

Fig 3d. Petiole length

Fig 3. Means of variables for the clusters of *Adhatoda*

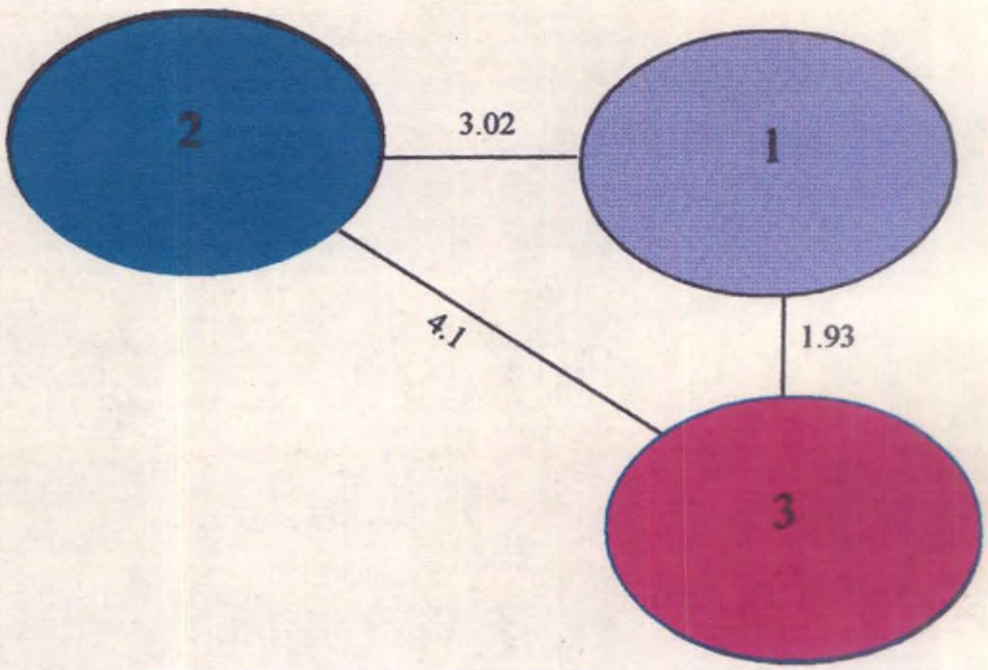

Fig 4. Distance between three cluster centroids

4.1.7. Classification based on morphological and histological data.

Morphological and histological studies conducted in the 52 types of *Adhatoda* were considered together and again grouping of the types was attempted considering vegetative and floral characters (in flowered types) and stomatal (amphistomatic/hypostomatic) features. It was seen that the types in cluster III possessed amphistomatic narrow leaves. Nine types in cluster I also had amphistomatic leaves in which six types had narrow leaves. Types with hypostomatic leaves were included in cluster I and II. Among the 16 types with hypostomatic leaves in cluster I, 10 types had broader leaves. Cluster I comprised of largest number of types (intermediate to the other two clusters) which were medium tall, bearing white flowers with and without purplish streaks inside, hypostomatic as well as amphistomatic narrowly as well as broadly elliptic lanceolate leaves. Cluster II consisted of tall types with larger hypostomatic broadly elliptic lanceolate leaves and flowers with purplish streaks inside. Cluster III included comparatively shorter types with smaller amphistomatic narrowly elliptic lanceolate leaves and flowers without purplish streaks inside.It was observed that types belonging to cluster II showed the characters of *A.zeylanica* , cluster III resembled *A.beddomei* and cluster I showed characters intermediate to both the species with respect to morphological and histological features.

4.2. Variability in representative accessions

After detailed study of the 52 types of *Adhatoda* for vegetative, floral and histological characters and subjecting them to cluster analysis, accessions representing three groups viz. *Adhatoda zeylanica*, *A. beddomei* and intermediate groups were selected for comparative evaluation of morphological, histological and biochemical characters. They were Acc.51 and 53 (*A.zeylanica*), Acc.28 and 34 (*A.beddomei*) and Acc.22, 23 and 27 (intermediate group).

4.2.1. Morphological variation

Besides the vegetative and floral characters described earlier, yield attributes were also noted and presented together in Tables 11 and 12.

4.2.1.1. Number of leaves

The number of leaves was lower in the *A.beddomei* types Acc.28 and 34 (65 and 66 respectively), medium in intermediate types Acc.22, 23 and 27 (112,96 and 105 respectively) and higher in *A.zeylanica* types Acc.51 and 53 (133 and 150 respectively).

4.2.1.2. Floral characters: *A.zeylanica* types possessed large white flowers with purplish streaks inside and large pubescent ovary. *A.beddomei* types had small white flowers without purplish streaks inside and small glabrous ovary. Among the intermediate types Acc.22 had flowers resembling *A. beddomei* but leaf and and stomatal characters of A. zeylanica,Acc.23 resembled *A.zeylanica* in floral and stomatal characters but *A. beddomei* in leaf characters, and Acc.27 had floral and stomatal characters of *A.beddomei* but leaf characters of *A. zeylanica*.

4.2.1.3. Root characters: The types did not vary much in the root length. It ranged from 29.6 to 40.6cm in Acc.34 and 22 respectively with maximum in intermediate types followed by *A.zeylanica*. *A.zeylanica* showed comparatively higher root girth ranging from 2.6 in Acc.51 to 5.5cm in Acc.53 respectively whereas the *A. beddomei* types had in general low root girth of 1.2 and 1.4cm. in Acc.28 and 34 respectively .The root girth ranged from 1.8 to 4.2cm in the intermediate types .

4.2.1.4. Fresh weight.

Fresh weight of whole plant, leaves, stem and root were recorded (Table 12,Fig.5a). Fresh weight of whole plant was lower for *A.beddomei* types with mean value of 115.6g compared to intermediate (217.2g) and *A.zeylanica* (314.0g) types. The same trend was recorded for the fresh weight of leaves, stem and root. Fresh weight of leaves was medium for the intermediate types (79.7g) compared to *A.beddomei* (43.1g) and *A.zeylanica* (123.1g) types. Similarly *A.beddomei* types had lower fresh weight of stem and root (52.5 and 20.0g respectively) than intermediate (93.1 and 44.4g) and *A.zeylanica* (140.3 and 50.7) types.

Table 11. Morphological and histological characters of representative types of *Adhatoda*

Type		Plant height(cm)	No.of branches	Leaf length(cm)	Leaf breadth(cm)	Leaf area(cm^2)	Petiole length(cm)	Leaf shape	Flower	Stomata
A.beddomei	Acc.28	26.00	4.0	11.2	4.4	36.40	1.5	NEL	*A.beddomei*	amphistomatic
	Acc.34	26.10	4.3	14.0	4.4	37.30	1.3	NEL	*A.beddomei*	amphistomatic
	Mean	26.05	4.2	12.6	4.4	36.90	1.4			
Intermediate types	Acc.22	53.10	5.3	17.1	4.7	46.22	1.50	NEL	*A.beddomei*	hypostomatic
	Acc.23	50.00	4.7	17.1	4.7	28.34	1.3	NEL	*A.zeylanica*	hypostomatic
	Acc.27	76.70	0.3	17.4	6.0	57.42	1.8	BEL	*A.beddomei*	amphistomatic
	Mean	59.90	5.4	16.1	4.8	43.97	1.5			
A.zeylanica	Acc.51	73.50	6.3	18.4	5.1	54.90	1.9	BEL	*A.zeylanica*	hypostomatic
	Acc.53	67.00	5.7	22.0	6.6	85.05	2.4	BEL	*A.zeylanica*	hypostomatic
	Mean	70.30	6.0	20.2	5.9	69.98	2.2			

NEL - Narrowly elliptic lanceolate
BEL - Broadly elliptic lanceolate

Table 12. Yield attributes of representative types of *Adhatoda*

Type		Leaf No.	Root length (cm)	Root girth (cm)	Fresh weight of whole plant(g)	Fresh weight of leaf(g)	Fresh weight of stem(g)	Fresh weight of roo(g)t	dry weight of whole plant(g)	dry weight of leaf(g)	dry weight of stem(g)	dry weight of root(g)
A.bedd-omei	Acc.28	65	30.5	1.2	120.6	44.1	54.3	22.1	32.1	12.2	14.5	5.4
	Acc.34	76	29.6	1.4	110.5	42.0	50.6	17.9	37.5	14.5	16.2	6.8
	mean	70.5	30.1	1.3	115.6	43.1	52.5	20.0	34.9	13.4	15.4	6.1
Interme-diate	Acc.22	112	40.6	1.8	186	69	78.2	38.8	47.5	16.1	22.2	9.2
	Acc.23	96	35.5	4.2	255	99.5	104.5	51.0	86.0	32.2	40.1	13.7
	Acc.27	105	36.3	2.7	210.5	70.6	96.6	43.3	66.7	21.0	21.2	20.2
	mean	104.3	37.5	2.9	217.2	79.7	93.1	44.4	65.3	23.1	27.8	14.4
A.zeyla-nica	Acc.51	133	31.3	2.6	265	97.1	124.5	43.4	115.3	41.7	51.8	18.8
	Acc.53	150	38.5	5.5	363	149	156.0	58	158	57.2	75.1	25.7
	mean	141.5	34.9	4.1	314.0	123.1	140.3	50.7	136.8	49.5	65.0	22.3

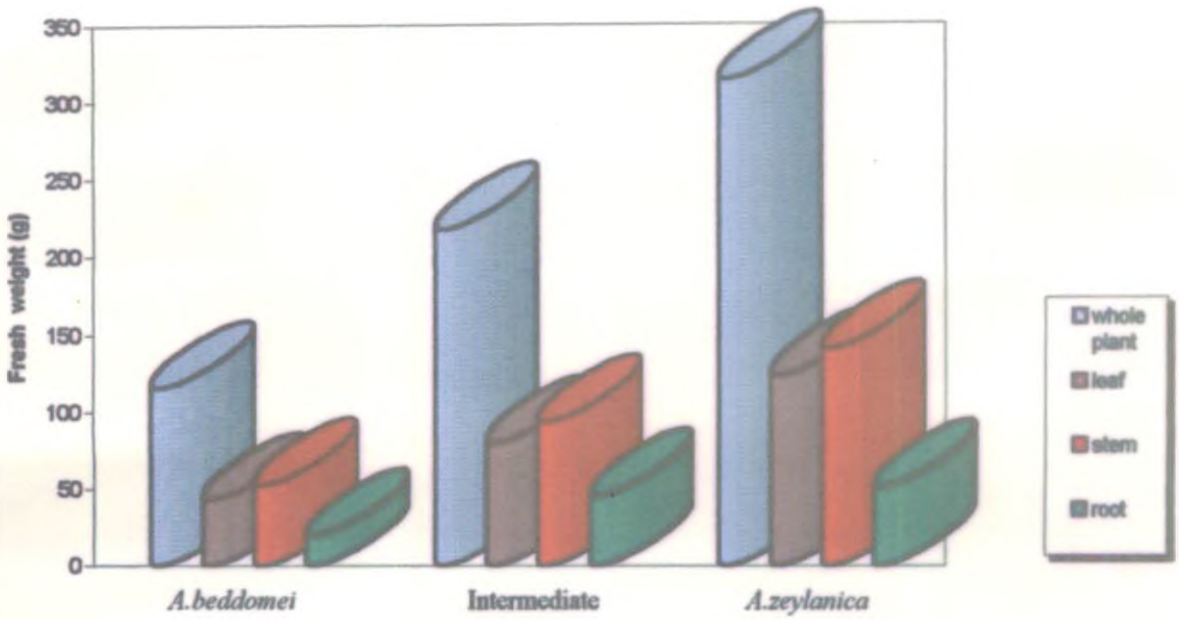

Fig 5a. Fresh weight of representative types of *Adhatoda*

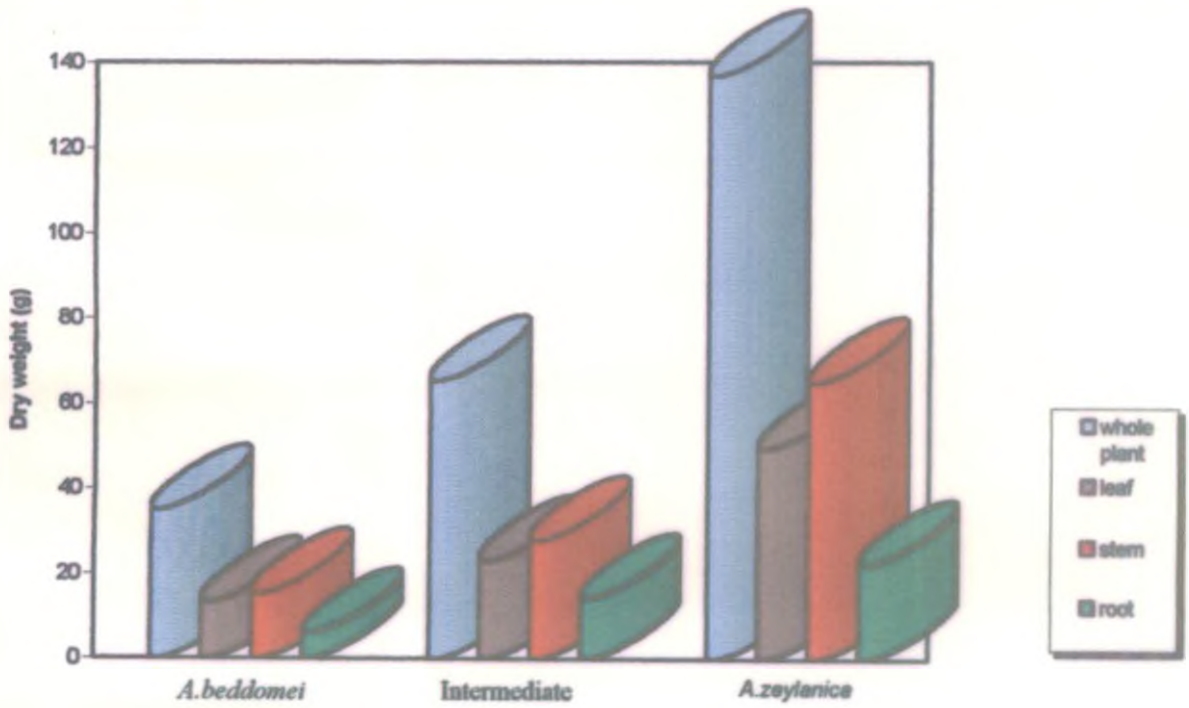

Fig 5b. Dry weight of representative types of *Adhatoda*

Fig 5a. Fresh weight of representative types of *Adhatoda*

Fig 5b. Dry weight of representative types of *Adhatoda*

4.2.1.5. Dry weight

Dry weight of whole plant, leaf, stem and root was recorded (Table12, Fig.6b) *A.beddomei* types had lower dry weight with a mean value of 34.9, 13.4, 15.4 and 6.1g for whole plant, leaf, stem and root respectively than intermediate (65.3, 23.1, 27.8 and 14.4g respectively) and *A.zeylanica* (136.8, 49.5, 65.0 and 22.3g respectively) types.

4.2.2. Stomata

A.zeylanica types had hypostomatic leaves, *A. beddomei* types had amphistomatic leaves and among the intermediate types Acc.22 and 23 had hypostomatic leaves and Acc.27 had amphistomatic leaves.

4.2.3. Biochemical variation

4.2.3.1. Phenols

In leaves a total of ten spots were resolved with Rf ranging from 0.04 to 0.99 with a maximum of seven spots in Acc.28 (Table 13,Plate 5a).All the types had first spot (Rf=0.04) in common. Acc.28 and 34 belonging to *A.beddomei* alone had ninth spot (Rf=0.90). *A.zeylanica* types Acc.51 and 53 had similar elution pattern with first (Rf=0.04), third (Rf=0.11), fifth (Rf=0.24) and tenth spots (Rf=0.99) in common. There was no similarity in the elution pattern among the intermediate accessions Acc. 22,23 and 27 .

In roots, eight spots were eluted with the eighth spot (Rf=0.98) common to all the accessions (Table13, Plate 5b). Acc.51 and 53 of *A.zeylanica* types had second (Rf=0.04), fifth (Rf=0.13) and sixth (Rf=0.33) spots which were absent in other accessions. Seventh spot (Rf=0.5) was common in Acc. 23 and 53 although they belong to different groups. Fourth spot (Rf-=0.11) was found in all the three intermediate types besides the *A.beddomei* type Acc.34.

4.2.1.5. Dry weight

Dry weight of whole plant, leaf, stem and root was recorded (Table12, Fig.6b) *A.beddomei* types had lower dry weight with a mean value of 34.9, 13.4, 15.4 and 6.1g for whole plant, leaf, stem and root respectively than intermediate (65.3, 23.1, 27.8 and 14.4g respectively) and *A.zeylanica* (136.8, 49.5, 65.0 and 22.3g respectively) types.

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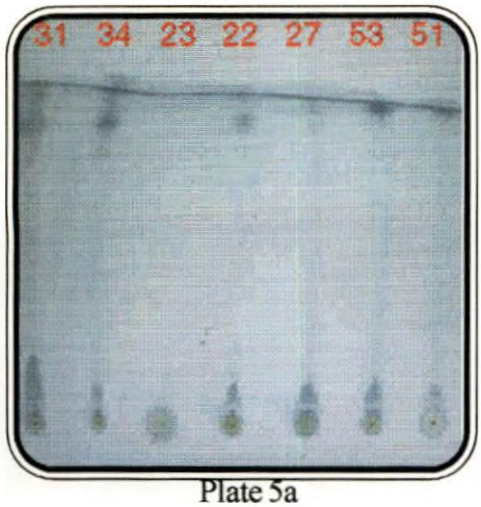

Plate 5a

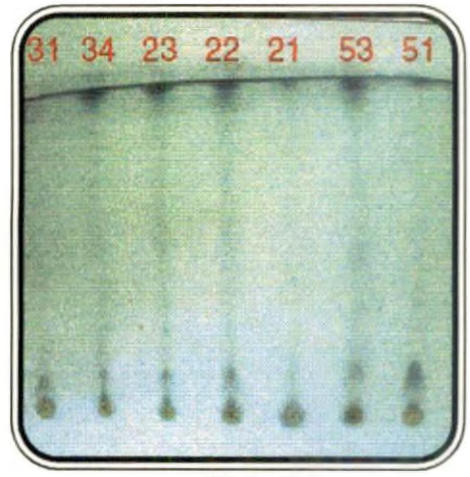

Plate 5b

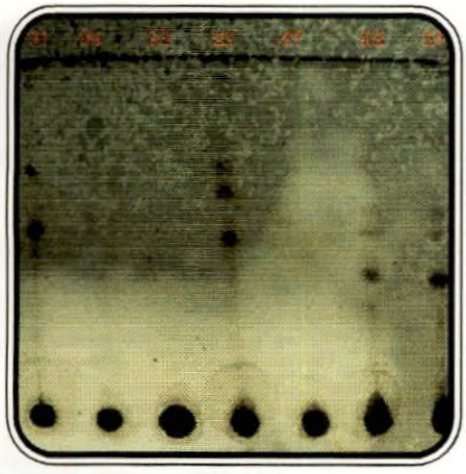

Plate 6a

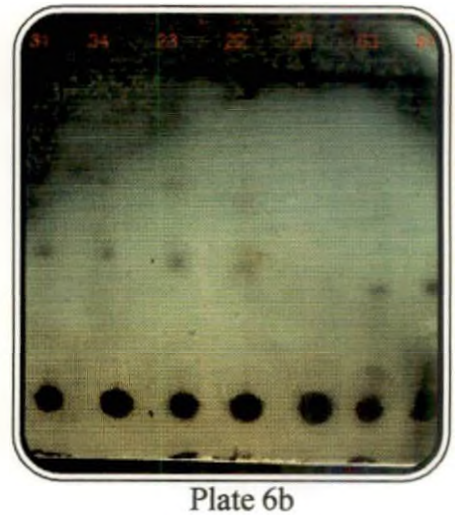

Plate 6b

Plate 7a

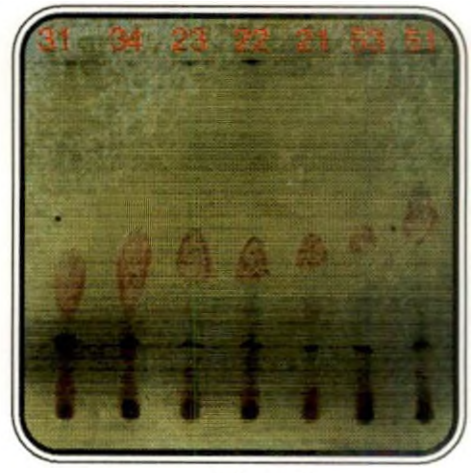

Plate 7b

4.2.3.2. Flavonoids

In leaves, two spots first with Rf=0.03 and second with Rf=0.11 were eluted (Table 14). None of the spots was found as a marker for identifying a specific group. Only Acc.23, 28 and 53 showed the presence of both the spots whereas Acc.22,27,34,38 and 51 had only the first spot (Rf = 0.04). In roots, a total of five spots were eluted with Rf = 0.03, 0.11, 0.83, 0.88 and 0.93 respectively for first, second, third, fourth and fifth spots (Table 14). Among these, third spot (Rf = 0.83) was found only in the intermediate types - Acc.22 and 23. All the accessions showed the presence of first (Rf = 0.03) and second spot (Rf = 0.11) in common. Fourth spot (Rf = 0.88) was found only in Acc.34 and 53 and fifth spot in Acc.28 and 51.

4.2.3.3. Terpenoids

In leaf, a total of eight different spots could be observed with a maximum of five spots in Acc.51. Third spot (Rf=0.52) was common in Acc.22, 28,51 and 53 (Table15,Plate 6a). Eighth spot (Rf=0.96) was found only in the *A.beddomei* type Acc.28. Fifth (Rf=0.65) and sixth spots (Rf=0.69) were found only in the intermediate type Acc.22. First, second and fourth spots (Rf=0.40, 0.44 and 0.59 respectively) were specific to *A.zeylanica* types Acc.51 and 53 and only Acc.51 showed the presence of seventh spot (Rf=0.77) in addition. No terpenoid spot could be detected in Acc.23, 27 and 34. In root, four spots could be identified (Table 15,Plate 6b). *A.beddomei* types 28 and 34 showed the presence of third (Rf=0.44) and fourth (0.67) spots in common. Only first spot (Rf=0.42) was seen in Acc.51 and 53 belonging to *A.zeylanica*. The intermediate types Acc.22 and 23 showed the presence of second (Rf=0.43) spot in common with an addition of fifth(0.67) spot in Acc.23. No terpenoid spot could be detected in Acc.27.

4.2.3.4. Alkaloids

In leaf samples, a spot with Rf = 0.99 and spots with closer Rf values of 0.24 and 0.20 respectively were common in Acc.28 and 34 (*A.beddomei* types). Acc.22 and 23 belonging to intermediate types had a spot with Rf=0.65 in common. Acc.22 had two more spots with Rf = 0.89 and 0.96 in addition. Acc.51 and 53 (*A.zeylanica* types) had two spots with Rf = 0.11 and 0.99 in common. The elution pattern is presented in Plate 7a .

Table 13. Phenol spot pattern of representative types of *Adhatoda*

Types	Rf value (leaves)										Rf value (roots)							
	0.04	0.07	0.11	0.16	0.24	0.32	0.48	0.56	0.9	0.99	0.03	0.04	0.08	0.11	0.13	0.33	0.5	0.98
Acc.22	+	–	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	+
Acc.23	+	+	–	–	–	–	–	–	–	–	+	–	–	+	–	–	+	+
Acc.27	+	–	+	+	–	+	–	–	–	+	+	–	–	+	–	–	–	+
Acc.28	+	–	+	–	–	+	+	+	+	+	–	–	+	–	–	–	–	+
Acc.34	+	+	–	–	+	–	–	–	+	+	+	–	–	+	–	–	–	+
Acc.51	+	–	+	–	+	–	–	–	–	+	–	+	–	–	+	+	–	+
Acc.53	+	–	+	–	+	–	–	–	–	+	–	+	–	–	+	+	+	+

Table 14. Flavonoid spot pattern of seven representative types of *Adhatoda* sp.

Type	Rf value (leaf)		Rf value (root)				
	0.03	0.11	0.03	0.11	0.83	0.88	0.93
Acc.22	+	–	+	+	+	–	–
Acc.23	+	+	+	+	+	–	–
Acc.27	+	–	+	+	–	–	–
Acc.28	+	+	+	+	–	–	+
Acc.34	+	–	+	+	–	+	–
Acc.51	+	–	+	+	–	–	+
Acc.53	+	+	+	+	–	+	–

Table 15. Terpenoid spot pattern of seven representative types of *Adhatoda* sp.

Type	Rf value (leaf)								Rf value (root)			
	0.40	0.44	0.52	0.59	0.65	0.69	0.77	0.96	0.42	0.43	0.44	0.67
Acc.22	–	–	+	–	+	+	–	–	–	+	–	–
Acc.23	–	–	–	–	–	–	–	–	–	+	–	+
Acc.27	–	–	–	–	–	–	–	–	–	–	–	–
Acc.28	–	–	+	–	–	–	–	+	–	–	+	+
Acc.34	–	–	–	–	–	–	–	–	–	–	+	+
Acc.51	+	+	+	+	–	–	+	–	+	–	–	–
Acc.53	+	+	+	+	–	–	–	–	+	–	–	–

Table 16. Essential oil and vasicine content of representative types of *Adhatoda*

Type	Essential oil (%)	Vasicine (%)
Acc.22	1.0	0.48
Acc.23	0.9	0.38
Acc.27	1.0	0.55
Acc.28	1.1	0.27
Acc.34	0.9	0.32
Acc51	1.0	0.66
Acc.53	1.0	0.66

In roots, all the accessions showed the presence of first spot with Rf=0.07 (Plate 7b). In addition, Acc.28 and 34 (*A.beddomei*) had spots with lower Rf value of 0.43 and 0.46 respectively. Acc.22, 23 and 27 had spots with Rf ranging from 0.50 to 0.53 and Acc.51 and Acc.53 had spots with Rf= 0.56 and 0.53 respectively besides the first spot (Rf=0.07). Acc.34 had another spot with Rf=0.19 in addition.

4.2.3.5. Essential oil content

The data collected on the essential oil content presented in Table 16 revealed not much variation among the types analyzed. It ranged from 0.9 to 1.1%.

4.2.3.6. Alkaloid content

The alkaloid content estimated as percentage of vasicine(on dry weight basis)was seen to be higher in the *A.zeylanica* types Acc.51 (0.66%) and Acc.53 (0.66%) compared to the intermediate types - Acc. 22, 23 and 27 (0.48, 0.38 and 0.58 % respectively). Acc.28 and 34 belonging to *A.beddomei* had the least content of vasicine of 0.27 and 0.32% respectively (Table 16).

4.3. Seasonal variation in morphological and biochemical characters

Among the 52 accessions of *Adhatoda*, 13 types belonging to *A.zeylanica*, *A.beddomei* aand intermediate types were selected for studying morphological and biochemical variation in the two seasons(dry and wet). They were Acc.35,51 and 53 (*A.zeylanica*), Acc.13,21 and 33 (*A.beddomei*) and Acc.3,6,12,20,29,41and 52 (intermediate types).

4.3.1. Morphological variation

Characters analyzed were leaf number, leaf area, root length, root girth, fresh weight of whole plant, leaf, stem and root, dry weight of whole plant, leaf, stem and root and were computed using pooled analysis of variance (Table 17). Interaction between character and season was significant for the characters except fresh weight of stem and dry weight of leaves. The variation between seasons was significant for all

the characters where as variation between accessions (within a season) was significant for the characters except leaf number, root length and root girth.

In general, all the accessions showed an increasing trend in the wet season for all the characters studied. Leaf number was doubled in the wet season (47.73 in dry season to 109.19 in wet season) with higher values for *A.zeylanica* and intermediate types. A slight increase in the leaf area was noted where the mean value increased from 46.03 in the dry season to 53.27 in the wet season. Mean root length and root girth increased from 29.4 to 43.45cm and 1.15 to 2.94cm respectively. Root length and root girth was the highest for *A.zeylanica* type, Acc.53 (46.63 and 3.4 respectively). Root length was lowest for *A.beddomei* type, Acc.33 (27.18cm) and root girth for Acc 21 (0.92cm), which is also a *A.beddomei* type.

4.3.1.1. Fresh weight

Fresh weight of whole plant, leaves and stem almost doubled in most of the accessions during the wet season. Mean fresh weight of whole plant was the highest (274.0g) for Acc.53 (*A.zeylanica* type) and lowest (78.33g) for Acc.13 (*A.beddomei* type). Fresh weight of leaves ranged from18.2g (Acc.12) to 85.3g (Acc.35) in the dry season and 41.05 (Acc.12) to 138.5g (Acc.53) during the wet season. Mean fresh weight of stem increased from 40.49g in the dry season to 73.8g in the wet season with a maximum of 98.5g and 162.0g for Acc.35 and Acc. 53 in the dry and wet season respectively. Both the accessions were *A.zeylanica* types. Mean fresh weight of roots among the accessions ranged from 12.8g (Acc.20) to 52.18g (Acc.53). Fresh weight of roots increased from 20.64 g during dry season to 33.08g in the wet season (Table.17).

4.3.1.2 Dry weight

Dry weight of whole plant, leaf, stem and root increased from dry season to wet season for all the accessions. Dry weight of stem and root was the maximum for Acc.53 for both seasons. (43.25 and 74.65g for dry weight of stem and 22.9g and 25.25g for dry weight of root for the dry and wet season respectively). Dry weight of stem was the lowest for Acc.12 for both the seasons (Table.17).

Table 17. Morphological variation of *Adhatoda* types in two seasons									
Type	Leaf no.			Leaf area(cm^2)			Root length(cm)		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Acc.3	54	130	92	67.55	71.35	69.45	26.65	59.5	43.08
Acc.6	31	133	82	36.95	44	40.47	33.3	51	42.15
Acc.12	49	128	88.05	46.45	47.15	46.8	23.45	45.05	34.25
Acc.13	31	67.5	49.25	35.5	39.6	37.55	36.1	46.65	41.38
Acc.20	26.5	55.5	41	34.4	46.3	40.35	20.55	44.35	32.45
Acc.21	56.5	89.5	73	35.5	37.6	36.55	26.6	33.5	30.05
Acc.29	42	59	50.5	32	45.85	38.93	30.2	37.5	33.85
Acc.33	39	82.5	60.75	41.6	43	42.2	23.05	31.3	27.18
Acc.35	71	129	100	69.3	70.8	70.05	26.9	43.05	34.97
Acc.41	60.5	266	163	46.1	43.7	44.9	41.55	45.1	43.32
Acc.51	62	132.5	97.25	42.95	47.3	45.13	31.3	41	36.15
Acc.52	20.5	37	28.75	52.95	74.65	6308	24.55	31.75	28.15
Acc.53	52	110	81	57.2	81.05	69.23	38.2	55.05	46.63
Mean	45.73	109.19		46.03	53.27		29.42	43.45	
CD for comparison of season(5%)			27.44			4048			11.79
CD for comparison of accession(5%)			70.7			11056			4.65

Table 17. (contd.)

Root girth(cm)			Fresh weight-whole plant(g)			Fresh weight-leaf(g)			Fresh weight-stem(g)		
S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
1.1	4.15	3.63	87.05	165.15	126.1	32.8	59.05	45.93	45.6	67.6	56.6
0.8	4	2.4	84.55	163.8	124.18	33.25	59.6	46.43	39.2	73.3	55.75
1.3	4	2.65	55.1	110.5	82.8	18.2	41.05	29.63	24.3	41.8	33.05
0.7	1.6	1.15	62.65	94.05	78.05	22.75	48.05	35.4	19.9	56.8	38.35
0.8	1.5	1.15	52.35	122.15	87.25	26.8	42.55	34.68	26.3	42.6	34.45
0.8	1.05	0.92	72.25	134.75	103.5	30.6	54.95	42.98	29.3	62.1	45.7
1.05	4.95	3	58	204.94	131.48	23.1	78.3	50.7	22.1	84.7	53.4
1.6	2.4	2	67.9	v	105.73	24.65	56.55	40.6	23.3	62.3	42.8
2	2.75	2.38	217.55	263.9	240.73	85.25	105.55	95.4	98.5	106.7	102.6
1.25	2.45	1.85	122.8	212.9	167.85	47.8	83.25	65.53	50.6	93.3	71.95
1.3	2.55	1.93	108.15	161.4	134.78	39.65	67	53.33	52.3	71.5	61.9
0.9	1.4	1.15	64.75	124.25	94.5	21.8	45.75	33.78	21	36	28.5
1.35	5.45	3.4	185.5	362.5	274.03	69.55	138.5	104.03	74	162	118
1.15	2.94		95.28	174.14		36.63	67.67		40.49	73.8	
		1.93			22.12			8.1			
		0.76			56			20.72			

Table 17. (contd.)

Type	Fresh weight-root(g)			Dry weight-whole plant(g)			Dry weight-leaf(g)			Dry weight-stem(g)		
	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean	S1	S2	Mean
Acc.3	12.75	37.75	25.25	34.75	53.75	44.25	14.20	23.00	18.70	20.65	21.70	21.18
Acc.6	13.65	32.55	23.10	20.05	29.00	24.53	7.95	23.05	15.50	19.25	14.00	16.63
Acc.12	16.30	27.05	21.68	20.50	40.10	30.30	4.30	8.40	6.35	4.95	12.05	8.50
Acc.13	12.65	19.00	15.83	28.65	54.10	41.38	11.34	25.40	18.38	9.85	24.05	16.95
Acc.20	12.15	13.50	12.83	37.55	57.25	47.40	12.25	23.80	18.00	14.80	22.25	18.53
Acc.21	11.25	18.50	14.88	33.00	52.85	43.20	17.35	27.15	22.25	15.05	29.95	22.50
Acc.29	16.30	45.50	30.90	33.60	70.30	51.90	8.05	27.35	17.70	7.45	27.05	17.25
Acc.33	22.35	27.80	25.08	44.90	65.50	55.20	15.05	25.75	20.40	13.80	29.65	21.73
Acc.35	37.85	43.60	40.72	118.90	76.40	97.65	21.40	31.00	26.20	24.05	22.85	23.45
Acc.41	25.25	39.05	32.15	73.30	103.05	88.18	22.15	37.19	30.03	28.05	42.65	35.35
Acc.51	20.30	30.80	25.55	29.55	55.55	44.05	5.40	12.25	8.83	6.15	12.50	9.33
Acc.52	27.55	30.55	29.05	25.20	45.00	35.10	8.75	15.95	12.35	7.50	13.90	10.70
Acc.53	40.00	64.35	52.18	106.35	166.05	136.20	41.55	56.60	49.07	43.25	74.65	58.95
Mean	20.64	33.08		46.68	67.07		14.61	25.97		16.52	26.71	
CD for comparison of season(5%)			50.77			12.32						5.30
CD for comparison of accession(5%)			12.77			31.53						13.44

s1:season1(dry) s2: season2 (wet)

Table 17. (contd.)

Dry weight-root(g)			Vasicine(%)		
S1	S2	Mean	S1	S2	Mean
5.45	14.85	10.15	1.41	0.19	0.80
4.95	5.00	4.98	0.96	0.85	0.91
8.55	15.05	11.80	0.90	0.19	0.55
7.18	8.95	8.05	0.99	0.28	0.64
7.20	6.15	6.67	1.10	0.85	0.98
6.80	12.00	9.40	1.00	0.28	0.64
5.50	14.35	9.00	1.10	0.85	0.98
13.55	18.70	16.13	1.15	0.28	0.72
10.15	16.50	13.30	1.22	0.47	0.85
10.20	14.30	12.25	1.10	0.85	0.92
8.30	12.70	10.50	1.40	0.66	1.03
11.45	10.45	10.95	0.88	0.38	0.63
22.90	25.25	25.08	1.36	0.66	1.01
904.00	13.40		1.12	0.45	
		1.88			0.10
		4.76			NS

4.3.2. Biochemical variation

4.3.2.1 Phenols

Elution pattern of phenols in the whole plant revealed that total number of spots was more during the dry season (13 spots) than the wet season (9spots). Ten groups could be obtained after grouping the accessions based on elution pattern in the dry season and nine groups in the wet season. Second spot (Rf = 0.15) was absent in *Adhatoda beddomei* types (Acc.13,21 and 33). Maximum number of spots were found in intermediate types Acc.6, 12 and 20 with ten spots and minimum for Acc.21 (*Adhatoda beddomei*) with only two spots. During the wet season also second spot (Rf = 0.08) was absent in all the *Adhatoda beddomei* types. The intermediate accessions Acc.3, 29 and 41 had first (Rf = 0.05) and second spots (Rf = 0.08) in common with an addition of seventh (Rf = 0.27), eighth (0.35) and sixth spots (Rf = 0.22) respectively. *Adhatoda beddomei* types, Acc.13 and 21 had first and third spots (Rf= 0.05 and 0.11 respectively) in common with an addition of ninth (Rf = 0.38) and fifth (Rf = 0.19) spots respectively. *Adhatoda beddomei* types differed in their elution pattern widely (Table 18 and 19, Plates 8a,b,c and d).

4.3.2.2. Flavonoids

The eluted plates as above on drying and viewing under UV betrachter gave yellowish green fluorescent spots indicating the presence of flavonoids (Table 20). Four spots were observed in both the seasons, however Rf value differed between the two seasons. Grouping of accessions based on the elution pattern of spots was not conforming to the cluster analysis in both the seasons. In the dry season Acc.3,21,29 and 41 had only the first spot (Rf = 0.20). Acc. 20 and 51 showed the presence of a third spot (Rf = 0.43) in addition. Acc 6,12,13,35 and 53 showed the presence of all the four spots. Acc.33 and 52 showed the presence of only the third spot (Rf = 0.43).

The elution pattern was different in the wet season where Acc. 3,6,12,13,20,29,33,41,51 and 52 showed similar pattern with first (Rf = 0.03) and second spots (Rf = 0.11). Acc.21 and 35 had a third (Rf = 0.17) and fourth spot (Rf = 0.21) in addition. Acc.53 showed the presence of first, second and third spot after elution.

Plate 8. Thin Layer Chromatogram of phenols in *Adhatoda* in two seasons

8a & 8b. Dry season

8c & 8d Wet season

Plate 9. Thin Layer Chromatogram of terpenoids in *Adhatoda* in two seasons

9a and 9b. Dry season

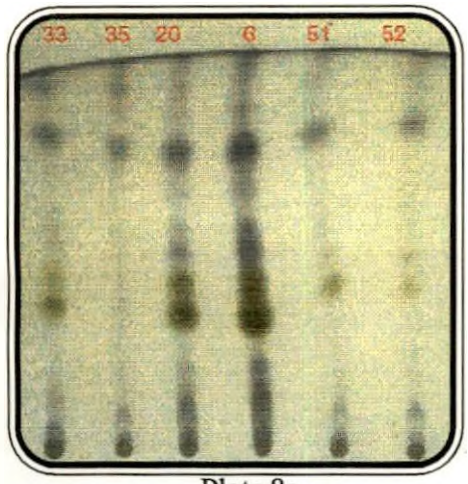

Plate 8a

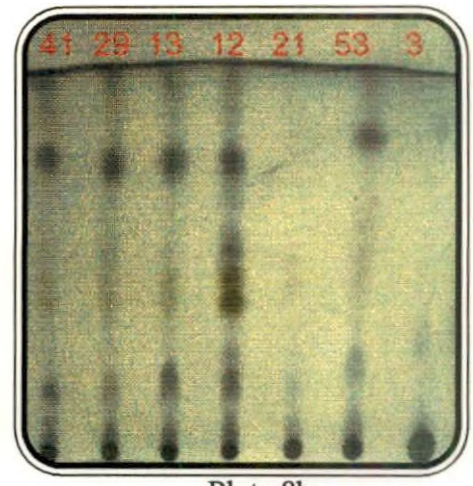

Plate 8b

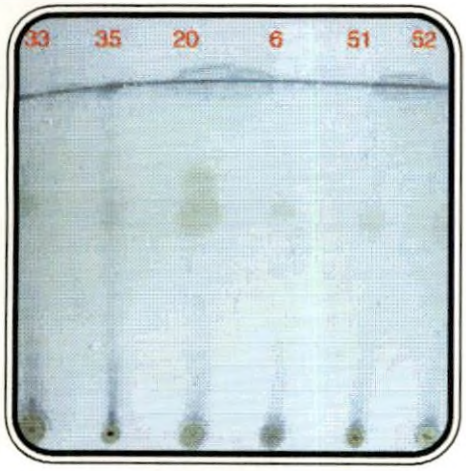

Plate 8c

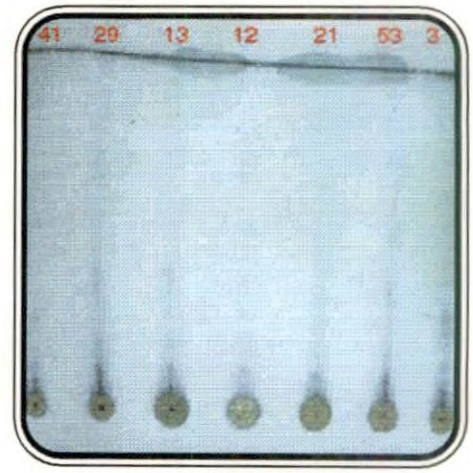

Plate 8d

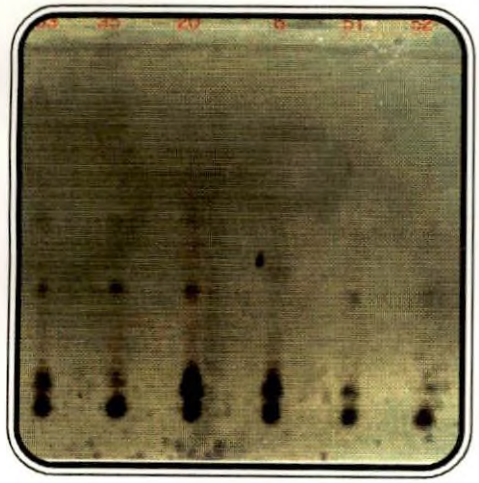

Plate 9a

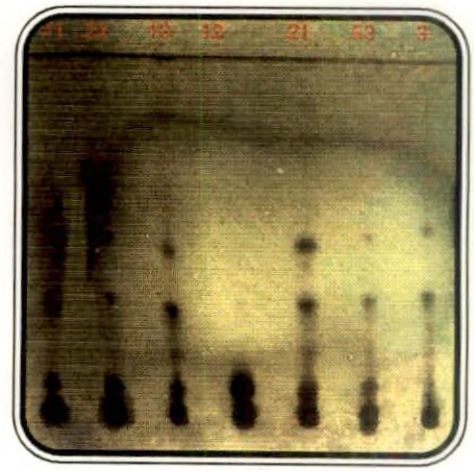

Plate 9b

Plates 9c and 9d. Thin Layer Chromatogram of terpenoids in *Adhatoda* in wet season

Plate 10. Thin Layer Chromatogram of alkaloids in *Adhatoda* in two seasons

10a and 10b. Dry season

10c and 10d Wet season

Plate 9c

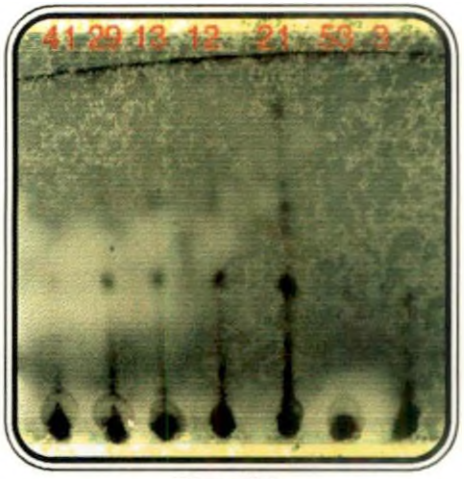

Plate 9d

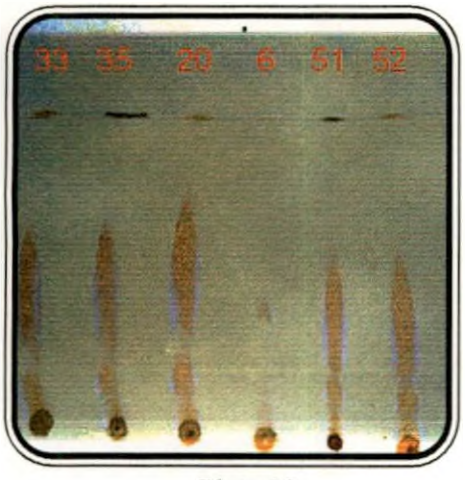

Plate 10a

Plate 10b

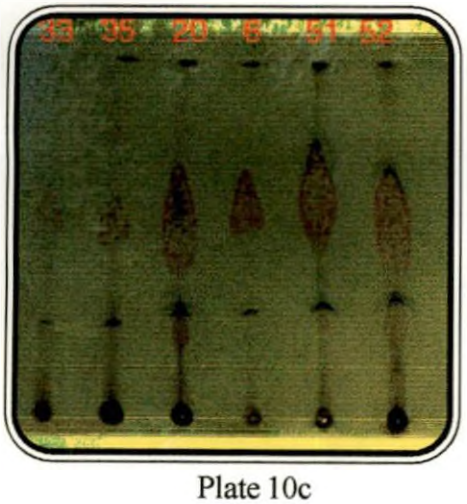

Plate 10c

Plate 10d

4.3.2.3. Terpenoids

Although the total number of spots was more in the wet season (9 spots) compared to the dry season (7 spots), number of spots in a type was found to decrease in the wet season. Acc. 20,33 and 35 belonged to the same group based on elution pattern of terpenoids in both the seasons although they represented three different types. During the dry season, first (Rf = 0.03) and second spots (Rf = 0.06) were common in all the types. Two of the *A.beddomei* types. Acc.13 and 21 showed the presence of sixth spot (Rf = 0.49). Among the three *A.zeylanica* types, two of them Acc.35 and 53 had similar pattern with first (Rf = 0.03), second (Rf = 0.06), third (Rf = 0.11), fifth (Rf = 0.32) and seventh spots (Rf = 0.52). During the wet season first spot (Rf = 0.33) was found only in two intermediate types Acc.3 and Acc.52. Fourth spot (Rf = 0.48) was also found in the intermediate types Acc.6 and 41. The intermediate type Acc.6 only showed the presence of sixth spot (Rf = 0.78). Only Acc.51 possessed seventh spot (Rf = 0.88). In short, no spot could be confirmed as a marker for identifying a specific type (Table 21,Plates 9a,b,c and d).

4.3.2.4 Alkaloids

Number of spots was more in the wet season than the dry season in some of *A.zeylanica* and intermediate types where as it decreased in the wet season for all the *A.beddomei* types. During the dry season all the types showed the presence of only three spots but with varying Rf values. First spot (Rf = 0.04) was common in all the types. During the wet season, spots with high Rf values - 0.93, 0.97 etc. were seen in intermediate types viz. Acc. 41 and 29 respectively (Plates 10 a,b,c and d).

4.3.2.5. Alkaloid content

Alkaloid content expressed as percentage of vasicine was found to decrease from January to July in all types studied with a content of 1.12 and 0.45 % in dry and wet season respectively In general, *A.zeylanica* types - Acc.35, 53 and 51 contained higher vasicine content of 1.22, 1.36 and 1.4% respectively during the dry season and *A. beddomei* types - Acc.13, 33 and 21 had comparatively lower alkaloid content of 0.99,1.15 and 1.0% respectively. In the intermediate types, the vasicine percentage ranged from 0.90 in Acc.12 to 1.41 in Acc.3. During the wet season also the same trend was followed where *A. zeylanica* accessions had more than 0.45% of vasicine

Table 18. Phenol spot pattern of *Adhatoda* types (dry season)

Type	Rf value												
	0.05	0.15	0.17	0.18	0.21	0.58	0.59	0.71	0.85	0.86	0.90	0.98	1.00
Acc.3	+	–	–	–	+	–	–	–	–	+	–	–	–
Acc.6	+	+	–	+	+	+	+	+	+	–	+	+	–
Acc.12	+	+	–	+	+	+	+	+	+	–	+	+	–
Acc.13	+	–	–	+	+	–	–	–	+	–	+	+	–
Acc.20	+	+	–	+	+	+	+	+	+	–	+	+	–
Acc.21	+	–	–	+	–	–	–	–	–	–	–	–	–
Acc.29	+	–	–	+	–	–	–	–	+	–	+	+	–
Acc.33	+	–	+	–	–	–	–	–	–	+	–	+	–
Acc.35	+	+	–	–	–	–	–	–	+	–	+	+	–
Acc.41	+	+	–	+	–	–	–	–	+	–	+	+	–
Acc.51	+	+	–	–	–	–	–	–	–	+	–	–	+
Acc.52	+	+	–	–	–	–	–	–	–	+	–	–	+
Acc.53	+	+	–	+	+	+	–	–	–	+	+	+	–

Table 19. Phenol spot pattern of *Adhatoda* types (wet season)

Type	Rf value								
	0.05	0.08	0.11	0.16	0.19	0.22	0.27	0.35	0.38
Acc.3	+	+	–	–	–	–	+	–	–
Acc.6	+	–	–	–	–	–	–	–	–
Acc.12	+	–	+	–	–	–	–	–	–
Acc.13	+	–	+	–	–	–	–	–	+
Acc.20	+	–	+	–	–	–	–	+	–
Acc.21	+	–	+	–	+	–	–	–	–
Acc.29	+	+	–	–	–	–	–	–	–
Acc.33	+	–	–	+	–	–	–	–	–
Acc.35	+	–	–	+	–	–	–	–	–
Acc.41	+	+	–	–	–	+	–	–	–
Acc.51	+	–	+	–	–	–	–	–	–
Acc.52	+	–	+	–	–	–	–	–	–
Acc.53	+	–	+	–	–	–	–	–	–

Table 20. Flavonoid spot pattern of *Adhatoda* types(dry and wet season)

Type	Rf value (first season)				Rf value (second season)			
	0.2	0.32	0.43	0.55	0.03	0.11	0.17	0.21
Acc.3	+	–	–	–	+	+	–	–
Acc.6	+	+	+	+	+	+	+	+
Acc.12	+	+	+	+	+	+	–	–
Acc.13	+	+	+	+	+	+	–	–
Acc.20	+	–	+	–	+	+	–	–
Acc.21	+	–	–	–	+	+	+	+
Acc.29	+	–	–	–	+	+	–	–
Acc.33	–	–	+	–	+	+	–	–
Acc.35	+	+	+	+	+	+	+	+
Acc.41	+	–	–	–	+	+	–	–
Acc.51	+	–	+	–	+	+	–	–
Acc.52	–	–	+	–	+	+	–	–
Acc.53	+	+	+	+	+	+	+	–

Table 21. Terpenoid spot pattern of *Adhatoda* types(dry and wet seasons)

Type	Rf value(first season)							Rf value(second season)								
	0.03	0.06	0.11	0.28	0.32	0.49	0.52	0.33	0.34	0.35	0.48	0.56	0.78	0.88	0.95	1.0
Acc.3	+	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−
Acc.6	+	+	+	−	+	−	−	−	−	+	+	+	+	−	−	+
Acc.12	+	+	+	−	−	−	−	−	−	+	−	+	−	−	−	+
Acc.13	+	+	+	−	−	+	−	−	−	+	−	+	−	−	−	−
Acc.20	+	+	+	−	+	−	+	−	−	+	−	−	−	−	−	−
Acc.21	+	+	+	−	+	+	−	−	+	−	−	+	−	−	+	+
Acc.29	+	+	+	+	+	−	+	−	−	+	−	+	−	−	−	−
Acc.33	+	+	+	−	+	−	+	−	−	+	−	−	−	−	−	−
Acc.35	+	+	+	−	+	−	+	−	−	+	−	−	−	−	−	−
Acc.41	+	+	+	+	−	+	−	−	−	−	+	−	−	−	−	−
Acc.51	+	+	−	−	+	−	−	−	−	+	−	−	−	+	−	+
Acc.52	+	+	−	−	+	−	−	+	−	−	−	−	−	−	+	+
Acc.53	+	+	+	−	+	−	+	−	+	−	−	−	−	−	−	−

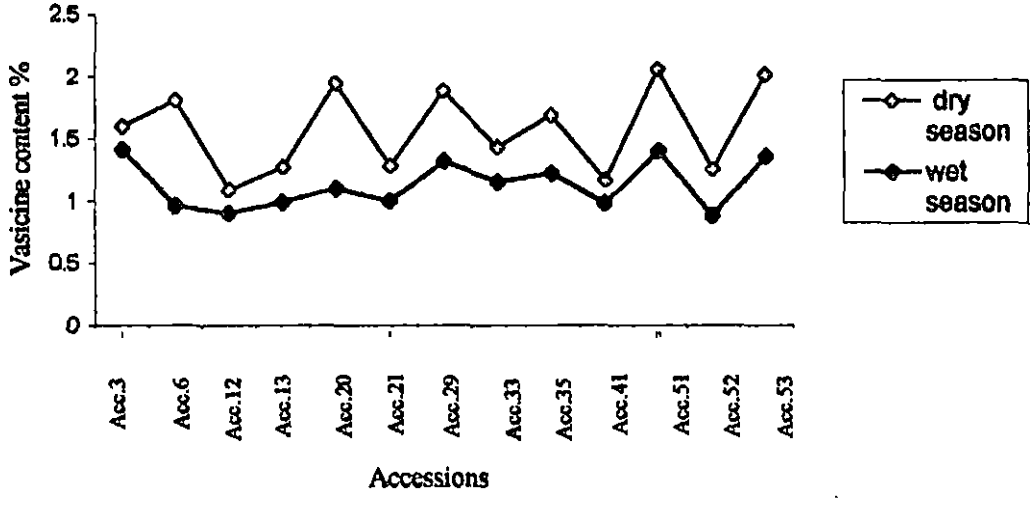

Fig 6. Vasicine content in *Adhatoda* in two seasons

and *A.beddomei* accessions with less than 0.3% of vasicine. Wide range of alkaloid content (0.19 to 0.85%) was visible in the intermediate types (Table 17, Fig. 6).

Discussion

DISCUSSION

The results on the "morphological and biochemical variation in adhatoda types (*Adhatoda* spp.)"are briefly discussed in this chapter.

5.1. Characterization of Adhatoda types

Although all the 52 accessions of *Adhatoda* spp. showed general characters of the genus Adhatoda there was distinct variation among them in many of the vegetative and floral characters.

Based on the vegetative characters viz. plant height, number of branches, leaf area and petiole length the types were grouped into three clusters and comparison was done among the clusters as it was found to be more convenient and reliable. The mean height was 63.87cm in cluster I, 70.42cm in cluster II and 39.96cm in cluster III. There was wide variation in number of branches between accessions (2.0 to 7.7) with a mean of 5.1, 5.4 and 3.9 in cluster I, II and III, so also petiole length -1.5, 2.5 and 1.4cm for cluster I, II and III respectively. The leaves were generally elliptic lanceolate with attenuate ends in which both narrow and broad types were observed. All the types in cluster III had narrowly elliptic lanceolate leaves, those in cluster II had broadly elliptic lanceolate leaves and cluster I included types with broad as well as narrow elliptic lanceolate leaves.

On detailed review of vegetative characters in *Adhatoda*, it was clear that the types in cluster II showed the characters of *Adhatoda zeylanica,* those in cluster III resembled *A. beddomei* and the types in cluster I showed characters intermediate to both the species. These results were conforming to the observations on floral characters. The inflorescence in general was prominently bracteate spike with axillary peduncle although two types of spikes-one long and spreading and the other short and compact were observed. Flowers were sub sessile in the axils of opposite bracts, the two stamens were attached near the top of the corolla tube, and had bicarpellary syncarpous bilocular ovary and bifid stigma. Two floral types could be observed one with large white flowers having purplish streaks in the inner side of corolla, large

pubescent ovary and another type with small white flowers without purplish streaks and small glabrous ovary. Stigma was bifid in all the types. All the flowered types in cluster II belonged to the former group, those in cluster III belonged to the latter and cluster I included both the floral types.

Many workers gave detailed description of the two species of *Adhatoda*, which agreed with the present observations. Hooker (1886) described *A.vasica* as a dense shrub 4-8 feet high, elliptic leaves of 8 by 3 inch size, petiole length of one inch, flowers with white transversely rose-barred lips and minutely hairy ovary. *A beddomei* has entire leaves with 6 by 1.5 inch size, petiole ½ inch and white flowers without rose barred lips and glabrous ovary.

Leaf and floral characters of *Adhatoda* types which represented the two species *A.zeylanica* and *A beddomei* also agreed with the description of Gamble (1957), Aiyer and Kolammal (1962) and Henry *et al.*(1987). An analysis of the vegetative characters showed that the intermediate types resembled *A.zeylanica* in plant height and number of branches and *A.beddomei* in leaf area and petiole length. Aiyer and Kolammal (1962) reported the number of secondary nerves in the leaves to be 10-12 and upto 15 in *A.zeylanica* and 8-10 in *A. beddomei.* Pundarikaksudu and Bhavsar (1988) observed 8-10 pairs of secondary nerves in small leaf variety of *A.vasica* and 13-15 pairs in big leaf variety. But among the 52 types studied except 5 types (two of them belonging to cluster III and others belonging to cluster I) all other types possessed more than 10 pairs of secondary nerves. Similarly, eventhough the leaf tip was described as acute in *A.vasica* and attenuate in *A.beddomei* (Gamble 1957, Aiyer and Kolammal , 1962) all the types possessed leaves with attenuate ends. All the flowered types of . *A.zeylanica* possessed bracts with more than five ribs with a range of 7-9 and *A.beddomei* types had bracts with only 5 ribs, which agreed with the description by Aiyer and Kolammal (1962). The leaf colour was observed as dark green on upper side in majority of the types and only six types possessed leaves with light green colour on the lower side. In the present study all the *A.zeylanica* types except Acc.40 had leaf colour which is in conformation with the description given by Gamble (1957) and Aiyer and Kolammal (1962) but the light green colour was observed in five intermediate types. There are no reports regarding the leaf colour of *A.beddomei* and all the *A.beddomei* types

showed dark green colour on the upper side. Pundarikakshudu and Bhavsar (1988) reported the small leaf variety of *A.vasica* to possess dark green leaves and big leaf variety of *A.vasica* to possess light green leaves.

One of the intermediate types (Acc.25) had flower characters similar to *A. beddomei* and leaves broadly elliptic lanceolate typical of. *A.zeylanica* but bracts possessed 8 ribs. Ovary was pubescent and large sized in *A.zeylanica* types but glabrous and small sized in *A.beddomei* types with bifid stigma in both the types which agree with the description given by Hooker (1886), Gamble (1957) and Aiyer and Kolammal (1962). But the style was hairy only at the base in both the species in contrary to their reports that the style was terminal hairy in *A.beddomei* and hairy at the base in *A.zeylanica*.

It was also observed that the major morphological characters like plant habit, leaf size and inflorescence characters of *A.zeylanicq* types agreed with the description of big leaf variety of *A.vasica* and that of *A.beddomei* types agreed with the description of small leaf variety of *A.vasica* given by Pundarikakshudu and Bhavsar (1988).

Both hypostomatic and amphistomatic types of leaves were observed in the 52 accessions of *Adhatoda*. All the *A.zeylanica* types had hypostomatic leaves and *A.beddomei* types had amphistomatic leaves, which agreed with the description by Datta and Mukherjee (1952) and Aiyer and Kolammal (1962). Both hypostomatic and amphistomatic types of leaves were found in the intermediate types. There was no significant vairiation in the stomatal index between the three groups. Pundarikakshudu and Bhavsar (1988) also observed similarity in stomatal index between the big leaf (BLV) and small leaf variety of (SLV) *A.vasica* but leaves of both the types were amphistomatic in contrast to the present finding. This probably suggests that SLV is a morphotype of *A. vasica*.

They observed more number of simple covering trichomes among which two celled ones were common in BLV compared to SLV, which has lesser number of simple covering trichomes with a majority of three celled ones. No such difference could be observed in the present study and both two celled as well as three celled simple covering trichomes were found in the two species. Stomatal frequency as well

as its distribution have been utilized to classify *Chlorophytum* (Naik and Nirgade, 1981) Malvales (Rao and Ramayya ,1981) and *Ocimum* spp. (Vadukoot, 1996) for comparative studies.

Among the intermediate types, three groups of accessions were observed. One group (Acc.25 and 27) had floral and stomatal characters of *A.beddomei* and leaf characters of *A.zeylanica*. Another group (Acc.23) had flowers and stomata of *A.zeylainca* types and leaf resembling that of *A. beddomei*. A thrid group (Acc.22) had flowers of *A.beddomei* but leaf and stomatal characters of *A. zeylanica*. Many reasons can be attributed to the occurrence of intermediate types in *Adhatoda*. Reddi *et al.* (1989) reported cross-pollination in *Adhatoda zeylanica* with *Xylocopa* sp. as the pollinating agent. Datta and Maiti (1968) suggested that the evolution of different cytotypes or biotypes within *Adhatoda* may be brought about by translocation, fragmentation, deletion as well as by polyploidy or aneuploidy. The chromosomal changes have probably selected out cytotypes best suited to their natural environments and the chromosomal differences may be related to the quantitative anatomical characters and to the quantity and quality of chemical substances available in this species.

Isozymes have been most often used to analyze taxonomic, genetic and evolutionary relationships of different plant populations. Electrophoretically exposed genetic markers can be used in selection-monitoring procedures during introgression of new germplasm. High amount of variation within *Adhatoda* types at electrophoretic loci of esterase and peroxidase has been observed in the present study. This could be due to vegetative propagation in the species where variability due to natural crossing once created gets fixed. Isozymes had been used for characterizing species by many workers like Athma *et al.* (1982) in castor bean, Suvachittanont (1991) in *Kaempferia*, Sebastian *et al.* (1996) in *Piper* and Joseph (1999) in turmeric. Although two groups could be obtained based on peroxidase banding pattern, no band was found specific to a species or accession in *Adhatoda*. Though there was variation between the accessions in peroxidase activity, grouping based on it was not in conformity to the cluster analysis. Variation in peroxidase activity between cultivars had been reported

by Reddy and Bhalla (1994) in *Portulaca grandflora*, Rossini *et al.* (1995) and Ortega *et al.* (1995) in soyabean.

In esterase, total of seven variable isozymes were observed. The accessions were grouped into thirteen based on the variation in banding pattern. Maximum number of accessions (24) belonged to the first group which has EST-1, 2,4,6 and 7 in common with Rm = 0.422, 0.400, 0.300, 0.222 and 0.089 respectively. Among these, 12 types belonged to cluster III (*A.beddomei* types), twelve types to cluster I which include intermediate types out of which eight had amphistomatic leaves and four types had hypostomatic leaves. It was observed that none of the *A.zeylanica* types showed the group I type of banding pattern.

Occurrence of different banding pattern within a species indicates that there could be alleles of same locus which only a genetical study can confirm(Wendel and Weeden,1989). However, among the organic molecules isozymes are very useful aids to compare genotypes and they are used as a supplementary tool along with morphological, genetic and other biochemical methods. There is no doubt that for proper isozyme finger printing the entire germplasm should be analysed for more enzymes before drawing conclusions.

5.2. Comparative study of representative accessions

Accessions typical to the three groups viz. *A.zeylanica* (Acc. 51 and 53), *A.beddomei* (Acc. 28 and 34) and intermediate types (Acc.22, 23 and 27) were compared for morphological and biochemical characters.

The lower number of leaves in *A.beddomei* types may be due to the short stature of the plant with lesser number of branches. The greater vigour expressed through number of branches and leaves and spread of *A.zeylanica* accounts for the dense foliage growth.

A similar trend was noted for fresh weight and dry weight of whole plant, leaf stem and root for the three groups in which *A.zeylanica* types recorded the highest value for all the above yield attributes and *A.beddomei* types recorded the lowest. The

greater fresh weight of leaves and stem in *A. zeylanica* is due to its tall stature with more number of branches, leaves and greater leaf area.

Root length and root girth showed slight variation between accessions though the root weight showed notable difference. The higher root weight of *A.zeylanica* may be due to the thicker inner bark region, which occupies 1/3 thickness of the bark. In *A. beddomei* the outer most cork layer is much thicker however the inner bark occupies only a quarter of the thickness of the bark. Also, the stone cells that constitute a characteristic annular band in the cortex are smaller in size in *A.beddomei* than those of *A.zeylanica* (Aiyer and Kolammal, 1962) .The increasing trend in the dry weight of whole plant, leaf, stem and root for *A.zeylanica* types may be due to the higher fresh weight and greater dry matter partitioning in the species.

Studies on chemical variation have been suggested as one of the principal growing points in the field of taxonomy and systematics. Phytochemical entities like phenolics, which include free phenols, phenolic acids and flavonoids, terpenoids and alkaloids can supplement the existing morphological variation and its importance in taxonomic studies is well documented.

Unidirectional separation of compounds using Thin Layer Chromatography (TLC) was found effective as the spot numbers were less and good separation was obtained in the solvent system. TLC is advantageous compared to paper chromatography because of its versatility, speed and sensitivity and gas liquid chromatography because of its simplicity in performance and cheapness.

The free phenols and phenolic acids are best considered together during plant analysis. On spraying Folin-Ciocalteu's reagent, which is a more specific reagent for phenols, on the eluted dried plates blue spots appeared which indicated the presence of free phenols in *Adhatoda* sp. When the accessions representative of the three groups were qualitatively anlaysed for free phenols it was seen that there was wide variation in the elution pattern between accessions. A total of ten spots with Rf value ranging from 0.04 to 0.99 were observed in leaves. As the ninth spot with Rf = 0.90 was found only in the *A.beddomei* types Acc.28 and 54 and this spot can be considered as a marker for this species. Similarly the *A.zeylanica* types had the simultaneous

occurrence of first (Rf = 0.04), third (Rf = 0.11), fifth (Rf = 0.24) and tenth spots (Rf = 0.99).

In roots, second (Rf = 0.04), fifth (Rf = 0.13) and sixth (Rf = 0.33) spots could be considered as markers in *A.zeylanica*. Harborne (1973) reported that with Folin-Ciocalteu's reagent phenols with catechol or hydroquinone nuclei appear as blue spots immediately after spraying. This suggests that the free phenols present in *Adhatoda* sp. may be having catechol or hydroquinone nuclei.

The taxonomic value of phenolic studies had been demonstrated by Bate-Smith (1948) who opined that the discontinuous distribution of rarer phenolics and the correlated occurrence or absence of commoner ones offer potentially valuable evidence to a taxonomist. Phenolic constituents as taxonomic markers had been studied earlier by Nageshwar *et al.* (1988) in Amaranthaceae and Esti *et al.* (1988) in olive.

Another group of phenolics viz. flavonoids are also effectively utilized for chemotaxonomical studies. On analysing flavonoid elution pattern in *Adhatoda*, yellowish green fluorescent spots were observed when viewed under UV light. In leaves, the two spots eluted had low Rf values of 0.025 and 0.108 but spots with higher values had been observed in roots. Although there was variation in elution pattern between accessions, no spot could be identified as a marker character in any of the species.

Rangaswami and Seshadri (1971) identified luteolin as the flavone present in *A.zeylanica* and Harborne (1973) reported bright yellow or yellow green fluorescent spots for luteolin when viewed under UV light. These reports confirm the occurrence of luteolin as the flavone in *Adhatoda*. Variation in flavonoid spot pattern had been used for studying phylogenetic relationships by Lopes and Monaco (1997) in coffee,Raveendran and Babu (1994) in *Piper* and Kade *et al.*(1997) in lotus. Similar flavonoid spot pattern was observed for the two species of *Plumbago* viz. *Plumbago rosea* and *P.zeylanica* by Menon (1999).

The reason for the variation in phenolics between species or cultivars within a species can be attributed to the fact that majority of the diagnostic plant phenolics are the end products of lengthy and complex metabolic pathways which are particularly

susceptible to environmental and ontogenic influences. Niral *et al.* (1998) reported that phenolics are found to play a role in resistance of plants to disease causing microorganisms and insect pests. This may be a reason for the rare incidence of pests and diseases in *Adhatoda.*

On analysing the terpenoid spot pattern in *Adhatoda*, dark brown spots were visualized on spraying 50% H_2SO_4 on silica gel coated plates with hexane: ethylacetate in 9:1 ratio as the solvent system which is used as a general procedure for separation of terpenoids. In leaves, first, second and fourth spots with Rf = 0.40, 0.44 and 0.59 respectively were found as markers for *A.zeylanica.* No such specific spot pattern could be found in the other two groups. No spot could be detected in Acc.23, 33 and 34 in leaves. In roots, among the four spots present, third and fifth spots (Rf = 0.44 and 0.67 respectively) could be found specific to *A.beddomei* types and first spot (Rf = 0.42) specific to *A.zeylanica* types, which can be considered as marker characters. No terpenoid spot could be detected in Acc. 27, which can be due to the failure of the solvent system used to separate the terpenoids or absence of the particular terpenoid group in this accession.

Mirov (1938) detected many chemical varieties in the *Pinus* species based on the presence of terpenoid in the gum turpentines and found that *Pinus jeffreyi* was a relict Californian species with no terpenes in its turpentine but *P.ponderosa* turpentines contained α and β pinene and limonene.Variation in terpenoids has been utilized for chemotaxonomical works by Fokina (1982), Holden and Mahlberg (1992) and Stevens *et al.* (1993).

Chromatographic separation of alkaloids was attempted using chloroform: methanol in 9:1 ratio as the solvent system and Dragendorff reagent as the spray reagent and showed spots with varying Rf value in the leaf. Orange spots with Rf value of more than 0.60 and pink spots with Rf value ranging from 0.20 to 0.25 were visualized. *A.beddomei* types had a spot with Rf = 0.99 and spots of closer Rf value of 0.24 and 0.20 (in Acc.28 and 34 respectively), intermediate types Acc.22 and 23 had a spot with Rf = 0.65 and *A.zeylanica* types Acc.51 and 53 had two spots with Rf = 0.11 and 0.99 in common. In roots, *A.beddomei* types had spots with lower Rf value (0.43 to 0.50) than the other two groups (0.50 to 0.56).

The total alkaloid content estimated as percentage of vasicine during July showed variation among the groups (0.27 to 0.66%). *A. zeylanica* types Acc.51 and 53 were seen to possess higher amount of the alkaloid (0.66%) than the intermediate (0.38-0.55%) and *A. beddomei* (0.27 to 0.32%) types.

Presence of vasicine and other alkaloids had been reported by many workers since 1920. Atal (1980) reported the presence of the alkaloid vasicine which contributed 75 to 90% of total alkaloids. Bhalla *et al.* (1982) reported 0.59% of alkaloids to be present in *A.vasica*. They separated vasicine and vasicinone from the alkaloidal mixture by thin layer chromatography using 8:2:1 mixture of choloroform : methanol : ethyl acetate. On spraying Dragendorff reagent, they obtained orange spots with high Rf value (0.62) for vasicinone and pink spots with low Rf value (0.22) for vasicine. Pandita *et al.*(1983) analysed total alkaloids, vasicine and other alkaloids quantitatively and qualitatively and observed a total alkaloidal content of 0.30 to 1.83% and vasicine content of 0.20 to 1.73%. They also separated vasicine, vasicinone and other alkaloids like deoxyvasicine by thin layer chroimatography.

Pundarikakshudu and Bhavsar (1988) observed that the leaves and roots of BLV of *A.vasica* plants had in general higher alkaloid concentrations as compared to those of SLV plants and vasicine amounts to 75-90% of the total alkaloids. Heltmann (1979) attempted systematic classifications in the genus *Atropa* by studying the alkaloid analysis of leaves and roots of *Atropa belladona, A.accuminata, A.baetica and A.pallidiflora, A.baetica* from Spain contained a higher percentage of alkaloid (0.91%) than European populations of *A.belladonna* (0.18-0.90%). However, the leaves of *A.pallidiflora* and *A.accuminata* contained largest amount of the alkaloid (upto 30%). Alkaloid profiles for classification were also studied by Maiti *et al.* (1979) in *Solanum*, Muzquiz (1994) in lupin and Mateos *et al.*(1988) in *Erythrina*.

The essential oil content in *Adhatoda* varied from 0.9 to 1.1 % on dry weight basis in leaves. The presence of essential oil in the leaves of *Adhatoda zeylanica* had been reported by D'cruz *et al.* (1979) and was shown to possess bronchodilatory activity. CSIR (1985) also reported the presence of a fragrant golden yellow essential oil (0.075% on dry basis) in leaves, roots and flowers of *A.zeylanica* on steam distillation.

5.3 Evaluation of biochemical attributes

The biochemical attributes such as vasicine content, number of phenol and terpenoid and banding pattern of esterase were having positive relation with the total biomass yield. Vasicine content is also found to be high in high biomass yielding plants (Acc. 51 and 53) which can be a valuable information for recommending better cultivation and management practices to increase both biomass and vasicine content. Highest biomass yielding type (Acc. 53) expressed maximum number of bands for esterase. Screening of esterase bands also revealed that EST- 4 (Rf = 0.300) and other identified bands (eg. EST- 3, Rf. 0.389) showed a positive relation to total biomass and vasicine production.

Nature and properties of phenols have a definite role in the plant metabolism. Based on the TLC analysis the phenols of leaf were different from that of roots. Low yielding plants expressed more number of phenols in leaves whereas more number of phenols was recorded in roots of the plants having high biomass and vasicine content. The TLC studies on terpenoids showed a marked difference in the number of terpenoids of high and low yielder. More number of terpenoids (four to five) was found in the leaves of high biomass yielders whereas the roots of the same types possessed only one terpenoid (Rf = 0.42).

Banding pattern of peroxidase revealed that number of bands was independent of yield and vasicine production. Since the essential oil content, peroxidase activity and number of flavonoids of all the accessions were not having much variation, the adaptability of the plant in varying environmental conditions may be stable.

The TLC results of alkaloids by Dragendorff spray expressed streaks of compounds which may be due to the mixing up of more than one closely associated alkaloids. Even though the mixing of compounds was there, the number of spots varied in low and high biomass yielders. In general the phenols and terpenoids present in the leaf and root can be consider as an index for identifying adathoda accessions.

5.4 Variablity analysis

Significant difference among genotypes for the characters such as plant height, number of branches, leaf area and petiole length was observed in *Adhatoda.* Phenotypic coefficient of variation (pcv) greater than genotypic coefficient of variation (gcv) for plant height, number of branches and leaf area suggest that apparent variation is not only due to genotype but also due to the influence of environment. But the slightly greater value for gcv than pcv for petiole length indicates that greater role is played by genotype than by environment. High heritability accompanied with high genetic advance for leaf area and petiole length indicates that most likely the heritability is due to additive gene effects. High genetic gain value for petiole length indicates that this character is least affected by environment.

Significant variation in characters like plant height, leaf area and petiole length had been obtained by Pandita and Bhan (1999) in *Asparagus*, Menon (1999) in *Plumbago* sp. and Paul (2000) in *Andrographis paniculata.*

5.5. Seasonal variation

The seasonal variation in biometric, yield and biochemical characters of 13 types of *Adhatoda* which were assumed to belong to the three groups namely *A.zeylanica, A.beddomei* and intermediate group was studied in two seasons - dry (first week of January) and wet (first week of July) at 6 months and 12 months after planting respectively.

5.5.1. Biometric and yield attributes

The pattern of growth was linear in all the three groups studied upto one year. There was progressive increase in leaf number, leaf area, root length and root girth upto July ie, after commencement of south west monsoon. The present study reveal that leaf number and leaf area that contribute the major assimilatory surface of plant is influenced by season. The increase in mean root length and root girth in the wet season in *A.zeylanica* types compared to the other types may be due to the vigorous growth of the plant during the wet season.

The fresh weight and dry weight of whole plant, leaf, stem and roots was higher in *A.zeylanica* types than the intermediate and *A.beddomei* types. In all the types fresh and dry weights increased progressively with the age of the crop upto 12 months.

KAU (1999) reported the influence of stage of harvest on biometric and yield characters of *Adhatoda* and found that characters like plant height, leaf area, whole plant, root and stem yield per plant showed significant difference with respect to stage of harvest. The root, stem and whole plant weight showed progressive increase with advance in age and the highest yield was observed at the harvesting stage of two years. The leaf yield was maximum at harvest interval of 1 1/2 year, which showed a decreasing trend at second year.

Granda *et al.* (1986) found that the total foliage and leaf area was markedly higher during wet season and minimum during dry season in *Rauvolfia tetraphylla*. Meera (1994) observed progressive increase in vine length, number of branches/vine, diameter of vine and internodal length with the age of the crop in *Holostemma annulare*. Number of leaves, total leaf area per plant, fresh weight and dry weight of leaf and stem showed an increasing trend upto 12 months after planting in July which coincides with the monsoon season and thereafter declined.

Menon (1999) observed a more vigorous growth for *Plumbago zeylanica* compared to *P.rosea*. *P.zeylanica* recorded higher total leaf area per plant after the commencement of southwest monsoon. In both the species number of roots and girth of roots tended to increase with age upto 16MAP. Fresh and dry weights of root also increased progressively with age of the crop and dry root weight was significantly higher in *P.zeylanica*. The fresh weight of leaf was significantly higher in *P.zeylanica*. Paul (2000) also reported variation in fresh weight and dry weight (total, leaf, stem and root) among different accessions of *Andrographis paniculata* collected from different parts of Kerala and a quantum increase was seen upto 4 MAP.

5.5.2. Biochemical variation

The elution pattern of secondary metabolites like phenols, flavonoids, terpenoids and alkaloids in the whole plant studied for two seasons (dry and wet) revealed that there was a decreasing trend in the number of spots in the wet season

than dry except flavonoids and alkaloids. For alkaloids, number of spots was found more in the wet season for *A.zeylanica* and intermediate types whereas more in the dry season for *A.beddomei* types.

The maximum number of spots in an accession decreased from ten to three for phenols during the wet season. Maximum number of spots was found in intermediate types Acc.6, 12 and 20 (ten spots) and minimum for *A.beddomei* type Acc. 21 (two spots) during the dry season. Second spot with Rf = 0.15 was absent in all the *A.beddomei* types during both the seasons which indicate the absence of a specific phenol group in the species which act as a marker character and needs to be identified.

For terpenoids also, the maximum number of spots in an accession decreased in the wet season with a maximum of six spots in Acc.29 during dry and five spots in Acc.6 during wet season. Although the maximum number of spots in an accession was four in both the season for flavonoids, there was variation in the elution pattern between accessions during dry and summer.

It is evident that dry conditions and or decrease in water supply cause an increase in the content and yield of secondary metabolites. This fact points towards the possibility of involvement of these compounds in the adaptability to drought conditions, their increased synthesis might be a part of the mechanism of osmotic adjustment, although they are not osmotically active substances it is important to consider in each case not only the presence of the active substances but also their total content which together will give us the final evaluation of the effect of water regime on the level of the desired metabolites (Atal and Kapur, 1982).

Seasonal variation in flavonoids of diploid and tetraploid camomile was studied by Letchamo (1996). He found marked differences in flavonoid accumulation in relation to harvest frequencies and the sowing seasons between the two types.

For alkaloids, the number of spots decreased for *A.beddomei* types and increased for *A.zeylanica* and intermediate types during the wet season. When alkaloid content was analyzed (as percentage of vasicine) a gradual drop was found in the wet season in all the types ranging from 0.19 to 0.85% compared to dry which showed an alkaloid content ranging from 0.90 to 1.41%. A higher alkaloid content was found in *A.zeylanica* types compared to *A. beddomei* types. Pundarikakshudu and Bhavsar

(1988) studied the variation in alkaloid contents of two types of Adha*toda vasica* designated as big leaf variety (BLV) and small leaf variety (SLV). The leaves and roots of SLV contained the highest amount of alkaloids in January, which showed a gradual drop till June and a rising trend thereafter. In BLV, the amount of alkaloids reached the optimum level first in September-October and again in January. After January, alkaloid concentrations started decreasing in a way similar to that seen in SLV plants.

Shah (1982) generalized that the content of alkaloid in wet and cold years is lower than in dry years. He observed a higher alkaloid content in *Datura stramonium* and *Hyoscyamus niger* when grown in arid conditions than in irrigated conditions. Sen and Datta (1986) pointed that the total alkaloid of *Catharanthus roseus* was the highest in winter. Seasonal variation in alkaloid have been reported by Kustrak *et al.* (1982) in *Chelidonium majus* where the alkaloid content was highest in summer, decreased during autumn and lowest at blossoming. Alkaloid content from different sites was also slightly different. Amador *et al.* (1996) found that in *Cuscuta* spp., alkaloids were found only during the wet season. Glowniak *et al.* (1999) reported highest amount of taxoids in December and January in *Taxus baccata.* KAU (1999) reported that the vasicine content increased progressively from 0.28% to 1.10% at the optimum harvest stage of 24 months after planting.

In view of the present investigation, differentiation of two species of *Adhatoda* reported viz. *A.zeylanica* and *A.beddomei* was possibile. Characterization of the 52 accessions of *Adhatoda* based on their morphological,histological and biochemical characteristics resulted in the classification of the types into three groups viz.*A.zeylanica*, *A.beddomei* and intermediate. The key morphological characters observed for the three groups are as follows:

Character	***A.zeylanica***	***A.beddomei***	**intermediate**
Habit	large dense shrub	small shrub	medium sized shrub
Leaf size	large	Small	medium
Leaf shape	broadly elliptic lanceolate	narrowly elliptic lanceolate	broadly or narrowly elliptic lanceolate
Leaf tip	attenuate	Attenuate	attenuate
Petiole	longer	Shorter	intermediate
Stomata	hypostomatic	amphistomatic	hypo/ amphistomatic
Flower size	large	Small	large/ small
Flower colour	white	White	white
Purplish streaks on corolla	present	Absent	present/ absent
Ovary	pubescent	Glabrous	pubescent/ glabrous
Style	hairy at base	hairy at base	hairy at base

There was some variation in the leaf tip and pubescence of style observed from the original description of the two species given by Hooker (1886) and Gamble (1957).

Since most of the vegetative and floral characters studied were conforming to the taxonomic descriptions, it is to be presumed that *A.beddomei* (chittalodakam) do exist in the homesteads of Kerala though it has escaped from natural habitat and got

domesticated. The presence of specific phenols and terpenoids in *A.beddomei* gives a clue that detailed investigations in this line may help to reaffirm the species identity. So also, electrophoretic studies employing more number of enzymes, molecular techniques like Restriction Fragment Length Polymorphisms (RFLPs) or Rapid Amplified Polymorphic DNAs (RAPDs) may be followed for supplementing the morphological and cytological studies for characterizing as well as confirming the identity of different types of *Adhatoda*.

Summary

SUMMARY

Investigations were undertaken in the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 1998-2000 to characterise the germplasm accessions of *Adhatoda* and to ascertain the species identity of chittatalodakam a rare and endangered species.

Wide variations were observed in vegetative and floral characters among the 52 accessions collected from various parts of Kerala under domestication. Based on the vegetative characters viz. plant height, number of branches, leaf area and petiole length, the accessions were grouped into three clusters and they were compared for all vegetative and floral characters. Maximum number of accessions belonged to cluster I (25) followed by cluster III (20) and cluster I (7). Types in cluster II showed the characters of *A.zeylanica*, cluster III resembled *A.beddomei* and cluster I showed characters intermediate to both the species. *A.zeylanica* types were generally taller (mean plant height of 70.42cm) with more number of branches (5.4), greater leaf area (70.08 cm^2), petiole length (6.1cm) and broadly elliptic lanceolate hypostomatic leaves. The flowers were large white with purplish streaks in the inner side of corolla and had large pubescent ovary. *A.beddomei* types were generally smaller (39.96cm) in habit with lesser number of branches (3.9), smaller leaf area (39.52), smaller petiole length (1.4cm) and narrowly elliptic lanceolate amphistomatic leaves. Flower was small, white without purplish streaks inside and with small glabrous ovary. The intermediate types were medium tall (63.87cm) with leaf area (43.71cm) petiole length (1.54cm) and number of branches (5.1cm) ranging between that of two species and included both the floral types. The observations regarding a few characters like leaf tip (attenuate in all types whereas *A.zeylanica* was reported to have acute ends) and pubescence of style (style was hairy at the base and glabrous towards the tip in all

types whereas in *A.beddomei* types, style was reported to be terminal hairy) were contrary to the description of the two species given by earlier workers.

Higher phenotypic coefficient of variation observed for plant height, number of branches and leaf area than genotypic coefficient of variation suggests that apparent variation is not only due to genotype but also due to the influence of environment. High heritability (broad sense) accompanied with high genetic advance for leaf area and petiole length indicates that most likely the heritability is due to additive gene effects and selection based on such traits may be effective.

The histological studies revealed that *A.zeylanica* types had hypostomatic leaves, *A.beddomei* types had amphistomatic leaves and intermediate types included both types of leaves. There was no significant variation in the stomatal index and trichome index between the accessions. Number of cells in the trichomes varied from one to four.

The electrophoretic studies involving two isoenzymes, peroxidase and esterase showed varied banding pattern between the accessions. Although grouping was done based on similarity in banding pattern, no specific band could be identified as a marker character for either of the species. Variation in banding pattern could be due to vegetative propagation in the species where variability once created get fixed.

Seven accessions representative of the three groups when analysed for morphological, biochemical and yield attributes showed considerable variation. *A.zeylanica* types were characterised by vigorous growth with higher fresh and dry weights for whole plant, leaf, stem and root. They also had higher alkaloid content (0.66%). *A.beddomei* types were less vigorous with lower fresh and dry weights of whole plant leaf, stem and root with lower alkaloid content (0.27-0.32%). The above characters ranged between the two species in the intermediate types.

Phenols and terpenoid spots specific to the two species were identified which could be used for confirming the characterization made using morphological traits. The essential oil content ranged from 0.9-1.1% but no difference was observed between the three groups. Morphological, biochemical and yield characters in the selected 13 accessions belonging to the three groups when analysed in two growth stages – 6 MAP (coinciding with dry season) and 12 MAP (coinciding with wet

season) showed wide variation in stages and accessions. Irrespective of the species differences, greater biomass in terms of fresh weight of whole plant, leaf stem and root was observed at 12 MAP where *A.zeylanica* types outranged the other types. The alkaloid content was higher at 6 MAP coinciding with dry season (0.88-1.41%) than 12 MAP which falls during wet season (0.19-0.85%). *A.zeylanica* types recorded a higher alkaloid content during the dry season (1.0-1.04%) compared to *A.beddomei* types (0.99-1.15%). In the wet season also *A.beddomei* types showed a lower alkaloid content (0.28%) compared to *A.zeylanica* (0.47%-0.66%). In both the seasons, intermediate types showed a wide range in alkaloid content. Similar trend was noticed for distribution pattern of secondary metabolites like phenols, terpenoids and alkaloids (for *A.beddomei* types) where number of spots decreased during the wet season. This shows that dry conditions favoured accumulation of secondary metabolites and optimum stage of harvest should be fixed considering these factors.

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MORPHOLOGICAL AND BIOCHEMICAL VARIATIONS IN ADHATODA TYPES (*Adhatoda* spp.)

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ABSTRACT OF A THESIS
Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture
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2001

ABSTRACT

A comparative evaluation of the morphological, histological and biochemical features of various *Adhatoda* accessions collected from different parts of Kerala was carried out in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1998-2000 for characterizing the germplasm accesions and to ascertain their species identity.

The 52 accessions after evaluating for their morphological characters were classified into three clusters, which represented *A.zeylanica* (7types), *A.beddomei* (20) and intermediate types (25). Key characters which served for distinguishing between the two species were identified as plant habit, leaf size and shape, stomatal distribution, streaks on corolla and pubescence of ovary. *A.zeylanica* types were taller in habit with more number of branches, long petiole, broadly elliptic lanceolate hypostomatic leaves and white flowers with purplish streaks inside and large pubescent ovary. *A.beddomei* types were small with lesser number of branches, short petiole, narrowly elliptic lanceolate amphistomatic leaves, with white flowers without purplish streaks inside and small glabrous ovary. Intermediate types showed both the leaf and floral types emphasizing the possible occurrence of natural hybrids in the genus. The variation in the elution pattern of phenols and terpenoids typical to the species makes them to be effectively utilized in the biochemical characterization of the species. *A.zeylanica* types showed a higher alkaloid content (0.66%) compared to intermediate types (0.38-0.55%) and *A.beddomei* (0.27-0.32%). Isozyme and enzyme studies also showed variation between accessions. However, further trials involving more enzyme systems is necessary for characterization of species.

High heritability (broad sense) accompanied with high genetic advance for leaf area and petiole length indicates that most likely the heritability is due to additive gene effects and selection based on such traits may be effective.The growth was found progressive upto 12 MAP when it yielded more fresh and dry weights of whole plant, leaf, stem and root compared to 6 MAP. A higher alkaloid content as well as number of spots for phenols, terpenoids and alkaloids in the dry season compared to wet season indicate the influence of moisture stress on accumulation of secondary metabolites and the need for scheduling harvesting time coinciding with this season.