COLLECTION, CATALOGUING AND EVALUATION OF *Rauvolfia spp.*

BY

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THESIS

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requirement for the degree

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DECLARATION

I hereby declare that this thesis entitled '**Collection, cataloguing and evaluation of Rauwolfia spp.**' is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any University or Society.

Vellanikkara,

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Certified that this thesis entitled 'Collection, cataloguing and evaluation of Rauwolfia spp.' is a record of research work done by **Mr.A.K.Narayanan**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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CONTENTS

LIST OF PLATES

LIST OF TABLES

LIST OF ILLUSTRATIONS

Introduction

INTRODUCTION

There is a renewed interest, worldwide, in herbal medicines which have few side effects. The genus Rauwolfia is important for its medicinal value and is of great demand in the international market. The drug ie., the dried root is used as an antidote to snakebite and hence called 'Sarpagandha' (means snake smell or repellent) in Sanskrit. It's use in lunacy or moon disease gave the name 'Chandrabhaga' in Hindi and called 'Amalpori' in Malayalam (Sahu, 1979).

The therapeutic properties of these plants were explained, even in the vedic literature, like Charaka Samhita and Susrutha Samhitha in 800-1000 BC itself. The root and root extracts of these plants were used in Ayurvedic and Unani systems of medicine as a 'panacea' for various types of physical and mental ailments. The drug is called 'Pagalkidawa' in India owing to its use in mental disorders. This is used in the treatment of epilepsy, fibrifuge, as a diuretic, to facilitate child birth, rheumatism, fits, eczema, fevers and insanity (CSIR, 1969). It is widely used in the treatment of hypertension (Kirthikar and Basu, 1975).

The chemistry and pharmacology of Rauwolfia was studied during 1930s and the alkaloid ajmalicine was isolated from the roots by S. Siddique and R.H. Siddique in 1931 (Gauniyal et al., 1988). In 1952, Emill Schlitler and H.J. Bain isolated the alkaloid 'Reserpine', from the crude root extract, which was regarded

as the greatest medical discovery after the "Antibiotic Era" triggered by the discovery of penicillin. Reserpine is being used in the modern allopathic medicines for the treatment of hypertension and as a tranquillizer.

Actually, the genus 'Rauwolfia' (also spelled 'Rauvolfia') was named, in honour of a 16th centuary German Physician and Botanist Leonhard Rauwolf of Augsburg, who made an extensive tour of Asia and Africa in quest of medicinal plants (Sahu, 1979). In 1703 Plumier, the pioneer Botanist of the Caribbean, dedicated to this early traveller, a genus of plants, in the family Apocyanaceae, which he described as 'Rauvolfia' in his book 'Nova Plantarum Americanarum Genera'. Burmann In 1755 changed the spelling to 'Rauwolfia'. At present both these spellings are adopted in the international literature.

The drug from Rauwolfia was obtained from herb - gatherers during earlier times. Now the natural wild resources are being depleted and to meet the increasing demand, we have to go for the cultivation of this plant. The Council of Scientific and Industrial Research publications say that, India requires 100-150 tonnes of drug per annum against which our production is only 30 tonnes per annum (CSIR, 1969). Maheswari et al. (1991) reported that a net income of Rs.26915 per ha in 18 months period, could be obtained by the cultivation of Rauwolfia serpentina. This shows the potential of Rauwolfia as a 'crop' plant. It can come

up well in partial shade conditions proving it's potentiality as an intercrop in the Coconut and Rubber plantations of Kerala.

Improved varieties of Rauwolfia, having higher alkaloid content and root yield, are essential for it's profitable cultivation. A study on the propagation methods and the nutritional requirements of Rauwolfia serpentina has already been conducted by Bhaskaran (1964), under Kerala conditions. Studies on crop improvement were not seen to be attempted, under Kerala conditions. The present study was conducted as a preliminary step in the crop improvement of Rauwolfia, with the following objectives.

i) Study the distribution of Rauwolfia spp. in different parts of Kerala.

ii) Detailed descriptive study on different plant characters and formulation of a descriptive blank.

iii) Preliminary comparative evaluation study for the total alkaloid content in the roots.

Further crop improvement and breeding programmes in future can be planned and formulated, based on the informations obtained from this study.

Review of Literature

REVIEW OF LITERATURE

There are a lot of literature available on the genus Rauwolfia, especially R. serpentina. The literature available related to the study is reviewed in this chapter.

2.1 Occurrence and distribution

2.1.1 World

Monachino (1954) reported that, Rauwolfia has an Indo-Malayan distribution, in India, Ceylon, Andaman Islands, Burma, Siare, Java and Sumatra.

CSIR (1969) reported that, Rauwolfia is a large genus of shrubs, undershrubs or trees distributed in the Tropical Asia, Africa and America and five species were reported in India.

Sahu .(1979) enlisted the names of 155 species of Rauwolfia found in different parts of the world, mainly Tropical Asia, Africa and America.

Gauniyal et al. (1988) reported 175 species found in tropical and subtropical regions of the world. They also reported that Rauwolfia was generally found in the moist regions, with annual rainfall of 150-375 cm and altitudes upto 1200 m in North India, East Pakisthan, Burma, Thailand, Ceylon, Malaya, Indonesia and was cultivated in Great Pakistan, India and Phiiippines.

2.1.2 India

Hooker (1882) described seven species of Rauwolfia found in India. They are

1. Rauwolfia serpentina (Distributed in - Tropical Himalayas, plains near the foot of the hills from Sirkind, Edgeworth, Moradabad to Sikkim, Assam, Pegu, Deccan, Western ghats, Travancore and Cochin)
2. Rauwolfia penguana (Pegu and Kurz)
3. R. densiflora (Khasi Mountains, Deccan peninsula, Western ghats)
4. R. micrantha (Malabar)
5. R. beddomei (Travancore)
6. R. microcarpa (Burma near Taong dong)
7. R. decurva (Deccan peninsula, Canara, Konkan and Poona)

Cook (1905) described three species occuring in the erst-while presidency of Bombay. They are Rauwolfia serpentina, R. densiflora and R. canescense.

Gamble (1921) reported five species present in the erst-while presidency of Madras. They are R. serpentina, R. densiflora, R. micrantha, R. beddomei and R. canescense. Sulochana (1959) also reported these five species in India.

Sahu (1979) reported the occurrence of five species of Rauwolfia in India viz., R. serpentina, R. densiflora, R. micrantha, R. beddomei and R. tetraphylla. He reported that, R. tetraphylla and R. canescense are synonymous.

Sarin (1982) reported the frequent occurrence of Rauwolfia serpentina in Goa, Coorg, North Canara and Shimoga of Karnataka.

Gauniyal et al. (1988) opined that, out of the 17 species seen in the world, only five species were reported in India. In India 'Rauwolfia' was widely distributed but occurred sporadically only, in the Sub-Hymalayan tract from Punjab eastwords to Sikkim, Gangetic plains, lower hills of Himalayas from Simla to Assam, Dehradun, Tripura, North Oudh, Gorakpur in U.P., Foot Hills of Darjeeling, Jalpaiguri, reserve forests of North Bengal, Howrah, Saralabhanga riverbank, Jowai, Jowgory, Goalpura district of Assam, Khasi and Janti Hills upto an elevation of 1300-1400 m in Meghalaya, Andaman, Western Ghats and in Deccan peninsula. He also reported the occurrence of Rauwolfia serpentina in parts of Orissa, Khasipur and Eastern ghats.

Oomachan and Masih (1991) reported the occurrence of R. serpentina in Madhya Pradesh.

2.1.3 Kerala

Hooker (1882) reported the occurrence of R. micrantha in Malabar, R. beddomei in Travancore and R. serpentina in Western ghats, Travancore and Cochin.

Gamble (1921) reported the occurrence of R. serpentina in Western ghats, R. densiflora in Travancore and Western ghats and R. micrantha in Malabar and Travancore.

Sahu (1979) reported the occurrence of R. densiflora and R. beddomei at erstwhile Travancore-Cochin states upto 2000', R. micrantha at South Malabar, Travancore and Cochin and R. tetraphylla in the coastal port towns of Kerala.

Sarin (1982) reported the occurrence of R. serpentina in Palghat, Calicut, Trichur and Wynad districts of Kerala. Palghat, Nilambur, Wynad and Trichur forest areas contributed one tonne of dried roots per annum.

Babu (1990) reported the occurrence of R. serpentina and R. tetraphylla in the Malappuram district of Kerala.

Sudhadevi (1992) reported the occurrence of R. serpentina and R. tetraphylla at Sholayar and Vazhachal forests of Trichur district of Kerala.

Kuriakkose (1992) reported that Rauwolfia serpentina plant was now becoming an endangered species in Kerala, due to the over exploitation.

2.2 Taxonomy

Hooker (1882) had described the systematic position of Rauwolfia in the plant kingdom, as follows

Division	:	Phanerogame
Subdivision	:	Angiosperm
Class	:	Dicotyledon

Sub-class	:	Gamopetaleae
Series	:	Inferea
Cohort	:	Asteralae
Natural order	:	Apocyanaceae
Genus	:	Rauwolfia

Gamble (1921) provided the taxonomical key for the identification of the members of the family Apocyanaceae and he has described five species found in erstwhile presidency of Madras, viz., R. serpentina, R. densiflora, R. beddomei, R. canescense and R. micrantha.

Sulochana (1959) and Sahu (1979) provided the key for five species of Rauwolfia found in India ie., R. serpentina, R. densiflora, R. beddomei, R. micrantha and R. tetraphylla.

Babu (1990) provided key for two species, Rauwolfia serpentina and R. tetraphylla.

2.3 Morphology

Gamble (1921) described the morphology of five species of Rauwolfia found in India and formulated a key for the identification of these species.

Monachino (1954), CSIR (1969), Srivasthava (1978), Sahu (1979) and Gauniyal et al. (1988) has given the morphological description of Rauwolfia serpentina. According to them R. serpentina

is an erect undershrub of 60-90 cm height, simple leaves, arranged in whorls of three, having a size of 7.5-18 cm x 3.5-6 cm, elliptic and lanceolate, with 7-16 pairs of nerves and 8 mm long petiole. Stem unbranched and slender. Roots greyish in colour long, thick, round on upper side and tapering to the base. Inflorescence, a cyme, with many flowers which are small, 1.2 cm - 2.5 cm long, white or pinkish white, regular and hypogynous. Calyx glabrous, bright red, and 3-6 mm long. Corolla with five petals of 1-1.3 cm long, tubular, swollen a little above the middle, androecium epipetalous with five stamens inserted in the middle of corolla tube, very short and cup shaped. Gynoecium with bicarpellary ovary, filiform style and large capitate stigma which is bifid. Fruit is a single or didynamous, oval and slightly flattened, drupe of size 5-6.5 mm x 4.5-5 mm which is green coloured at early stage, turning to purplish black on ripening. Seeds are ovoid, single in each carpel, oval and flattened.

Sahu (1979) described the morphology of the other species also. According to him R. densiflora is a large shrub of 12' height. Roots are hard, woody, brown coloured and leaves are in whorls of 3-4, oblanceolate, with 8-16 pairs of veins. Inflorescense is a bifurcating 1"-2" long corymbose cyme. Flowers are white, with elongated sepals and tubular 0.5" long, large round lobed corolla. Stamens are epipetalous, five in number and gynoecium is with a bicarpellary ovary, and large capitate stigma. Fruit

is a brownish purple drupe and seeds are pyrenes pointed at the top.

R. beddomei is a glabrous shrub of height 5'-6' with minutely lenticillated stem and leaves of size 3"-6" x $1\frac{1}{2}$-$1\frac{3}{4}$". Inflorescense is a laxcyme with peduncles of length 2"-4". Calyx is triangular and corolla is 0.5" long with pinkish white petals. Androecium with five stamens and gynoecium with bicarpellary ovary are also pertains to this species.

Rauwolfia micrantha is a slender shrub with woody, hard, yellowish brown roots. Leaves are elliptic in shape narrowed to an often slender petiole, with 10-12 pairs of main nerves. Inflorescense is a cyme with very few flowers of size 0.25"-0.4". Calyx lobes are triangular and corolla is with a short, narrow tube, dialated at the top, yellowish to lilia coloured at the throat and 0.2" long.

R. tetraphylla is a woody shrub of 4' height with woody hard roots. Leaves 3-5 nately whorled, mostly four in each whorl, unequal in size 0.5"-3.5" x 0.5"-1.5", elliptic, with about 10 pairs of main nerves. Inflorescense is a few flowered cyme with peduncles of size 0.25"-0.75" and small creamy white flowers. Calyx is short and ciliate, corolla 0.15" long, broad and dialated at the top.

Babu (1990) described the morphological aspects of R. tetraphylla and R. serpentina.

2.4 Cytology

Raghavan (1957) and Tapadar (1964), confirmed that R. serpentina is diploid with a basic chromosome number 2n = 22.

Tapadar (1963) induced tetraploidy in R. serpentina with 2n = 44 by colchicine treatment.

Rajkhova (1964) reported a series of polyploids in the genus Rauwolfia. R. serpentina is diploid with 2n = 22, R. densiflora is tetraploid with 2n = 44 and R. tetraphylla is hexaploid with 2n = 66.

Dnyansagar and Torne (1967) has conducted mitotic analysis of Rauwolfia serpentina roots. Dnyansagar and Torne (1969) reported the procedure for meiotic analysis of R. serpentina.

Sahu (1979) also reported the cytological details of R. serpentina.

Bedi and Gill (1982) studied meiosis in Rauwolfia serpentina and reported the gametic chromosome number of n = 11 and the existence of a heterozygous interchange during meiosis.

Banerjee and Sharma (1989) reported that R. serpentina and R. vomitoria are diploids with 2n = 22 while R. canescense is hexaploid with 2n = 66.

2.5 Anatomy

Chandra (1956) studied anatomy of R. canescense and Sulochana (1959) prepared a key for the identification of the five reported species of Rauwolfia, based on root anatomy.

Sahu (1979) explained the anatomical details of the roots of Rauwolfia species. He reported that, eventhough the genus was described as lacticiferous, in none of the roots clear lacticiferous tubes were found. He also reported that hypodermal periderm was highly schlerenchymatous. In R. serpentina, cork was indefenitely fissured while in R. tetraphylla it was flaky. In all the species it was radial in orientation and the cells were irregularly polygonal with thinly suberised walls. Based on the anatomical characters he had prepared a key for the identification of the different species.

Gupta and Lamb (1985) described the developmental anatomy of the fruit in R. serpentina. They reported that the epicarp is 8-9 layered with tannin in their cells, endocarp is uniquely made up of various type of schlereids and on the pericarp, anomocytic, paracytic and abnormal types of stomata are present.

No studies on the anatomy of the stem and leaf of Rauwolfia species were reported.

2.6 Cultivation

According to Gauniyal et al. (1988) experimental cultivation of R. serpentina has been taken up in different regions all over India including Mysore, Maharashtra, Madras and Kerala. Reports of the cultivation of R. serpentina in Bihar (Verma, 1969), Uttar Pradesh (Verma, 1970), West Bengal (Biswas, 1955), Orissa (Sahu, 1979), Sourashtra of Gujarath (Ahluwalia, 1963), Rajasthan (Mathur, 1961), Pondichery (Homji, 1965), Punjab (Singh, 1964), Jammu and Kashmir (Sobti et al., 1957 and Parkashi and Achari, 1968), Assam and Madhya Pradesh (Sahu, 1979) are available.

2.6.1 Propagation methods

2.6.1.1 Seeds

Santapau (1956), Hedayathullah (1959), Dutta et al. (1962) and Maheswari et al. (1982) reported low germination of less than 10 per cent, if direct sowing was practised, in Rauwolfia serpentina.

Chandra (1956) reported that heavy and mature seeds had a higher germination percentage of 43, while light seeds showed low germination percentage.

Sobti et al. (1957) reported that seeds that sink in 10 per cent brine solution showed 10-30 per cent germination.

Badhwar et al. (1957 and 1963) recommended propagation of Rauwolfia serpentina by seeds, for better economic yield and observed 25-50 per cent germination.

Nair (1956) opined irregular and sporadic germination of 38 per cent and 29 per cent in the seeds collected from Dehradun and South India respectively.

Dutta et al. (1962) reported maximum germination of seeds of Rauwolfia serpentina, when collected in January.

Bhaskaran (1964) reported that seed propagated plants had maximum height, maximum root yield (346.9 kg/ha) and maximum root alkaloid content, in Rauwolfia serpentina.

Maheswari et al. (1984) proposed seed treatment of overnight soaking in water and thiram, captan or ceresan before sowing, for maximum germination.

Gauniyal et al. (1988) reported that the best time for seed collection is July in Deccan, May in West Bengal and Dehradun and October in Bhubaneswar.

Gauniyal et al. (1988) also opined that the low viability of the seeds was either due to the stony endocarp, parthenocarpy,

differed somatoplastic sterility or the presence of cinnamic acid derivatives.

2.6.1.2 Stem cuttings

Chandra (1956) reported that the hard wood cuttings of size 5" to 8" length produced roots within 15 days after planting with hormonal treatment.

Sahu (1979) reported that 7.5 cm long stem cuttings with two buds was the best material for vegetative propagation and noticed 66 per cent germination. According to him 100-125 kg of stem cuttings were required for 1 hectare of land.

Gauniyal et al. (1988) suggested that the stem cuttings of 6-7 cm with 2 buds is best suited for propagation and the hard wood cuttings performed better than the soft wood cuttings.

2.6.1.3 Root cuttings

Monachino (1954) reported that the quickest way of propagation in Rauwolfia serpentina was by using the divided root stock.

Badhwar et al. (1956) reported that cuttings from fresh or green roots, of length 1"-2" are the best material for propagation.

Chandra (1956) reported the highest percentage of success using root cuttings of length 2" having a thickness of 0.2"-0.3" supplemented with normal treatment.

Sobti et al. (1959) reported that dormant root cuttings, planted in nursery, in February, sprouted in April and May.

Sahu (1969) reported that 7.5 cm long root cuttings gave 72 per cent germination and about 100 kg of this was required for one hectare planting.

2.6.1.4 Grafting

Kaul (1956) proposed graft propagation in Rauwolfia with R. serpentina as root stock and R. canescense as scion. This complex plant was multiplied by cutting a few inches below the soil containing the element of R. serpentina.

2.6.2 Transplanting

Chandra (1956) reported the potentiality of intercropping R. serpentina in Mango orchards which will provide shade and best conditions for growth.

CSIR (1969) reported that June to July is the best time for transplanting and seedlings with 7.5-12 cm height are best suited for transplanting.

2.6.3 Manuring and fertilizer application

Bhaskaran (1964) reported that 'N' has predominent influence on root yield and alkaloid content of roots. 'P' is essential for seed production and 'K' has found to enhance the alkaloid content of roots. Hence he recommended manuring with complete fertilizers with 'N' predominent, in Rauwolfia serpentina, under Kerala conditions.

Nandi and Chatterjee (1975), Sahu (1979) and Maheswari et al. (1984) recommended application of Farm Yard Manure at the rate of 45-90 quintals per hectare, supplying 22.5 to 45 kg Nitrogen for increasing the yield from 18.47 per cent to 41.39 per cent.

Huq et al. (1986) reported that the application of Potassium Naphthenate at the rate of 1000 ppm on 56th day after planting increased plant height by 26 per cent, number of leaves by 36 per cent, leaf area by 29 per cent, number of shoots by 63 per cent, number of inflorescence by 84 per cent, number of fruits by 75 per cent, total alkaloid content by 23 per cent, leaf alkaloid by five per cent and root reserpine, ajmalin and sterol contents by 11 per cent, six per cent and three per cent respectively.

Gauniyal et al. (1988) recommended application of Farm Yard Manure at the rate of 25-30 tonnes per hectare at the time of land preparation.

Maheswari et al. (1988) reported that in semi arid subtropical conditions 30 kg N per hectare and 60 kg P per hectare gave maximum root yield in R. serpentina.

Misra (1992) reported that N, P and K at the rate of 80, 60 and 30 kg per hectare with a plant to plant spacing of 15 x 50 cm gave maximum yield in R. serpentina. The application of chemical fertilizers were not found to increase the root yield significantly. He also recommended a basal dose of 30 kg K_2O, 30 kg P_2O_5 and 20 kg NO_3 per hectare before planting and two top dressings with 20 kg N per hectare during the growth period for better root yield in R. serpentina.

2.6.4 Irrigation

Gauniyal et al. (1988) recommended irrigation at fortnightly intervals during hot dry season and twice in a month during winter to Rauwolfia serpentina.

Maheswari et al. (1991) reported that alkaloid content was not affected by irrigation schedule and maximum return per hectare (rooted yield) was obtained at the irrigation water to cumulative pan evaporation ratio of 0.75, in Rauwolfia serpentina, with 17 irrigations during 18 months period.

2.6.5 Weeding and interculture

Sahu (1979), Singh (1964) recommended two weedings and one hoeing in the 1st year and one weeding and one hoeing during the monsoon of the second year in R. serpentina.

2.6.6 Intercropping

Ahluwalia (1963) reported that plants were more robust in open than in shade.

Sahu (1970) and Biswas and Badhuri (1975) reported that R. serpentina comes up well under Banana, Papaya and Mango orchards.

Maheswari et al. (1985) reported that in R. serpentina pure crop was giving maximum yield but intercropping with soybean in wet season and garlic or onion in winter was more remunerative.

2.6.7 Harvesting and storage

Monachino (1954) reported that the plant reaches full maturity with in two years.

Bhaskaran (1964) reported that seed setting stage ie., 180 days after planting is the proper period for harvest of the crop to get maximum yield of alkaloid.

Gauniyal et al. (1988) reports that best time for harvest varies with places and opines that after air drying the roots, artificial driers are to be used to reduce the moisture to less than eight per cent, cut the roots into 15-20 cm long pieces and pack in air tight containers, for long term storage.

2.6.8 Plant protection

Sahu (1979) and Gauniyal et al. (1988) have given the different pests and diseases affecting the Rauwolfia serpentina, their nature of damage and control measures.

2.7 Alkaloids

Cook (1905) reported that total alkaloid content of Rauwolfia differs with location and season.

Biswas (1955) reported that there was no significant difference in the alkaloid content of roots of R. serpentina under irrigated agriculture and forestry conditions.

Bajpai and Sharma (1956) reports that seeds of R. serpentina contains 0.2-0.3 per cent alkaloids.

Sulochana (1959) also reported that the alkaloid content of various geographical areas varied considerably.

Chatterjee and Ray (1962) divided the alkaloids of Rauwolfia into two groups, Ajmaline group and Reserpine group.

Dutta et al. (1963) and Gupta (1972) reported that bark of the root, which constitute 40-50 per cent had 90 per cent of the total alkaloids.

CSIR (1969) reported the alkaloid content of R. serpentina in different states such as Assam (2.57%), Bihar (2.24%), Madhya

Pradesh (2.99%), Orissa (1.73%), Uttar Pradesh (2.05%), West Bengal (1.76%) and Kerala (1.86%).

Sahu (1979) reported that maximum alkaloid content was observed during winter in wild conditions, when the plants shed their leaves.

Amar and Court (1980 and 1981) isolated 19 indole alkaloids, from R. vomitoria leaves, comprising of E-secoindole, sarpagan, picrinine, akvamniline, heteroyohimbine, oxindole, yohimbine and indolenine types. They isolated 39 indole alkaloids from root bark of R. nitida, of which the principal alkaloids were reserpine, serpentine, pseudo-reserpine and reserpiline.

Sahu (1983) provided a detailed record of the chemistry of the Rauwolfia alkaloids. He also described different methods for the estimation of alkaloids.

Shimolina et al. (1984) reported about 204 alkaloids isolated from different species of Rauwolfia.

Antipova et al. (1988) reported that the total alkaloid content in the roots, stem and leaves of Rauwolfia caffra, R. canescense, R. heterophylla, R. serpentina and R. verticillata ranged from 0.78-2.08 per cent, 0.17-0.51 per cent and 0.32-2.55 per cent respectively. They also observed that alkaloids of ajmaline group accumulated mainly in the roots where as those of reserpine group accumulated both in aerial parts and roots.

They proposed R. casescense for commercial cultivation due to it's higher alkaloid content.

Belem et al. (1988) isolated the alkaloids reserpiline, sarpagine and B-yohimbine from root bark of R. sellowii.

Gauniyal et al. (1988) reported that the total alkaloid content in R. serpentina was 0.7-3 per cent in roots. 2.4 per cent in root bark, 0.4 per cent in root wood, 0.45 per cent in stem and 0.54 per cent in leaf. They also reported that there was no striking effect of plant age in the alkaloid content of the roots, upto 3-4 years of growth.

Hamp and Zenk (1988) purified the enzyme strictosidine synthase responsible for inducing alkaloid biosynthesis, from cell suspension cultures of R. serpentina.

Ruyter et al. (1988) isolated a novel gluco-alkaloid Acetyl rauglucine and related glucosides from cell suspension culture of R. serpentina.

Martinez et al. (1989a) isolated six indole alkaloids' tetrahydroalstonine, aricine, 16-epiaffinine, ajmaline, amerovolfine and amerovolficiné from the stem bark of Rauwolfia cubana.

Martinez et al. (1989b) isolated a new sarpagine type alkaloid N(a) - dimethyl accedine from the stem bark of R. tetraphylla and R. cubana.

Nikolaeva et al. (1990) reported that an alkaloid ajmaline was present in the root bark of Rauwolfia vomitoria, R. cambodiana, R. canescense, R. serpentina, R. verticillata and R. littoralis.

Schmidt et al. (1990) presented the enzymatic pathway to ajmaline and raucaffricine in Rauwolfia serpentina cell cultures.

Ruyter et al. (1991) reported that in Rauwolfia serpentina although the total alkaloid content of the roots of callus regenerated plants were higher, the content of individual alkaloids like ajmaline, serpentine and reserpine were lower, compared to the parental stock. They identified the glucoalkaloid, raucaffricine as a constituent of all the samples, providing the first evidence for its occurrence in the roots of R. serpentina.

Endre et al. (1992) identified and isolated a novel alkaloid '6 alpha-hydroxy raumacline' from the cultivated R. serpentina cells.

2.8 Crop improvement

Autotetraploidy has been successfully induced by many workers such as Tapadar (1963), Ammal (1962), Badhuri and Biswas (1965), Parkashi and Achari (1968), Sahu (1979), Krishnan et al. (1985) and Gauniyal et al. (1988), in Rauwolfia serpentina. They opined that there was difference in the root yield and alkaloid content between the diploids (1.81%) and tetraploids (2.01%) but there

was no difference in the reserpine content (0.11%).

Bhagat et al. (1980) reported that there is wide variation in the R. serpentina genetic resources in India. Uttar Pradesh population represents the widest range of diversity for most of the traits and the populations from Assam possess higher root yield potential.

Chatterjee et al. (1988) studied the growth, development and biochemical parameters and alkaloid formation in relation to light, nutrition and high temperature stress in R. serpentina and a relationship was established between certain biochemical parameters and the formation of alkaloids.

Fuentes et al. (1988) screened 51 medicinal plants for salinity tolerance and found that R. caffra, R. nitida and R. tetraphylla were tolerant to high salinity level.

Sethi et al. (1991) studied the variation of chemobotanical characters in the indegenous collections of R. serpentina in India. They observed the highest range of variation for total root weight and number of secondary roots per plant in the collections from Choondapoon region of Karnataka and Concana region of Goa. These two characters contributed to higher yield of alkaloids. The Choondapoon region gave highest range of 1.58-2.03 per cent total alkaloids with a mean of 1.81 per cent and 0.08-0.24 per cent reserpine with a mean of 0.16 per cent. Such chemobotanical

variation, according to them, was due to the geographical, ecological and topographical differences.

2.9 Tissue culture works

There are several reports on the *in vitro* propagation studies on *Rauwolfia serpentina* by Vollosovich and Butenko (1968), Vollosovich and Tsarenko (1968), Mitra (1969), Shchizelskii *et al.* (1974), Chadha (1983), Akram and Ilahi (1985), Ilahi and Akram (1987) and on the production, accumulation and isolation of alkaloids in the callus tissues of *R. serpentina* by Ohta and Yatazava (1979) and Kaukhova *et al.* (1984).

Nikolaeva and Vollosovich (1972) studied the effect of auxin on the morphology and organogenesis of *R. serpentina* in tissue culture.

Shchizelskii *et al.* (1974) reported addition of 'Cu' and 'Mn' for the stimulation of the total alkaloid yield and ajmaline in the tissue culture of *R. serpentina*, in MS medium. They also reported that the increased 'P' and 'S' levels stimulated indole alkaloid synthesis.

Nikolaeva *et al.* (1977) reported that the maximum accumulation of the biomass and alkaloids in tissue culture of *R. serpentina* growing in nutrient suspension was on the 40th day of the growth.

Nikolaeva et al. (1978) reported that higher auxin levels in the nutrient medium caused dissociation of the cell aggregates but depressed the alkaloid production.

Kaukhova et al. (1981) described the composition of an alternative inorganic medium for the deep tissue culture of R. serpentina.

Cheng and Liang (1981) reported conditions for inducing and culturing callus of R. vunnanensis.

Vollosovich and Vollosovich (1982) described the possible use of high 'green syrup' (The first mother liquor in glucose production) as a promising industrial waste for the commercial production of tissue biomass.

Vollosovich et al. (1982) reported that a ratio of 1:60, NH_4NO_3 and sucrose in the nutrient medium produced optimum alkaloid yield in R. serpentina during culturing.

Chadha (1983) reported that shoot culture of R. serpentina was producing significantly higher doses of indole alkaloids.

Gorodnyansskaya et al. (1984) found out specialised structures of nonsegmented lacticiferous cells in callus tissue of R. serpentina and the main centre of alkaloid accumulation was secretory cells and alkaloids were also distributed in the

parenchymatous cells, lacking starch. Calcium oxalate crystals have been detected in 75 day old callus tissue which was a rare phenomenon in tissue culture.

Schubel and Stockigt (1984) isolated raucaffricine from the suspension cultures of R. serpentina grown in AP medium.

Akram and Ilahi (1985) suggested stem as a suitable material for clonal propagation of R. serpentina.

Yamamoto and Yamada (1986) have maintained R. serpentina cultures for more than 13 years under in vitro conditions and noticed that these cells produced more quantity of the pharmacologically important alkaloids ajmaline (0.005-0.12%) and reserpine (0-0.003%). They also selected a high reserpine producing cell strain using U-V light techniques.

Ilahi and Mehmood (1987) reported propagation of R. serpentina plants in vitro using lateral bud culture, root callus culture and leaf callus culture. They screened out five alkaloids of which major one was ajmaline with a maximum percentage of 0.0573.

Mathur et al. (1987) have developed a tissue culture procedure for the establishment and propagation of Colchiautotetraploids of R. serpentina for its commercial exploitation.

Roja et al. (1987 and 1990) reported multiple shoot culturing of R. serpentina. They reported that leaf and stem explants gave

actively growing callus tissues on MS medium supplemented with 2,4-D at the rate of 2 mg l^{-1} and BA at the rate of 1 mg l^{-1}. Multiple shoots developed from explants on MS medium containing BA 1 mg in combination with either NAA 0.1 mg or IAA 2 mg. Sustained growth of shoot cultures was achieved in MS liquid medium containing BA (1 mg) and NAA (0.1 mg). The shoot cultures yielded significantly higher levels of alkaloids ajmalidine, yohimbine, 3-epi -yohimbine and ajmaline (0.15%) in Zenk's production medium and contained compounds found in the roots as well as in the shoots of intact plants. The shoot culture showed stability of growth and alkaloid production characteristics over a period of five years.

Singh (1987) reported the response of excised roots of R. serpentina to growth regulatory substances, IAA, IBA, NAA and 2,4-D. These at low concentration of 0.5 mg l^{-1} promoted root apex growth, 2,4-D was efficient for callus formation and MH, 2,4,5-T and IBA inhibited root apex growth.

Solovyan et al. (1987) reported that there was a significantly higher DNA content per nucleus per haploid genome, in cultured R. serpentina cells than in the intact plants.

Ilahi et al. (1988) worked on in vitro studies on Rauwolfia serpentina for masspropagation and alkaloid synthesis. They found callus formation in root, leaf and stem explants and plant regeneration from root and stem callus in different media such as Murashige

and Skoog, Whites and Abou-Mandour with different hormonal concentrations. They found that ajmaline was the main alkaloid produced by cultures and alkaloids were higher in plants from leaf and stem cultures than in parent plants.

Grill et al. (1988) reported induction of heavy metal binding phytochelatins by inoculation of cell cultures of Rauwolfia serpentina in standard media.

Manilov et al. (1988) opined about the development of stable cell lines having higher yields of ajmaline in R. serpentina cultures.

Nikolaeva et al. (1988) and Vollosovich et al. (1977) described highly sensitive, accurate and rapid methods for quantitative determination of alkaloids in tissue culture of R. serpentina employing chloroform as solvent.

Ruyter et al. (1988) isolated acetyl rauglucine and related glucosides from the Rauwolfia culture.

Akram et al. (1990) reported that root callus of R. serpentina was induced to differentiate buds with 0.8 mg l^{-1} NAA and 2 mg l^{-1} BAP. Buds were rooted with 24 hour treatment of IAA and IBA (3 mg l^{-1}). Rooted buds differentiated with autotrophic plantlets and the plants on transfering to soil thrived well. The karyotypic analysis showed 2n = 22.

Chaturvedi et al. (1990) reported the conservation of plant genetic resources of Rauwolfia through excised root cultures. Direct embryogenesis took place in segments of about 12 year old root cultures of R. serpentina and a large number of plantlets were obtained by he proliferation of the segments in nutrient medium, supplemented with plant regulators. The regenerated plants survived well under the field conditions.

Guiller and Chenieux (1991) reported somatic embryogenesis from leaf protoplasts of R. vomitoria shoot cultures. Mesophyll protoplasts from axenic shoots, cultured at the rate of 10^5 - 10^6 protoplasts ml^{-1} in Mursahige and Tucker liquid medium containing growth regulators. With 6-8 weeks callus growth and proembryos were seen. Calli on transferring to solid medium produced shoots but no roots. But somatic embryos achieved different patterns of development and plantlets were obtained either directly through germination of proembryos or via embryogenic calluses.

Schutte (1991) reported the biosynthetic mechanism of secondary plant substances like monoterpene indole alkaloids in in vitro cultures of R. serpentina.

Gundlach et al. (1992) reported that Jasmonic acid was a signal transducer in elicitor induced plant cell cultures. Their study showed that endogeneous jasmonic acid and its methyl ester, accumulated rapidly after the treatment of plant cell suspension

cultures of R. canescense (R. tetraphylla) with yeast elicitor.

Sharma and Chandel (1992) reported low temperature storage of R. serpentina on a standard MS shoot culture medium supplemented with 1 mg BAP and 0.1 mg l^{-1} NAA. Nodal cultures of two varieties, Delhi local and Indore local, were maintained for nine months at 25°C by replacing cotton plugs with polypropylene caps as enclosures for the culture tubes and 33.3 per cent survival was noticed. In vitro cultures stored at the lower temperature of 15°C exhibited normal health, even after 15 months and showed 66.6 per cent survival. Storage temperature of 10°C and 5°C were found deleterious for growth.

Materials and Methods

MATERIALS AND METHODS

The investigations reported herein were carried out in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the period 1991 to 1993. The materials used and the methods adopted for the study are described in this chapter.

3.1 Collection of the study materials

The survey was mainly concentrated in two localities viz., Peechi Range of Thrissur and Palode Range of Thiruvananthapuram. In addition to these,other locations of the State, as detailed below were also surveyed.

1. Thirunelly (Wynad)
2. Alakkod (Kannur)
3. Kanhirappuzha (Palakkad)
4. Mannarkkad (Palakkad)
5. Edapal (Malappuram)
6. Malappuram (Malappuram)
7. Munderi-Nilambur (Malappuram)
8. Peechi (Thrissur)
9. Vellanikkara (Thrissur)
10. Palode (Thiruvananthapuram)

Collections were made with

collected plants brought to the Co

in earthern pots of size 1' x 1', filled with potting mixture, with proper labelling. The intact plants at the Tropical Botanical Garden, Palode and Herbal Garden of Aryavaidyasala, Kottakkal were also used for the study of different characters.

3.2 Study of the morphological characters

The description of the morphological characters was based on the terms proposed by Vasishta (1974) and Dutta (1971). Five randomly selected plants from each species were used for the descriptive study of the following characters. The descriptions used are noted on the right side.

1. General habit

i. Appearance	:	Herb/Shrub/Undershrub
ii. Nature of branching	:	High/Low/medium
iii. Height (cm)	:	Mean of five randomly selected plants

2. Stem

i. Nature of stem	:	Woody/Herbaceous/Succulent Cylindrical/Polygonal/Triangular
ii. Colour of young stem	:	Dark green/Medium dark green/ Pale green
iii. Colour of old stem	:	
iv. Appearance of stem surface	:	Pubescent/Glabrous/Smooth/ Shining
v. Internodal length (cm)	:	Mean of 25 internodes

3. Leaf

i. Number of leaves per node	:	Mean of 25 nodes
ii. Types of leaves per node	:	Different types
iii. General shape of leaf	:	
iv. Appearance of lamina	:	Smooth/Pubescent/Glabrous
v. Colour of young leaves		
Upper side	:	Dark green/Medium
Lower side	:	Dark green/Pale green
vi. Colour of old leaves		
Upper side	:	Dark green/Medium Dark green/Pale green
Lower side	:	
vii. Size		
Length		Mean of 25 leaves
Width		central portion measured
viii. Number of glands on leaf axil	:	Mean of 25 axils
ix. Length of petiole	:	Mean of 25 petioles
x. Shape of leaf base, leaf margin and leaf tip	:	
xi. Number of pairs of veins	:	Mean of 25 leaves
xii. Interveinal length	:	Mean of 25 measurements

4. Inflorescence

i. Position	:	Terminal/Axillary
ii. Orientation	:	Erect/Parallel/Drooping/ Sub-horizontal
iii. Branching habit	:	Very low/Low//Medium/High/ Very high

iv. Length of peduncle : Mean of 10 peduncles

v. Number of flowers/cyme : Average of 10 observations

vi. Presence of hairs : Absent/Present

5. Flower

i. General

Length of pedicel : Mean of 25 flowers

Length of flowers (Excluding pedicel) : Mean of 25 flowers

Colour :

ii. Calyx

Length : Mean of 25 observations

Colour of sepals of young flower :

Colour of sepals after fruitset :

Apex of sepals (shape) :

Length of sepals : Mean of 25 observations

iii. Corolla

General shape :

Length of corolla tube

Length of petal lobe

Proportion of corolla tube to lobe : Mean of 25 flowers

Proportion of corolla to calyx

Extend of hairiness : Low/Medium/High

Region of constriction from the base of the corolla tube	:	Mean of 25 observations

iv. Androecium

Colour of anther lobe	:	
Length of anther lobe	⎫	
Width of anther lobe	⎬	Mean of 25 observations
Size of filament	⎭	

v. Gynoecium

Number of locules	:	
Shape of ovary	:	
Size of ovary	⎫	
Length of style	⎬	Mean of 25 observations
Colour of stigma	⎭	
Length of stigma		

6. Fruit

i. Shape	:	
ii. Colour of young fruit	:	
iii. Colour of mature fruit	:	
iv. Length	⎫	
v. Breadth	⎭	Mean of 25 fruits

7. Seed

i. Colour of seed coat	:	
ii. Length	⎫	
iii. Breadth	⎭	Mean of 25 seeds

8. Root

i. Colour :

ii. General appearance : Hard/Soft/Woody/Thick

iii. Presence of lateral roots : High/Medium/Low

3.3 Study of the pollen grains

Ten flowers from five randomly selected plants were taken for the study. The anther lobes were crushed in acetocarmine and cytologically examined. Observations from 10 microscopic fields were taken on the following aspects.

3.3.1 Viability

Observations at 10 X magnification were taken. Counts on healthy and stained pollen grains, malformed and broken ones, were taken and viability was calculated using the formula

$$\text{Viability (\%)} = \frac{\text{Number of healthy and stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.3.2 Shape : Shape of pollen grains noted

3.3.3 Size

Using ocular and stage micrometers, the diameter of the pollen grains was measured. Fifty pollen grains from 10 microscopic fields at 40 X magnification were measured and the mean was calculated.

3.4 Anatomical studies

3.4.1 Stomata

Thin peelings from the lower side of the leaf were taken and viewed under the microscope. Counts on the number of stomata from 25 microscopic fields of five randomly selected plants were taken at 40 X magnification and the mean was calculated.

The length of the stomata and the size of the guard cells were measured using ocular and stage micrometers at 40 X magnification. Fifty stomata from 10 microscopic fields were measured and the mean was found out.

3.4.2 Stem, leaf and roots

Safranin stained (2-3 minutes) hand sections (cross section) of stem, leaf and roots were microscopically examined for anatomical comparison.

3.5 Estimation of the total crude alkaloid content

Total crude alkaloid content was estimated by the method described by Cromwell (1955) and Sahu (1983).

The samples for the alkaloid estimation were collected during January-March 1993 from 10 locations. Three samples at random were taken for analysis and the mean was calculated. Flowered plants of approximately one year old were taken, roots

and aerial parts separated and dried in an oven at 60°C till constant weight was obtained. The dried samples were powdered and stored.

Five gram of the powdered sample was taken in a 250 ml conical flask along with 100 ml chloroform and three ml of 10 per cent Ammonia solution. Mixture was shaken for 12 hours using a mechanical shaker for complete extraction of the alkaloids. The extract was first filtered through a vaccum funnel having Whatman No.41 filter paper and then through of column of silica gel (100 mesh). The residue was washed using chloroform. The washing was continued till a negative reaction to Dragandroff's Reagent was observed.

After the second filtration, the extract was dried for removing the solvent. The alkaloid content was calculated using the equation.

$$\text{Total crude alkaloids (\%) (on dry weight basis)} = \frac{W_2 - W_1}{5} \times 100$$

W_1 = Weight of the container alone

W_2 = Weight after the complete evaporation of the solvent

3.6 Estimation of the total chlorophyll content in aerial parts

The method described by Arnon (1949) and Witham et al. (1971) was adopted.

The extraction was conducted using 0.5 g powdered sample in acetone (80%). The extract filtered through a funnel having glass wool. The residue was repeatedly washed with acetone, to extract chlorophyll completely. The extract was made upto 100 ml and absorbance was read by spectrophotometer at 645 nm and 663 nm. Total chlorophyll content was calculated using the following formula.

$$\text{Total chlorophyll content (\%) (on dry weight basis)} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{V}{1000 \times W} \times \frac{1}{10}$$

where

A = Absorbance at specific wave lengths

V = Final volume of the chlorophyll extract in 80% acetone

W = Weight of the sample used for extraction

3.7 Regression analysis of total root alkaloids, chloroform extract and chlorophyll content

Regression analysis was done in MSTAT-C software.

RESULTS

The results of the investigation are presented in this chapter.

4.1 Occurrence and distribution

4.1.1 Thirunelli forests (Wynad District)

Among the five species only Rauwolfia serpentina was present in this locality. The frequency of occurrence was very low and mainly, it was concentrated in partially shaded slopy areas. The species was absent in densely shaded areas of the forest.

4.1.2 Alakkod (Kannur District)

R. serpentina was present in open places. Only a few plants were present. Other species were not available.

4.1.3 Munderi Forests of Nilambur (Malappuram District)

R. serpentina was the only species available here. It occured in partially shaded areas. Frequency of occurrence was low.

4.1.4 Edapal and Malappuram (Malappuram District)

R. serpentina was found in Edapal while R. tetraphylla occured in Malappuram, quite high occurrence was noticed here.

4.1.5 Kanhirappuzha forest areas and Mannarkkad (Palakkad District)

In the forest lands around Kanhirappuzha dam site R. serpentina was present under partially shaded conditions. But R. tetraphylla was present in the plain lands and was absent in forest area. Frequency of occurrence was low in both the cases.

R. serpentina occurred in Mannarkkad while other ------ were absent.

4.1.6 Peechi forests and Vellanikkara (Thrissur District)

R. serpentina and R. tetraphylla were present in these areas. In the forests, R. serpentina was the only species found while R. tetraphylla was found in the plain areas of these two localities. R. serpentina occurred less frequently and was absent in the interior of forests.

The frequency of occurrence of R. tetraphylla was high and was restricted to roadsides, waste lands and the open lands of human inhabitation.

4.1.7 Palode Range (Thiruvananthapuram District)

Eventhough extensive survey in the forest area was conducted R. serpentina was the only species present here. It was found at the top of the hills (Peringanmala) where a calm, cool and

partially shaded condition prevailed. But the frequency of occurrence was very low.

The other species R. densiflora, R. beddomei and R. micrantha were not available in any of the localities surveyed.

It was also observed that the change in colour of the persistent calyx of R. serpentina, on fruit set (green to red) was mistaken by the local people as a red flowered plant of Amalpori.

4.2 Study of the variation of different characters

The observations are presented using the accession codes A, B, C, D, E with each code representing a particular species.

4.2.1 Morphological variations

Morphological variability is high in Rauwolfia spp. The observations are presented in Table 1.

4.2.2 Variation of pollen grains

The observations are presented in Table 2. The viability of pollen grains ranged from 65.5 per cent to 88.5 per cent and mean diameter from 0.015 to 0.028 cm.

4.2.3 Anatomical variations

It is noticed that anatomical variations are high in Rauwolfia spp. as far as root, leaf and stem are concerned. Variation in the

length of stomata from 0.0125 to 0.0150 cm and size of guard cells from 0.0030 to 0.0061 cm is observed. The observations are presented in Table 3.

4.2.4 Variation of total crude alkaloids (roots), chloroform extract (aerial parts) and total chlorophyll content (aerial parts)

The observations are presented in Table 4. The total crude alkaloids of roots in Rauwolfia spp. varied from 1.03 to 2.65 per cent. The range was 1.03 to 1.35 per cent in R. tetraphylla and 1.31 to 2.65 per cent in R. serpentina.

The chloroform extract of the aerial parts showed a variation of 2.09 to 8.20 per cent in Rauwolfia spp. while it was 4.34 to 6.76 per cent in R. tetraphylla and 2.09 to 3.84 per cent in R. serpentina.

The total chlorophyll content of the aerial parts varied from 0.206 to 0.944 per cent in Rauwolfia spp. In R. tetraphylla this range was 0.543 to 0.944 per cent and in R. serpentina it was 0.206 to 0.599 per cent.

4.3 Regression analysis

The results of the pooled regression analysis of all the samples are presented here.

4.3.1 Total crude alkaloid content of the roots (y) Vs chloroform extract of the aerial parts (x) (on dry weight basis)

Regression model : $y = a+b\ x$

Mean chloroform extract of the aerial parts (%) $\bar{x}$	= 4.117
Mean total crude alkaloid content of the roots (%) $\bar{y}$	= 1.609
Variance of x	= 3.518
Variance of y	= 0.175
Coefficient of correlation (r)	= -0.477
Regression line intercept (a)	= 2.047
Regression line slope (b)	= -0.106
Standard error of slope (S)	= 0.052
t test value (t)	= 2.030
Probability (p)	= 0.062

4.3.2 Total crude alkaloid content of t
chlorophyll content of the aerial parts (x) (on dry weight basis)

Regression model: $y = a+b\ x$

Mean of x	= 0.485
Mean of y	= 1.609
Variance of x	= 0.041
Variance of y	= 0.175
Coefficient of correlation (r)	= -0.697

Regression line intercept (a)	= 2.304
Regression line slope (b)	= -1.434
Standard error of slope (S)	= 0.394
t test value (t)	= 3.641
Probability (p)	= 0.003

Table 1. Variation in Morphological characters of Rauwolfia spp.

Characters	Accession A	Accession B	Accession C	Accession D	Accession E
	R. tetraphylla	R. serpentina	R. densiflora	R. beddomei	Rauwolfia sp
1	2	3	4	5	6
. GENERAL HABIT					
i) Appearance of the plant	Woody shrub	Herbaceous undershrub	Woody shrub	Woody shrub	Woody shrub
ii) Nature of branching	Profuse	Very few branches	Few branches	Highly branching	Few branches
iii) Mean height (cm)	132.56	47.83	81.00	93.13	65.00
.. STEM CHARACTERS					
i) Nature of stem	Woody cylindrical	Herbaceous cylindrical	Woody cylindrical	Woody cylindrical	Woody cylindrical
ii) Colour of young twigs	Dark green	Pale green	Dark green	Pale green	Dark green
iii) Colour of old stem	Ashy white green	Pale grey	Ashy brown	Ashy whitish brown	Ashy brown
iv) Appearance of stem surface	Pubescent	Glabrous	Smooth	Shining	Smooth
v) Mean Internodal length (cm)	7.23	1.73	4.89	1.34	3.52

Contd.

able 1. Continued

1	2	3	4	5	6
. LEAF CHARACTERS					
i) Mean number of leaves/node	3-4	3	3	3	3
ii) Number of types of leaves/node	2-3	1	1	1	1
iii) General shape	Oval	Long elliptic lanceolate	Broad oblanceolate	Lanceolate	Long lanceolate
iv) Appearance of leaf lamina					
Upper side	Glabrous	Smooth	Smooth	Smooth	Smooth
Lower side	Pubescent	Smooth	Smooth	Smooth	Smooth
v) Colour of young leaves					
Upper side	Pale green	Medium dark green	Dark green	Pale green	Dark green
Lower side	Pale green	Pale green	Pale green	Pale green	Pale green
vi) Colour of old leaves					
Upper side	Dark green	Dark green	Dark green	Medium dark green	Dark green
Lower side	Pale green	Pale green	Pale green	Pale green	Pale green
vii) Size of leaves	(Long) (Medium) (Short)				
Mean length (cm)	8.59 5.43 2.72	9.64	14.11	12.24	16.45
Mean width (cm)	3.99 2.98 1.82	2.8	6.16	3.75	4.78

Contd.

Table 1. Continued

1	2	3	4	5	6
viii) Number of glands on leaf axil and petiole (Mean)	24-32	12-15	17-20	18-20	14-18
ix) Mean length of petiole (cm)	0.72 0.35 0.20	1.16	1.85	0.93	1.65
x) Shape of leaf base	Broad acute	Sharp acute	Broad acute	Acute	Sharp acute
xi) Leaf margin	Wavy	Wavy and entire	Wavy	Wavy	Wavy
xii) Leaf tip	Mucronate	Acuminate	Broad acuminate	Acuminate	Acuminate
xiii) Mean number of pairs of veins	14.00	12.00	14.50	14.36	17.00
xiv) Mean interveinal length (cm)	0.66 (long and medium leaves) 0.26 (short leaves)	1.11	1.06	0.62	1.22
4. INFLORESCENCE					
i) Position	Axillary and terminal	Terminal	Terminal	Axillary	Terminal
ii) Orientation	Erect	Erect	Erect	Subhori-zontal	Erect
iii) Branching habit	Medium	Low	Low	High	Low
iv) Mean length of peduncle (cm)	1.16	4.20	4.75	7.00	4.00
v) Mean number of flowers/cyme	8.71	39.1	51.8	11.0	21.6

Contd.

Table 1. Continued

1	2	3	4	5	6
vi) Presence of hairs	Hairy	Smooth	Smooth	Non-hairy	Smooth
5. FLOWER CHARACTERS					
i) Mean length of pedicel (cm)	0.59	0.49	0.74	0.46	1.06
ii) Mean length of flowers (excluding pedicel) (cm)	1.42	2.85	1.90	1.60	2.18
iii) Colour of flower	Greenish white	Purplish white	Creamy white	Pinkish white	White
iv) Calyx					
Mean length (cm)	0.17	0.30	0.24	0.16	0.34
Colour of young sepals	Pale green	Pale green	Pale green	Pale green	Pale green
Colour of old sepals	Dark green	Red	Green	Green	Green
Apex of sepals (shape)	Saccate	Acute	Narrow	Acute	Curved
Mean length of sepals (cm)	0.12	0.25	0.20	0.10	0.30
v) Corolla					
shape	Small Broad Tubular	Long Narrow Tubular	Medium Broad Tubular	Medium Narrow Tubular	Medium Broad Tubular
Mean length of corolla tube (cm)	0.37	1.79	0.73	0.58	0.76
Mean length of petal lobes (cm)	0.12	0.76	0.47	0.24	0.70

Contd.

ble 1. Continued

1	2	3	4	5	6
Proportion of corolla tube to lobe	3.08	2.36	1.55	2.42	1.09
Proportion of corolla to calyx	2.88	8.50	5.00	5.13	4.29
Extend of pubescence in corolla	High	Low	Medium	Medium	Low
Region of constiction of corolla tube from base (Mean) (cm)	0.21	1.15	0.41	0.52	0.58
vi) Androecium					
Colour of anther lobes	Creamy white with yellow tinge	Yellow	Yellow	Pale creamy yellow	Pale yellow
Mean length of anther lobes (cm)	0.13	0.14	0.15	0.15	0.15
Mean width of anther lobes (cm)	0.078	0.065	0.055	0.060	0.035
Mean size of filament (cm)	0.025	0.027	0.100	0.075	0.085
vii) Gynoecium					
Number of locules	2	2	2	2	2
Shape of ovary	Oval	Oval	Oval	Oval	Oval

Contd.

Table 1. Continued

1	2	3	4	5	6
Mean size of ovary (cm)	0.10	0.15	0.15	0.15	0.15
Mean length of style (cm)	0.20	1.00	0.10	0.30	0.10
Colour of stigma	Pale greenish white	Greenish white	Creamy brown	Greenish white	White with pale green tinge
Mean length of stigma (cm)	0.05	0.10	0.10	0.10	0.10
6. FRUIT CHARACTERS					
Shape	Round elliptical	Round elliptical	Eliptical and elongated	Round conical and triangular	No fruit set noticed
Colour of young fruits	Pale green	Green	Green	Green	-
Colour of mature fruits	Brownish red	Blackish purple	Brownish purple	Dark brownish black	-
Mean length of fruits (cm)	0.76	0.75	0.89	0.85	-
Mean breadth of fruits (cm)	0.77	0.56	0.55	0.57	-

Contd.

Table 1. Continued

1	2	3	4	5	6
7. SEED CHARACTERS					
Colour of seed coat	Pale yellowish	Yellowish brown	Pale creamy white	Dark brown	-
Mean length (cm)	0.65	0.70	0.81	0.63	-
Mean breadth (cm)	0.40	0.48	0.44	0.42	-
8. ROOT CHARACTERS					
Appearance	Woody	Soft	Woody	Woody	Woody
Presence of lateral roots	Medium	Low	Very high	Low	Low

Table 2. Variations in pollen grains of Rauwolfia spp.

aracters	Accession A R. tetraphylla	Accession B R. serpentina	Accession C R. densiflora	Accession D R. beddomei	Accession E Rauwolfia sp
) Viability (%)	77.40	88.50	81.40	79.50	65.50
) Shape	Round	Triangular	Elliptically round	Round to oval	Round
) Mean diameter (cm)	0.015	0.028	0.022	0.020	0.023

Table 3. Anatomical variations of Rauwolfia spp.

aracters	Accession A	Accession B	Accession C	Accession D	Accession E
	R. tetraphylla	R. serpentina	R. densiflora	R. beddomei	Rauwolfia sp
1	2	3	4	5	6
STOMATA					
i) Stomatal count (40 x magnification)	18.90	33.30	16.80	12.75	20.90
i) Mean length of stomata (cm)	0.0150	0.0131	0.0150	0.0125	0.0144
i) Size of guard cells (cm)	0.0048	0.0030	0.0061	0.0036	0.0035
ROOT (Cross section)					
i) Thickness of Cork Cambium	Medium (6-8 layers)	High (10-12 layers)	Low (4-6 layers)	High (10-12 layers)	Medium (7-9 layers)
i) Secondary cortex	Small sized	Large sized	Large sized	Medium sized	Medium sized
i) Number of secondary xylem vessels	High	Low and compactly arranged	High	Low	Very high
v) Pith	Almost absent (filled with secondary xylem)	Prominent	Almost absent (filled with secondary xylem)	Almost absent (filled with secondary xylem)	Almost absent (filled with secondary xylem)

Contd.

Table 3. Continued

1	2	3	4	5	6
3. STEM (Cross section)					
i) Presence of epidermal hairs	Prominent	Absent	Absent	Absent	Absent
ii) Hypodermis and cortex	Medium sized and highly chlorophyllous	Large sized and less in chlorophyll content	Very large and no clearcut demarkation between the two	Medium sized with less chlorophyll content	Large sized with medium chlorophyll content
iii) Number of schlerenchymatous stone cells	Low	Medium	High	Low	High
iv) Compactness of arrangement of secondary xylem	Medium	High	Medium	Medium	High
v) Pith	Highly prominent	Prominent	Highly prominent	Prominent	Prominent
4. LEAF (Cross section)					
i) Epidermal hairs	Present	Absent	Absent	Absent	Absent
ii) Proportion of palisade to spongy tissues	1:3	1:4	1:5	1:3	1:3
iii) Presence of air spaces in mesophyll	Almost nil	Very few	High in number	High in number	Very high
iv) Presence of chlorophyll in mesophyll tissues	High	Low	Medium	Low	Medium

Table 4. Variations in total crude alkaloid content (root), chloroform extract (aerial parts) and total chlorophyll content (aerial parts) of Rauwolfia spp. (on dry weight basis)

District	Localities	Total crude alkaloid content of roots (%)	Chloroform extract of aerial parts (%)	Total chlorophyll content of aerial parts (%)
1	2	3	4	5
I. Accession A (Rauwolfia tetraphylla)				
Thrissur	Peechi	1.09	4.34	0.944
Thrissur	Vellanikkara	1.03	6.76	0.543
Palakkad	Kanhirappuzha	1.35	5.52	0.616
Malappuram	Malappuram	1.18	6.04	0.873
	Mean	1.16	5.54	0.729
II. Accession B (Rauwolfia serpentina)				
Wynad	Thirunelli	2.65	2.09	0.333
Kannur	Alakkod	1.33	2.74	0.517
Malappuram	Munderi	1.58	2.19	0.346
Malappuram	Edapal	1.63	2.46	0.268
Palakkad	Kanhirappuzha	1.31	3.84	0.548

Contd.

Table 4. Continued

1	2	3	4	5
Palakkad	Mannarkkad	1.91	2.68	0.372
Thrissur	Peechi	1.92	2.55	0.206
Thrissur	Vellanikkara	2.01	2.87	0.382
Thiruvananthapuram	Palode	1.53	3.48	0.599
	Mean	1.76	2.77	0.397
III. Accession C (*Rauwolfia densiflora*		1.49	8.20	0.482
IV. Accession D (*Rauwolfia beddomei*)		1.77	3.91	0.352
V. Accession E (*Rauwolfia* sp.)		1.96	6.20	0.372

Discussion

DISCUSSION

5.1 Distribution of the genus

The study on the occurrence and distribution of Rauwolfia revealed that the species Rauwolfia serpentina was uniformly distributed in almost all the localities surveyed. Their occurrence was not limited to forest lands of Thirunelly, Munderi, Kanhirappuzha, Peechi and Palode only, but they were distributed in plain lands of Alakkode, Edapal, Mannarkkad and Vellanikkara, showing their wide adaptability to varying environmental conditions.

The occurrence of this species in the partially shaded regions of the forest lands, shows it's adaptability to partial shade conditions. Eventhough this species of Rauwolfia was widely distributed, their frequency of occurrence was comparatively less. This might be due to the uncontrolled collection and over exploitation by the people. If this is not checked, the extinction of this species may happen, as reported by Kuriakkose (1992).

The occurrence of Rauwolfia tetraphylla was almost sparse in the forest lands surveyed. But they were widely distributed in non-forest areas, road sides and waste lands. This points to the fact that this species might be an introduced one to Kerala from other places. But the rapid multiplication of this, in the human inhabited areas, was mainly due to it's ability to produce large

number of attractive fruits which are dispersed by birds. Sahu (1979) also agrees to the view that this species might be an introduced one.

R. densiflora and R. beddomei are the two species which were not found in any of the localities surveyed, eventhough Gamble (1921) reported their occurrence in Kerala. Specimen plants are available at the Tropical Botanical Garden, Palode. The taxonomists of the Tropical Botanical Garden, Palode are of the opinion that the occurrence of these species is restricted to Agasthyavanam Biological Park of Thiruvananthapuram. The poor adaptability and the over exploitation might have caused the almost near disappearance of these species from Kerala.

R. micrantha was reported to be present in the erstwhile Malabar areas by Gamble (1921). Eventhough extensive survey of the various locations of the erstwhile Malabar area was conducted, this species was not found. The unavailability of even a single specimen indicates the almost complete extinction of this species in Kerala.

5.2 Morphological variations

Study of the morphological characters has shown that accession A has the characters described for the species R. tetraphylla (earlier called R. canescense) by Gamble (1921), Sahu (1979) and

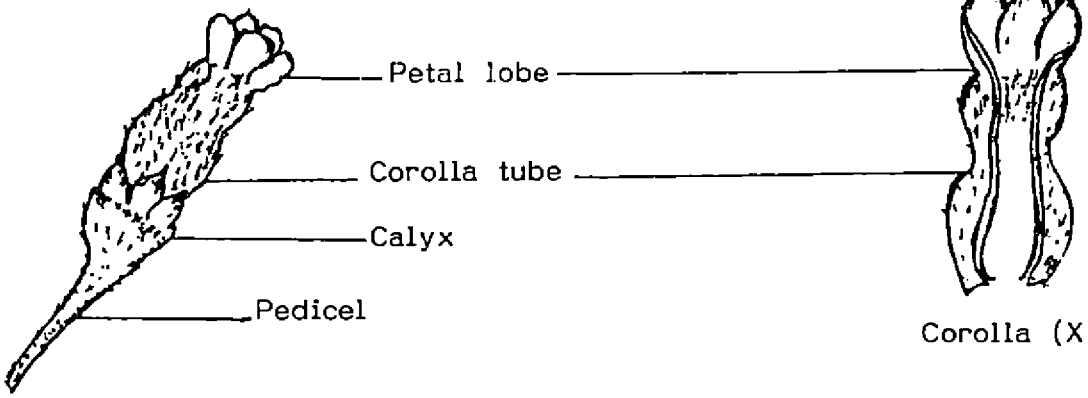
Babu (1990). The characters of accession A such as woody shrub nature, mean height of 132 cm, 3-4 nately whorled unequal leaves with 14 pairs of main nerves, few flowered inflorescence (8.71) with 1.16 cm long peduncle, greenish white flower, with short calyx (0.17 cm) and 0.37 cm long corolla, were almost similar to their reports and hence accession 'A' is the species R. tetraphylla (Plate 1).

From this study, a lot of other characters pertaining to this species were observed. These include dark green young twigs, ashy white old stem, mean internodal length of 7.23 cm, pubescent leaves which are of three types, larger (8.59 cm x 3.99 cm) medium (5.48 cm x 2.98 cm) and smaller (2.72 cm x 1.82 cm) ones, with a mean petiole length of 0.72 cm, 0.35 cm and 0.2 cm respectively. There were 24-32 glands per leaf axil, leaf base was broad acute, margin was wavy and tip mucronate. The mean interveinal length was 0.66 cm (larger leaves) and 0.26 cm (smaller leaves). The inflorescence was hairy, erect and medium branched with 1.42 cm long flowers, having a pedicel of length 0.59 cm and calyx of 0.17 cm length. The sepals were 0.12 cm long and saccate at the apex. Ratio of corolla tube to lobe was 3.08 and ratio of corolla to calyx was 2.88 with a constriction at 0.21 cm from the base. The anther lobes were creamy white with a length of 0.125 cm and width of 0.078 cm. Size of the filament was 0.025 cm and length of ovary was 0.1 cm with 0.2 cm long style. Fruits were round

and elliptical having a mean size of 0.76 cm x 0.77 cm, pale green coloured when young and brownish red on ripening. The roots were medium branched and woody in nature (Fig. 1, Plate 1).

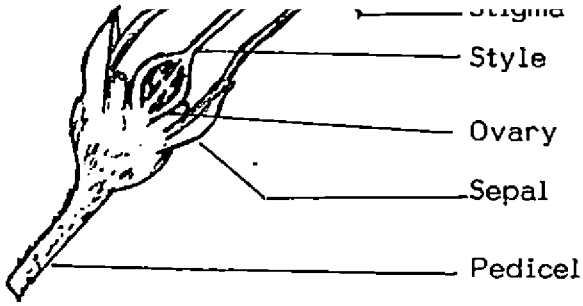
Accession 'B' was found to be having similar characters of Rauwolfia serpentina, described by Gamble (1921), Monachino (1954), Sahu (1979) and Gauniyal et al. (1988), such as presence of simple leaves in whorls of three, long elliptic and lanceolated single type of leaves having a size of 9.64 cm x 2.8 cm with 12 pairs of main nerves and unbranched stem. Inflorescence was many flowered (39.1) cyme with small, (2.85 cm) purplish white flowers, having 0.49 cm long pedicel, large narrow and tubular corolla (1.79 cm). Stamens were five in number and gynoecium was with bicarpellary ovary, filiform style (1 cm long) and large capitate stigma. Fruit was a drupe of size 0.75 cm x 0.56 cm, green at early stages and turning to blackish purple on ripening. Seeds were ovoid and flattened. Roots were long, thick and grey brown in colour.

In addition to these characters R. serpentina can be identified from it's herbaceous cylindrical stem, pale green young stem, pale grey old stem, mean inter nodal length of 1.73 cm, medium dark and dark green upper sides of younger and older leaves, presence of 12-15 glands on leaf axil, sharp acute leaf base, wavy and entire leaf margin, acuminate leaf tip, mean interveinal



A flower (X 8)

Corolla (X 10)



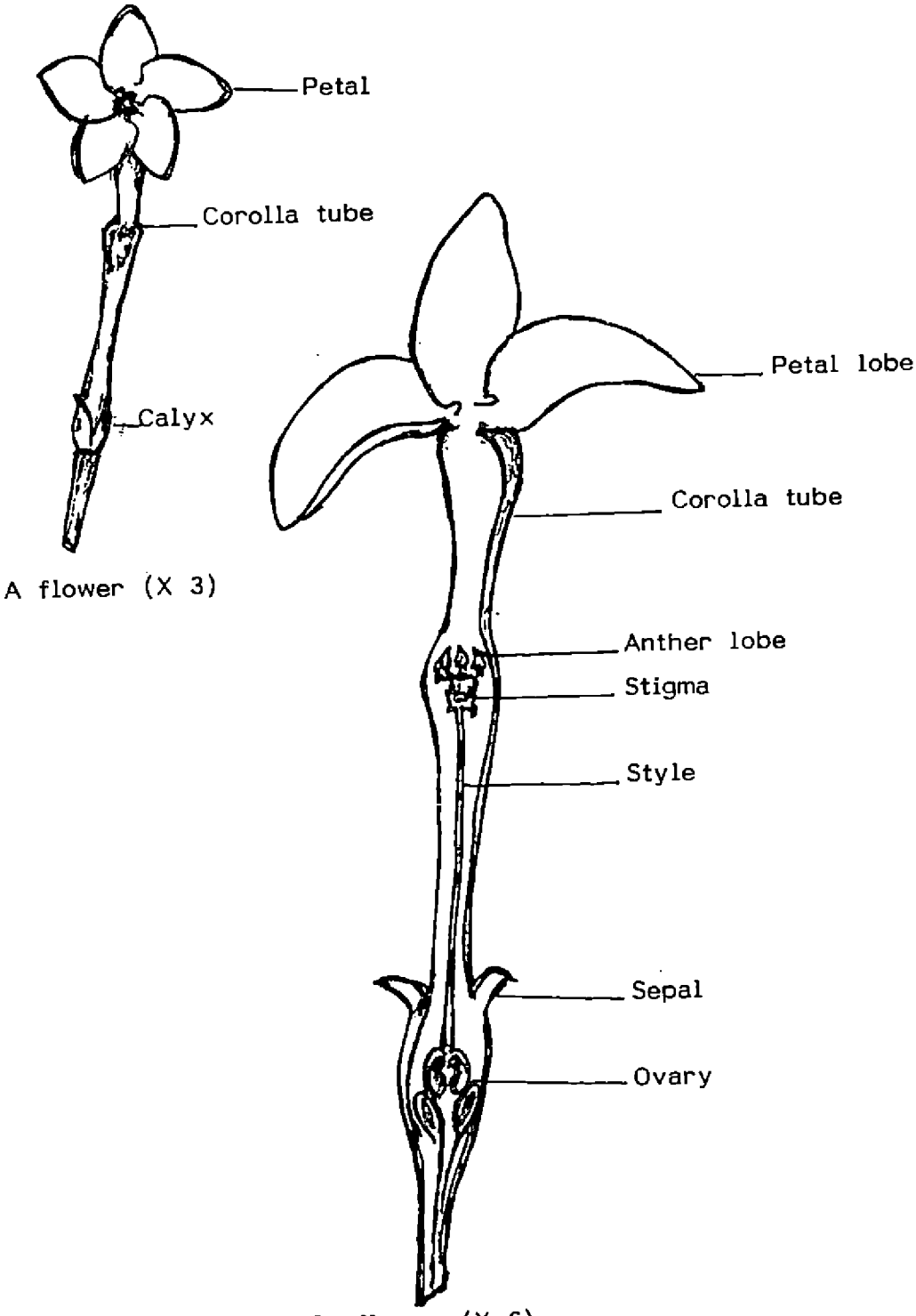
L.S. of flower (X 10)

C.S. of ovary (X 20)

length of 1.11 cm, terminal, erect and 4.2 cm long peduncle, 0.49 cm long pedicel, calyx of length 0.3 cm, pale green young calyx lobes turning to red on maturity. Sepals were having a size of 0.25 cm, with acute apex. Corolla was long and tubular with petal lobes of length 0.76 cm. Ratio of the corolla tube to lobe was 2.36 while ratio of the corolla to calyx was 8.5. Androecium was with yellow anther lobes of size 0.140 cm x 0.065 cm,, having a mean filamental size of 0.027 cm. Ovary was oval, 0.15 cm long and style was creamy white with 0.1 cm long greenish white stigma. The fruits were round elliptical in shape, green in colour while young and yellowish brown on ripening. Roots were pale brownish black with few laterals (Fig. 2, Plate 2).

Accession 'C' had similarity with the characters specified for Rauwolfia densiflora by Gamble (1921) and Sahu (1979). This includes the shrubby nature of the plant and the presence of three whorled broad, oblanceolate leaves with 14-15 pairs of veins. Inflorescence was a bifurcating cyme of size 4.75 cm. Flowers were creamy white with elongated sepals, 0.73 cm long corolla tube, with large (0.47 cm) and round lobes. Stamens were five in number and gynoecium was with bicarpellary ovary and large capitate stigma. Fruits were brownish purple drupes with seeds pointed at the top. Hence the accession 'C' is Rauwolfia densiflora.

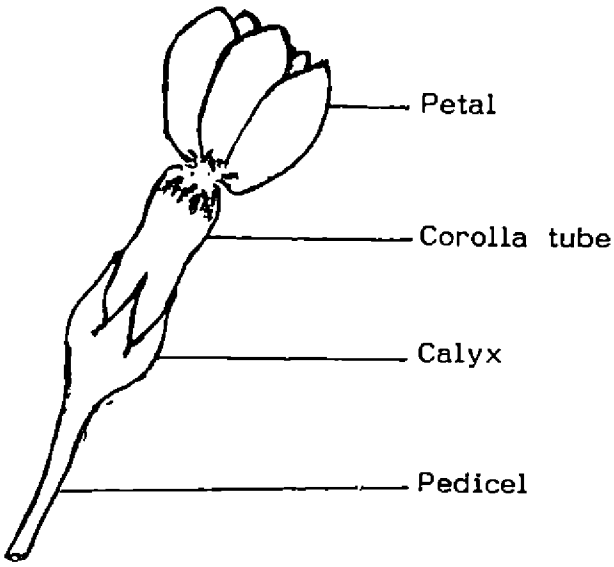
Fig. 2. Floral biology of Rauwolfia serpentina



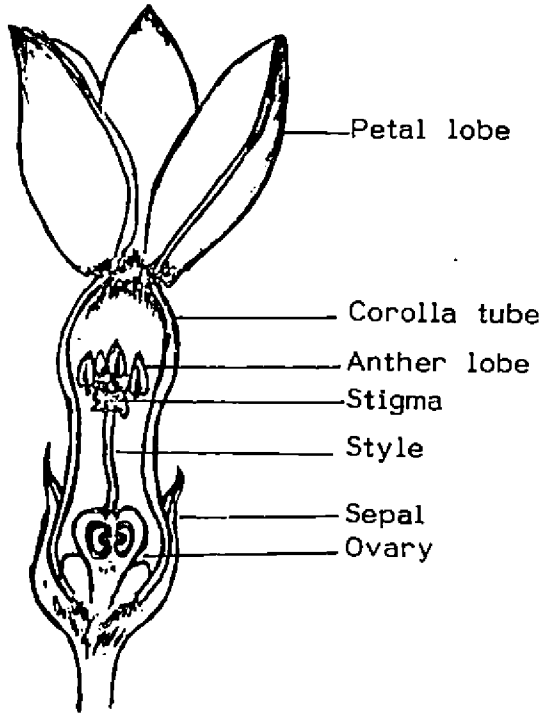
Additionally the following characters were observed specific to this species (Table 1). They were the presence of few branches, pale green young stem, pale grey old stem and main internodal length of 4.89 cm. Leaves were smooth with dark green upper and pale green lower sides, having a mean size of 14.11 cm x 6.16 cm and petiole of 1.85 cm length. Leaf base was broad acute, leaf margin was wavy and leaf tip was broad acuminate. The mean interveinal length was 1.06 cm and there were 17-20 glands in the leaf axil. Inflorescence was a terminal, erect, highly branched cyme with large number of flowers (51). Flowers were 1.902 cm long with 0.74 cm long pedicel. Calyx was green coloured while young and old, having a size of 0.24 cm. Sepals were narrow tipped and 0.2 cm long. Petals were having a length of 0.47 cm and the ratio of corolla tube to lobe was 1.55, while that of corolla to calyx was 5.00. The region of constriction was 0.41 cm from base of corolla tube. The anther lobes were having a size of 0.15 cm x 0.055 cm with 0.1 cm long filament. Gynoecium was with an oval, 0.15 cm long ovary, with 0.1 cm long style and 0.1 cm long, creamy brown, stigma. Fruits were elliptically elongated having a mean size of 0.89 cm x 0.55 cm. Seeds were pale creamy white with a mean size of 0.81 cm x 0.44 cm. Roots were hard and woody with numerous laterals (Fig. 3, Plate 3).

Accession 'D' has got similarity with R. beddomei. The glabrous and shrubby nature, leaves of size 12.24 cm x 3.75 cm

Fig. 3. Floral biology of Rauwolfia densiflora



A flower (X 5)



L.S. of flower (X 8)

C.S. of ovary (X 18)

with 14 pairs of veins, inflorescence, a subhorizontal laxcyme, with 7 cm long peduncles, corolla with 0.58 cm long pinkish white petals, five stamened androecium and bicarpellary ovary were the characters similar to that reported by Sahu (1979), for R. beddomei and so the accession 'D' is R. beddomei.

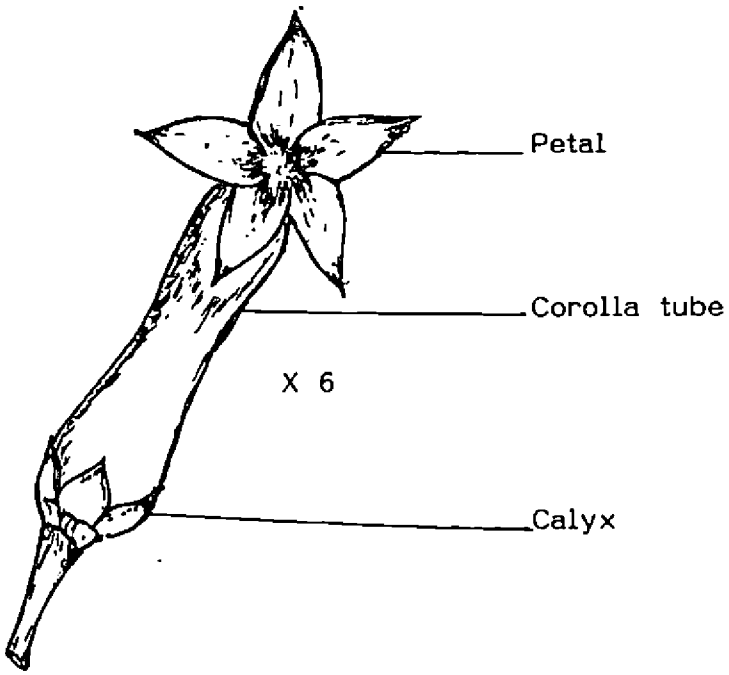
Additional characters observed from the study, for the species R. beddomei were, the highly branched, woody and shining nature of the stem, ashy whitish brown old stem, mean internodal length of 1.34 cm, smooth lanceolate leaves which were pale green while young and turning to dark green on aging. There were 18-20 glands per leaf axil and mean petiole length was 0.93 cm. Leaf base was acute and margin of leaf was wavy with acuminate tip. Mean interveinal length noticed was 0.62 cm. Inflorescences were terminal or axillary subhorizontal cymes with few branches and peduncles were of size 7 cm. Average number of flowers per cyme was very few (11). Flowers (length 1.6 cm) were with 0.46 cm long pedicel and 0.16 cm long calyx, which were pale green with acute tip. Mean length of sepals was 0.1 cm. Corolla tube was 0.58 cm long and petal lobes were 0.24 cm in length. The tube to lobe ratio of of corolla was 2.42 while tube to calyx ratio was 5.13. Region of constriction was 0.52 cm from the base of the corolla tube. Anther lobes were pale creamy yellow coloured, having a size of 0.15 cm x 0.06 cm with 0.075 cm long filament. Gynoecium was with 0.15 cm long ovary and 0.3 cm long style

with a greenish white, 0.1 cm long stigma. Fruits were round to conical or triangular in shape, green in colour while young, turning to brownish black on maturity, with a mean size of 0.85 cm x 0.57 cm. Seeds were dark brown coloured having a size of 0.63 cm x 0.42 cm. Roots were hard with only few laterals (Fig.4, Plate 4).

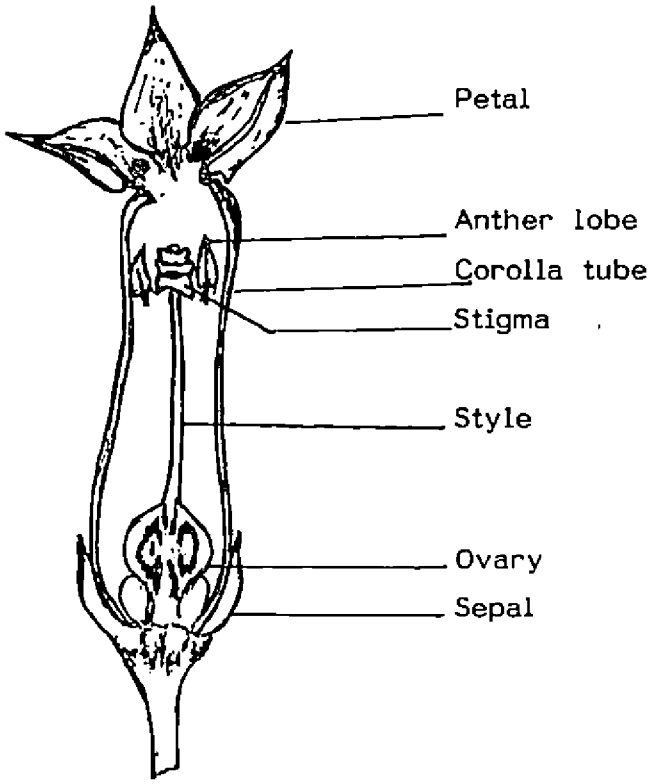
The accession 'E' showed characters similar to those of R. densiflora, such as woody shrub nature, presence of few branches, cylindrical and non-hairy stem, dark green coloured young stem, ashybrown old stem, presence of three leaves per node arranged in whorled condition and presence of only a single type of leaves,, which were dark green in colour. The leaf margin was almost straight. The inflorescence was a terminal cyme with white flowers, green coloured calyx, pale greenish white and medium sized, broad, tubular corolla, with a tube length of 0.76 cm (0.73 cm in R. densiflora). Anther lobes were yellow with a mean size of 0.15 cm and filament of length 0.01 cm. Ovary was bicarpellary and style was long (0.1 cm) and filiform with dumb bell shaped, 0.1 cm long stigma. Hence there might be a chance that accession 'E' belongs to the species R. densiflora (Fig. 5, Plate 5).

But on detailed examination, it was found that accession 'E' differed from R. densiflora (C) in certain other characters. Mean height was only 65 cm in 'E' while it was 81 cm in 'C'.

Fig.4. Floral biology of Rauwolfia beddomei



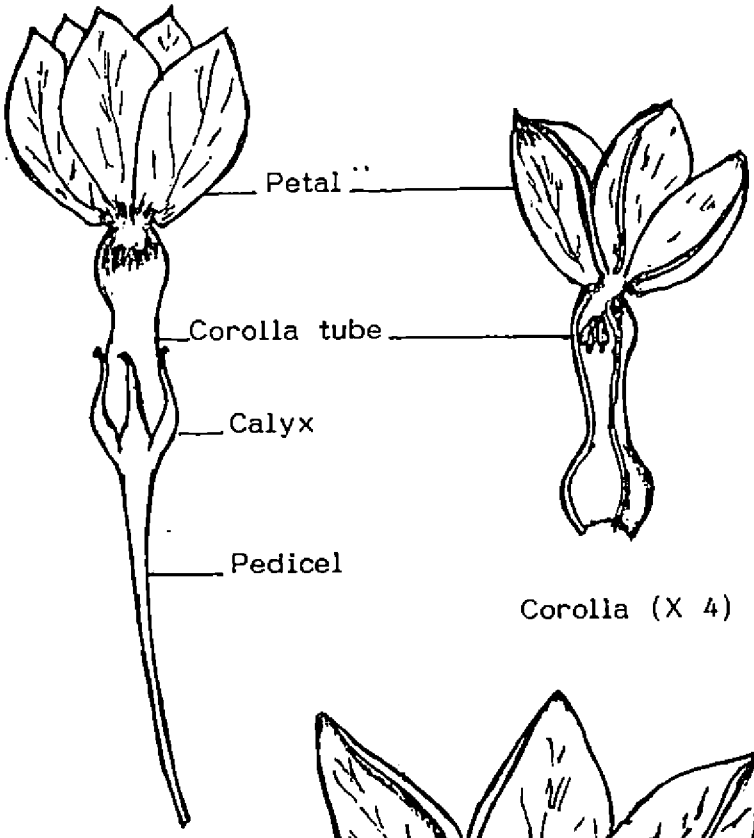
A flower (X 6)



L.S. of Flower (X 6)

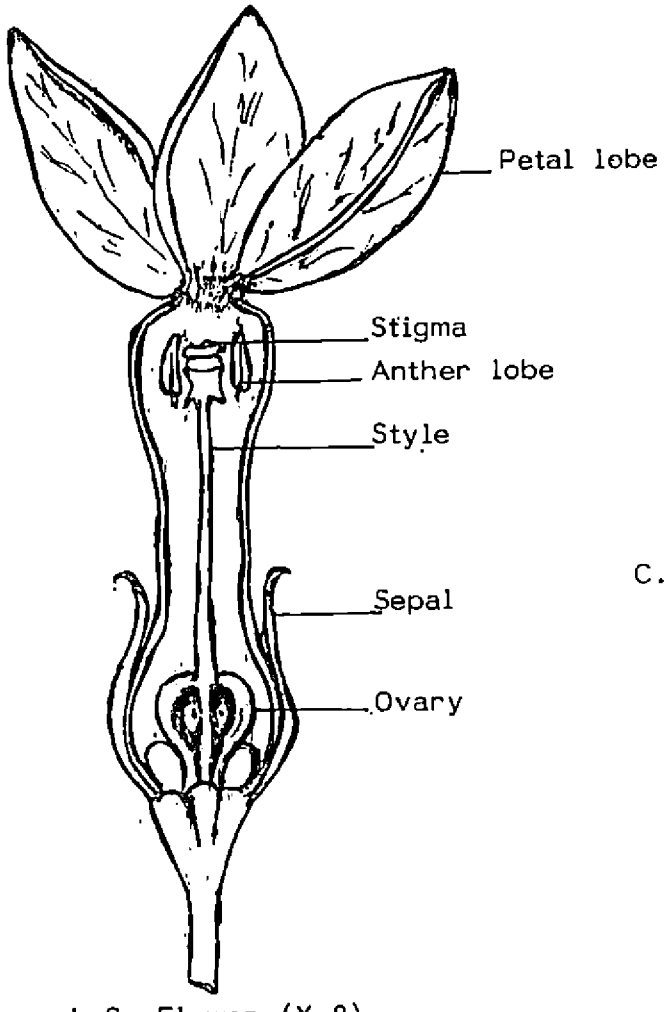
C.S. of Ovary (X 15)

Fig.5. Floral biology of accession E (Rauwolfia sp.)



A flower (X 4)

Corolla (X 4)



L.S. Flower (X 8)

C.S. of ovary (X 8)

Mean internodal length was 3.52 cm in E while, it was 4.89 cm in 'C'. These two characters show the lower growth rate of the accession 'E' compared to accession 'C'. The mean length of leaves was higher (16.45 cm) in 'E' than 'C' (14.11 cm). But the width of leaves was more in 'C' (6.16 cm) compared to E (4.78 cm). Thus 'E' has longer leaves while 'C' has broader leaves. Comparing the presence of glands in leaf axil, 'E' has got lesser number (16) than 'C' (20). The petiole length was also lower in 'E' (1.65 cm) than 'C' (1.85 cm). In 'C' the leaf base was broad acute while it was sharp acute in 'E', whereas the leaf tip was long acuminate in 'E' and broad acuminate in 'C'. Mean number of veins were higher (17) in 'E' than 'C' (14.5) and interveinal length was also more (1.22 cm) in 'E' than 'C' (1.06 cm). The leaves were long lanceolate in 'E' but oblanceolate in 'C'. The inflorescence was highly branched in 'C' while it was less branched in 'E'. Thus a lower growth rate was observed in 'E', but the floral characters were seen to be at a higher degree in 'E' compared to 'C'. Though the mean number of flowers per cyme was high in 'C' (52) than 'E' (21), length of pedicel was higher (1.06 cm) in 'E' than 'C' (0.74 cm) and the length of the flowers was also higher in 'E' (2.18 cm) than 'C' (1.902 cm). Size of the calyx was more (0.34 cm) in 'E' compared to 'C' (0.24 cm). The apex of the sepals was pointed and straight in 'C' while it was curved back in 'E'. Longer sepals were seen in 'E' (0.3 cm) than 'C' (0.2 cm). Similarly, length

of the petal lobes was also more (0.7 cm) in 'E' than 'C' (0.47 cm). Proportion of the corolla tube to lobe and corolla to calyx was lower in 'E' (1.09 and 4.29) than 'C' (1.55 and 5). The region of constriction was also at a higher level (0.58 cm) in 'E' than in 'C' (0.41 cm). Stigmatic surface was creamy brown in C' while it was white with a greenish tinge in 'E'. Fruit set as not noticed in 'E', eventhough flowering occurred. Study of the root morphology showed that 'C' has got more prominent tap root and lateral roots compared to accession 'E', which were having very few lateral roots. This shows that the two accessions are morphologically different.

The difference in morphology might presumably be due to the fact that the two accessions are different at species level. R. micrantha was the species reported in India, other than that discussed above. Certain characters were observed in 'E', similar to that of R. micrantha, described by Gamble (1921) and Sahu (1979), such as elliptic lanceolate leaves, slender petiole, peduncle of size 4 cm, presence of few flowers, calyx of quarter of the length of the corolla tube and white coloured corolla. But there were characters in which dissimilarity was observed, such as presence of more number of veins in 'E' (17 pairs) than R. micrantha (10-12 pairs), long sepals in 'E' which were curved back at the tip as against the triangular sepals in R. micrantha and shorter corolla tube in 'E' (0.76 cm) than in R. micrantha

(1 cm). Hence the accession 'E' is unlikely to be the species R. micrantha.

Another chance for this morphological difference is that 'E' might be a higher polyploid. Tapadar (1963) studied the characters specific for the diploids and their tetraploids in R. serpentina and he has put forward certain morphological characters specific to the higher ploidy level. He noticed slower growth rate, shorter internodes, reduction in height, shorter branches, dark greenish colour of leaves, shorter petiole, reduction in the number of inflorescences per plant and number of flowers per inflorescence, and larger floral parts like, increased size of calyx, and petal limbs, as the characters of higher ploidy level.

When the morphological characters of 'E' and R. densiflora (accession C) were critically examined and analysed it was found that 'E' has got slow growth rate, lower mean height (65 cm for E and 81 cm for C) dark green coloured leaves, shorter petiole (1.65 cm for E and 1.85 cm for C), lower number of glands per leaf axil (14-18 in E and 17-20 in C) and very few flowers per inflorescence (21 in E and 52 in C) which, as per Tapadar (1963) are the characters for the higher ploidy level.

Floral characters were also found to be that of a higher ploid in 'E'. The pedicel length was more (1.06 cm in E and

0.74 cm in C), the length of flowers was more (2.18 cm in E and 1.902 in C), the calyx was longer (0.34 cm in E and 0.24 cm in C), the length of sepals was more (0.3 cm in E and 0.2 cm in C) and the length of petal lobes was also more (0.7 cm in E and 0.47 cm in C). These floral characters were also in accordance with the view of Tapadar (1963) for higher ploids. Thus the morphological study infers that accession 'E' might be a higher ploid of R. densiflora. Since R. densiflora was reported to be a tetraploid with 2n = 44, the accession 'E' might be a still higher ploid of R. densiflora.

5.3 Variation of the pollen grains

Studies on the viability of pollen grains (Table 2) showed maximum viability in R. serpentina (88.5%) followed by R. densiflora (81.4%), R. beddomei (79.5%) and R. tetraphylla (77.4%). The viability was lowest in the accession E, (65.5%). According to Tapadar (1963) reduction in viability of pollen grains can be considered as a character of higher level of ploidy in Rauwolfia. Comparing the diploid R. serpentina, tetraploid R. densiflora and hexaploid R. tetraphylla, the viability of pollen grains of which was 88.5 per cent, 81.4 per cent and 77.4 per cent respectively, accession 'E' showed a lower viability of 65.6 per cent only. This also indicates the higher ploidy level of 'E' than the hexaploid R. tetraphylla.

The shape of the pollen grains also differed in all the accessions. It was small and round in R. tetraphylla, triangular in R. serpentina, elliptically round in R. densiflora, oval in R. beddomei and round in accession 'E'. According to Tapadar (1963) the variation in the shape of pollen grains can also be considered as the character for identification of diploids and polyploids. The observed difference in the shape of the pollen grains of 'E' and 'C' also supports the view that the two are different in ploidy level.

The mean diameter of the pollen grains also varied with the highest in R. serpentina (0.028 cm) followed by R. densiflora (0.022 cm), R. beddomei (0.020 cm) and R. tetraphylla (0.015 cm). It was 0.023 cm in accession 'E'.

5.4 Anatomical variation

Comparative study of the stomata showed that stomatal count varied with the highest in R. serpentina (3 ed by R. tetraphylla (18.9), R. densiflora (16.8) and R. beddomei (12.75), while that of accession 'E' was 20.9 (Table 3).

Comparing the stomatal frequency of R. densiflora (a tetraploid), R. tetraphylla (a hexaploid) and accession 'E', ie., 16.8, 18.9 and 20.9 respectively, the chance for the accession 'E' to be a higher ploid, is more because it has the highest stomatal count of 20.9, which is in line with the increasing trend of

R. densiflora (0.015 cm) followed by R. serpentina (0.0131 cm) and R. beddomei (0.0125 cm). It was 0.0144 cm in accession 'E'.

The size of the guard cells was maximum in R. densiflora (0.0061 cm) followed by R. tetraphylla (0.0048 cm), R. beddomei (0.0036 cm) and R. serpentina (0.0030 cm) while it was 0.0035 cm in accession 'E'.

Anatomical variations of the root, stem and leaf (cross sections) were observed among the different species of Rauwolfia (Table 3). In the roots, variations were noticed in cambium, secondary cortex, secondary xylem and pith regions. Stem anatomy showed variations in hypodermis, cortex, secondary xylem and pith, while leaf anatomy showed variations in the proportion of palisade to spongy tissues, presence of chlorophyll and airspaces, in the mesophyll. Presence of epidermal hairs and higher chlorophyllous tissues was specific to the species R. tetraphylla (Pls.6-20).

5.5 Variations in the alkaloid content

The comparison of the mean total crude alkaloid content in the roots of different species of Rauwolfia (Table 4) showed the highest value in R. beddomei (1.77%) followed by R. serpentina

(1.76%), R. densiflora (1.49%) and R. tetraphylla (1.16%). The accession 'E' had the value of 1.96 per cent. Tapadar (1963) observed a higher root alkaloid content in higher ploids of R. serpentina. Comparing the root alkaloid content of R. densiflora and accession 'E', it can be presumed that 'E' might be a higher ploid of R. densiflora. The morphological, cytological and anatomical evidence also supports this view.

The range of the mean root alkaloid content in the five accessions was from 1.16 per cent to 1.96 per cent. Antipova et al. (1988) reported that the root alkaloids of R. serpentina and R. canescense (R. tetraphylla) ranged from 0.78 per cent to 2.08 per cent. The range of total root alkaloids observed (1.16% - 1.96%) also comes within the range suggested by Gauniyal et al. (1988) for Rauwolfia (0.7% - 3%).

Eventhough R. beddomei has a higher alkaloid content in the roots, its importance as a drug may be limited, due to its rare availability compared to R. serpentina which is widely distributed. R. serpentina also containes an almost equal level of root alkaloids as that of R. beddomei.

But the accession 'E' had a still higher root alkaloid content (1.96%) than that of R. beddomei, which might be due to it's higher ploidy level.

Variation in the root alkaloid content ranging from 1.33 per cent to 2.65 per cent was observed in R. serpentina from nine geographic locations of Kerala. Same trend was reported by Cook (1905) and Sulochana (1959).

Samples from Thirunelly forests of Wynad showed maximum root alkaloid content (2.65%) followed by those from Vellanikkara (2.01%), Peechi (1.92%), Mannarkkad (1.91%), Edapal (1.63%), Munderi (1.58%), Palode (1.53%), Alakkod (1.33%) and Kanhirappuzha (1.31%) respectively. The samples from central Kerala, ie., Thrissur, Palakkad and Malappuram districts showed higher root alkaloid content than the samples from Southern Kerala (Palode) and Northern Kerala (Kannur) excluding Wynad. The higher elevation and the resultant lower mean temperature might have influenced in the higher root alkaloid content of the samples from Thirunelly. In Thrissur and Palakkad districts, samples collected from the non-forest areas (Vellanikkara and Mannarkkad) showed higher root alkaloids than those from the forest areas (Peechi and Kanhirappuzha). The samples from forest lands of Malappuram district namely, Munderi showed lower root alkaloid content than the samples from Edapal, which is a non-forest area. Based on this result no clear inference could be drawn about the relationship between root alkaloid content and geographical distribution. But it appears that, in addition to inherent variability, the environmental parameters such as altitude, rainfall, and soil conditions

might have some role in the variation of the alkaloid content.

Samples of R. tetraphylla, from Kanhirappuzha, showed the highest content of root alkaloid (1.35%) followed by those from Malappuram (1.18%), Peechi (1.09%) and Vellanikkara (1.03%). But in R. serpentina the contents of total root alkaloids in the above locations were 1.31, 1.63, 1.92 and 2.01 per cent respectively, showing a negative trend compared to R. tetraphylla. From this it is clear that, in the same geographic location, these two species behaved oppositely, as far as root alkaloid content is considered. Hence it can be assumed that the alkaloid content in the roots is a highly complex phenomenon involving environmental factors, species difference and their interaction. The conditions suited for the higher root alkaloid production in R. serpentina may not be favourable for the root alkaloid production in R. tetraphylla.

5.6 Variations in the chloroform extract of aerial parts

Variability was observed in the chloroform extract of the aerial parts of the samples from various geographic locations of Kerala. This variation may be due to the presence of alkaloids and other metabolites like, tannins, terpenes, chlorophyll etc., in the aerial parts. The highest value (8.2%) was observed in R. densiflora followed by R. tetraphylla (5.54%), R. beddomei (3.91%) and R. serpentina (2.77%). The chloroform extract was

6.2 per cent in accession 'E'. It can be presumed that the more woody and shrubby natured species showed higher percentage of chloroform extract than the succulent herbaceous species, R. serpentina.

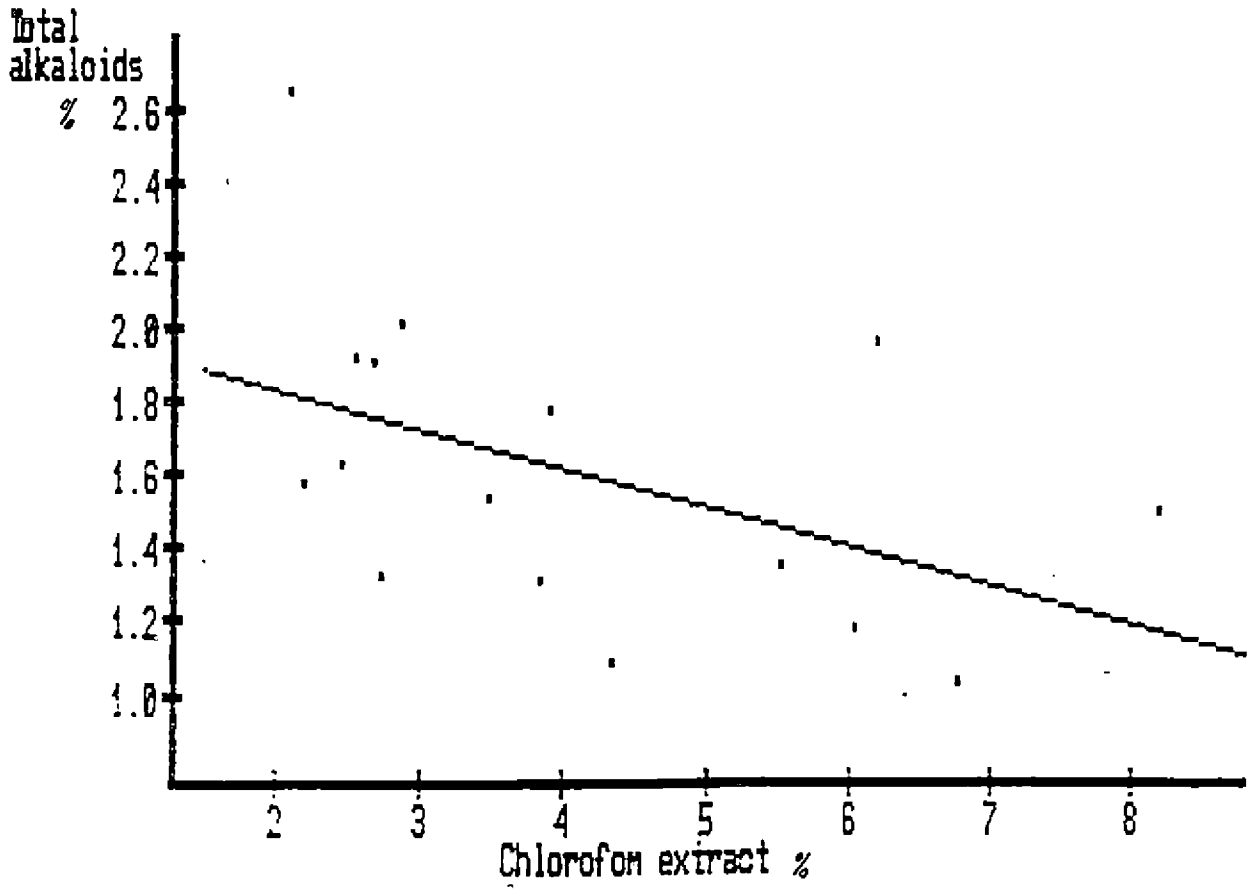
The percentage of chloroform extract of the samples of R. serpentina from different localities also showed variation. The highest value of chloroform extract (3.84%) was noticed in the sample from Kanhirappuzha which showed the lowest content of the total root alkaloids (1.31%). The lowest value of chloroform extract (2.09%) was noticed in samples from Thirunelly, where the highest root alkaloid content (2.65%) was observed. This shows the inverse relationship of the root alkaloid content and the percentage of chloroform extract of aerial parts (Fig. 6).

A pooled regression analysis of all the samples in respect of chloroform extract and total root alkaloids, showed a negative correlation with a correlation coefficient of -0.477. A relationship of

"Root alkaloid content = 2.047 - 0.106 x chloroform extract content"

was derived from the regression analysis. This relationship can be effectively utilised for the determination of total root alkaloid content. Thus the percentage of chloroform extract of aerial parts can be used as an index of selection for higher root alkaloid.

Fig. 6. Regression line showing the relationship between total alkaloids (roots) and chloroform extract (aerial parts) of Rauwolfia spp.



5.7 Variations in the chlorophyll content

Variation in the chlorophyll content was noticed in the collected samples there by showing the variation in photosynthetic efficiency. The highest content of chlorophyll was observed in R. tetraphylla (0.729%) while the lowest in R. beddomei (0.352%). But the total root alkaloid content was high in R. beddomei (1.77%) and low in R. tetraphylla (1.16%), showing a negative correlation of the alkaloid content in the roots and chlorophyll content in the aerial parts.

The variation of chlorophyll content was observed among the samples of R. serpentina collected from different localities.

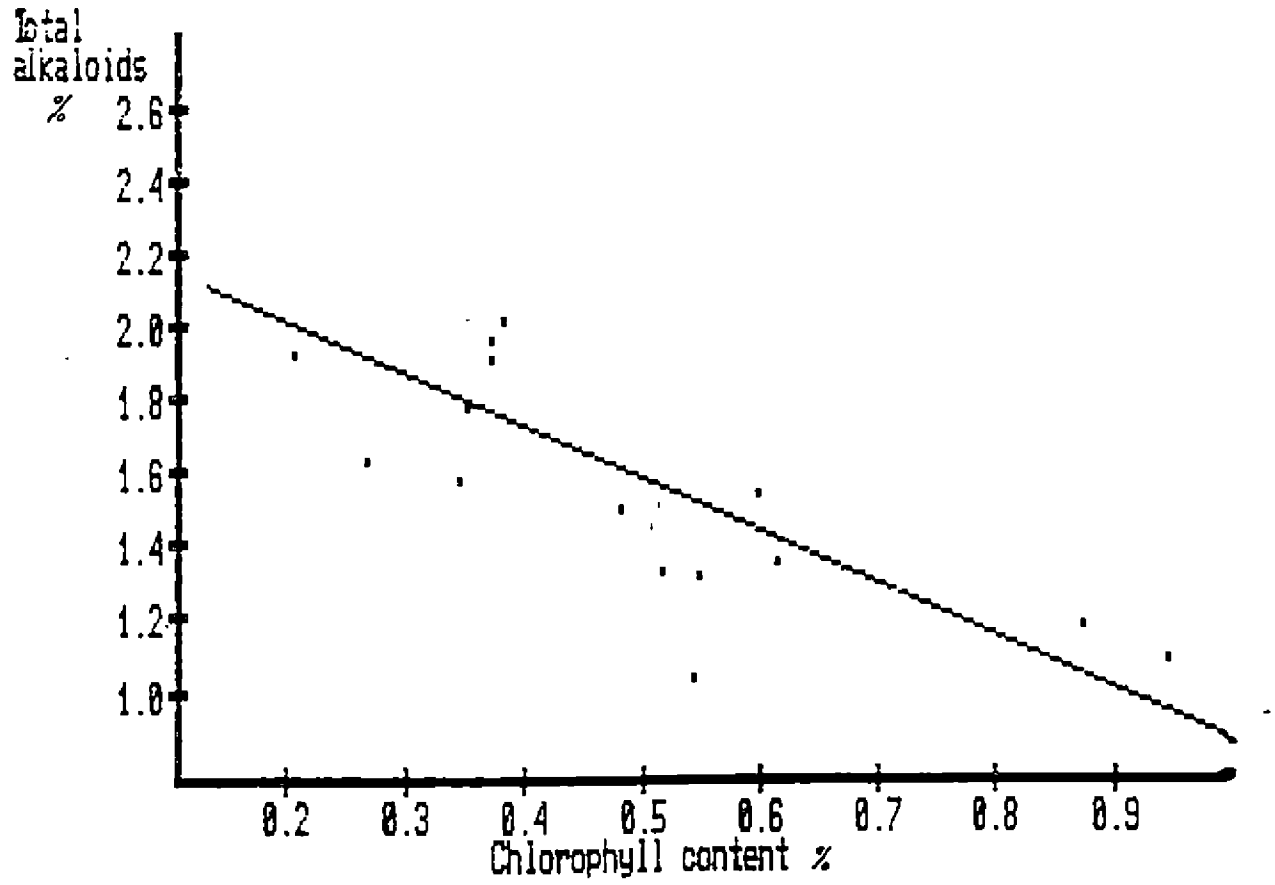
A pooled regression analysis of all the samples, to find out the relationship between root alkaloid content and chlorophyll content, revealed that these two were negatively correlated with a correlation coefficient of -0.697. A relationship of

"Total root alkaloids = 2.304 - 1.434 x chlorophyll content of aerial parts"

was derived from the regression analysis, which was highly significant (P = 0.003) (Fig. 7).

This relationship can be effectively utilized in the evaluation studies for total root alkaloids. The estimation of chlorophyll content is comparatively easier, accurate and less time consuming

Fig. 7. Regression line showing the relationship between total alkaloids (roots) and total chlorophyll content (aerial parts) of Rauwolfia spp.



than the estimation of root alkaloids which is expensive and time consuming. Hence in the screening trials for the total root alkaloids in Rauwolfia, the chlorophyll content of the aerial parts can be used as an index, for the selection of superior types, even at the early stages of growth, without uprooting the plants.

Plate 1. Rauwolfia tetraphylla - A portion of the twig

Plate 2. Rauwolfia serpentina - A general view of the plant

Plate 3. Rauwolfia densiflora - A close view of the flowering twig

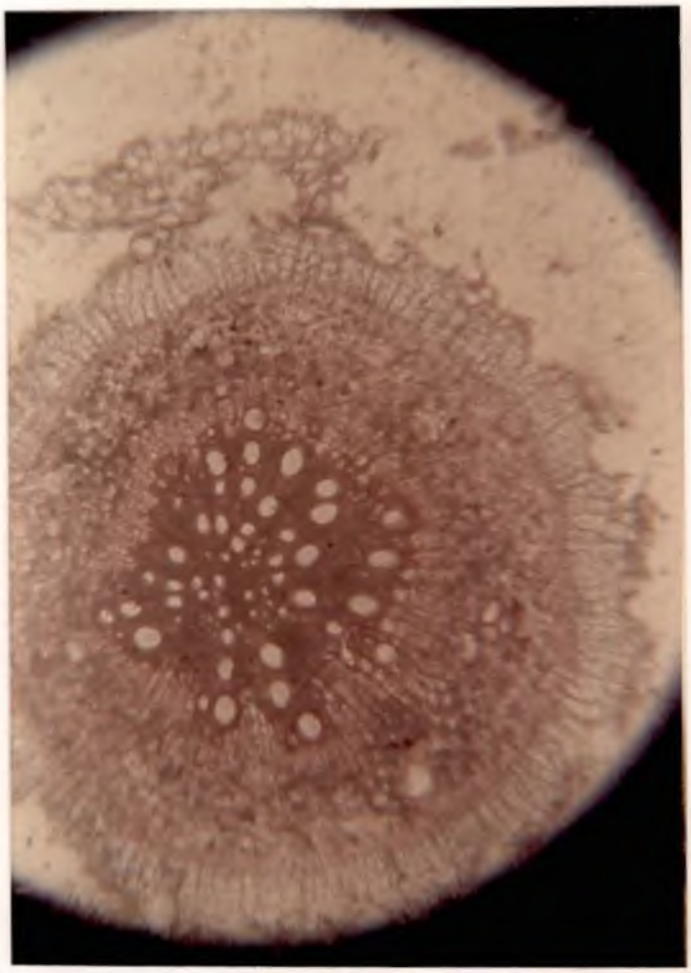
Plate 4. Rauwolfia beddomei - A close view of the flowering twig

Plate 5. Accession E (Rauwolfia sp.) - A close view of the flowering twig

Plate 6. R. tetraphylla - C.S. of Root (X 50)

Plate 7. R. tetraphylla - C.S. of Stem (X 50)

Plate 8. R. tetraphylla - C.S. of Leaf (X 50)



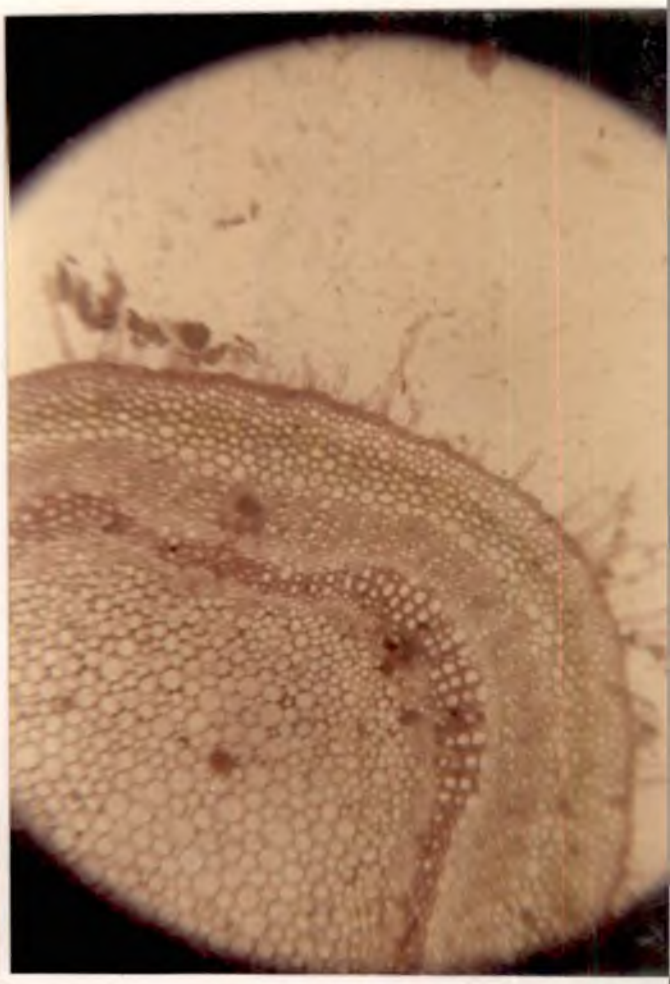
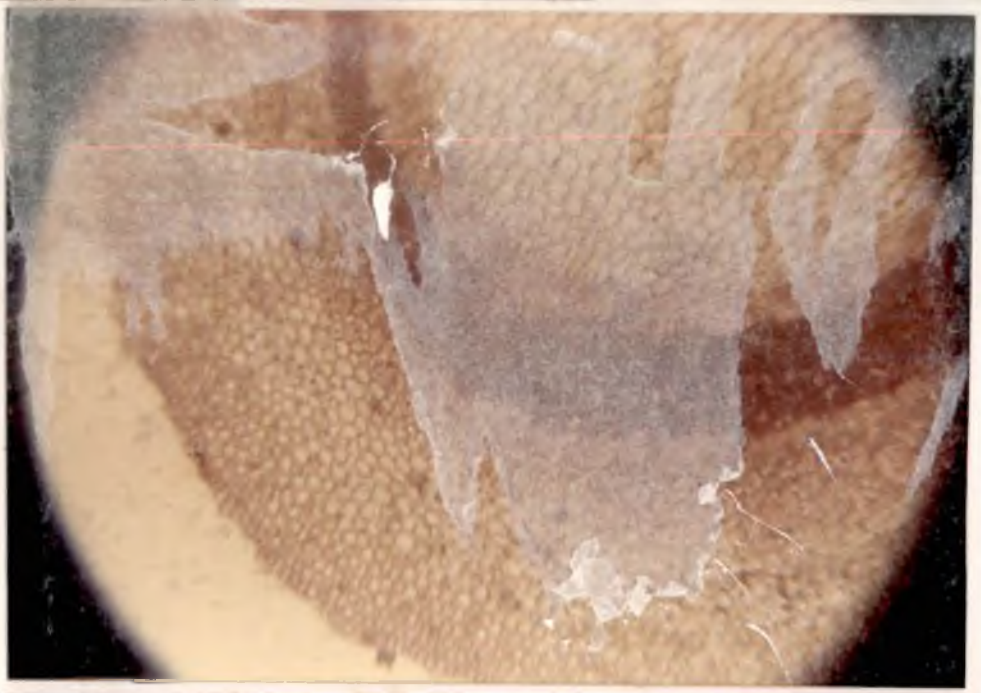
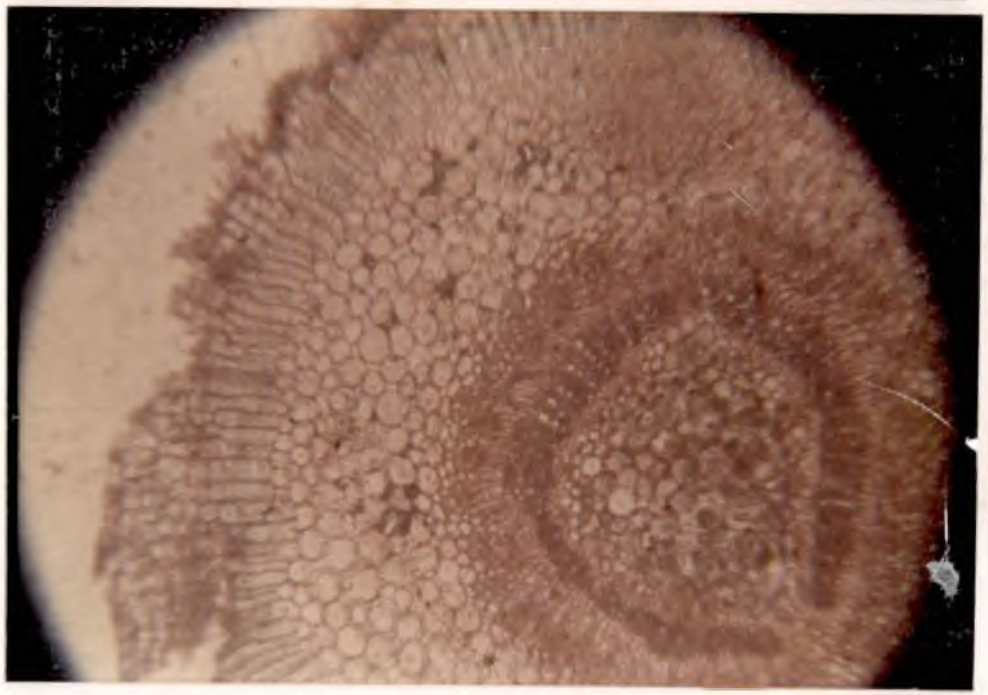


Plate 9. R. serpentina - C.S. of Root (X 50)

Plate 10. R. serpentina - C.S. of Stem (X 50)

Plate 11. R. serpentina - C.S. of Leaf (X 50)





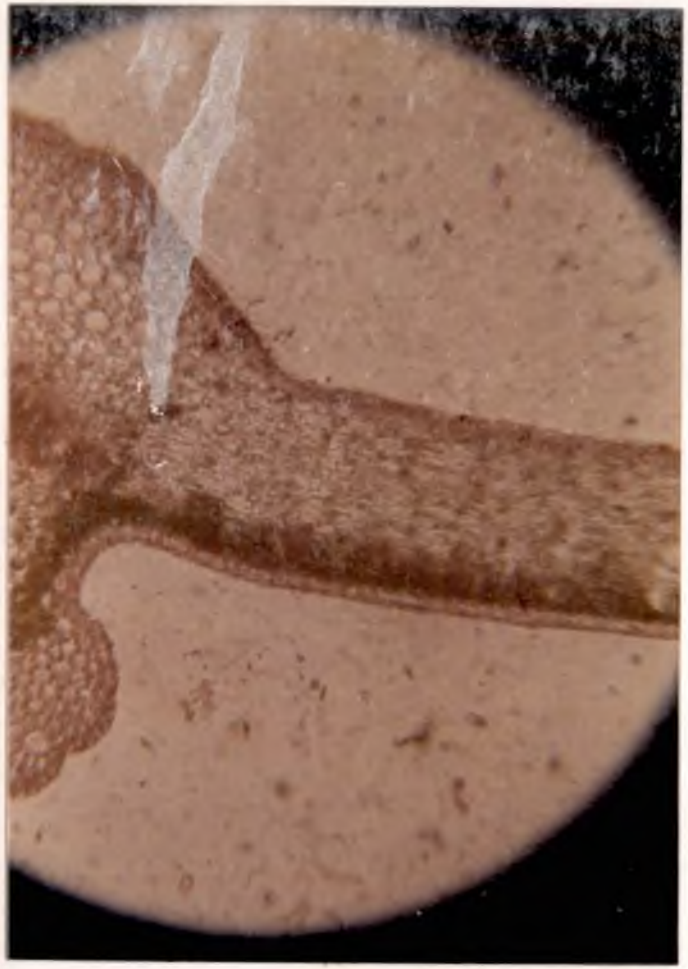


Plate 12. R. densiflora - C.S. of Root (X 50)

Plate 13. R. densiflora - C.S. of Stem (X 50)

Plate 14. R. densiflora - C.S. of Leaf (X 50)

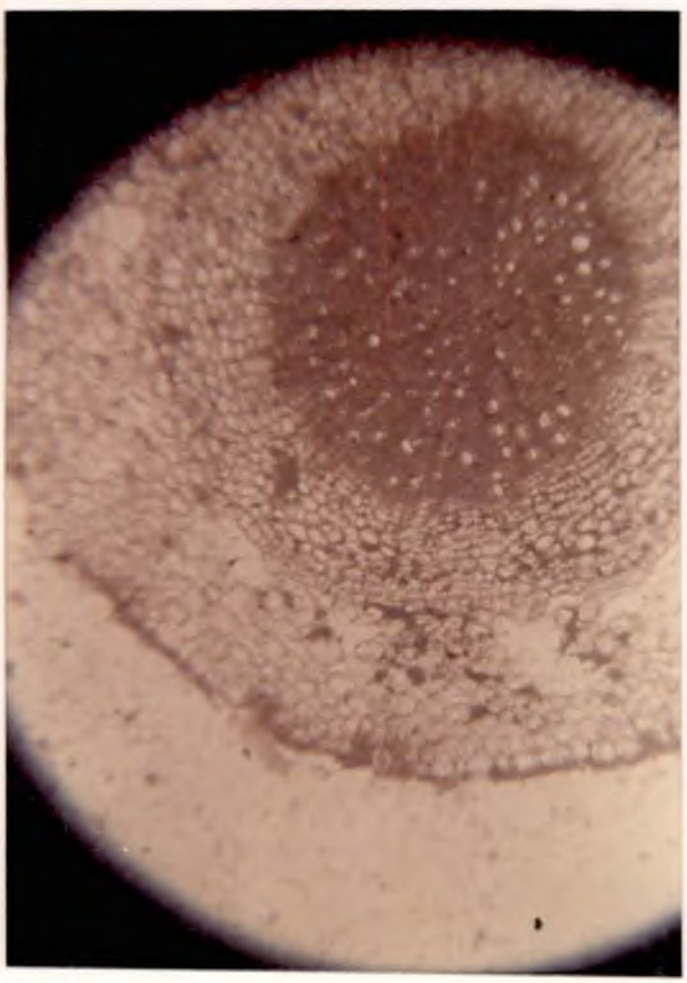
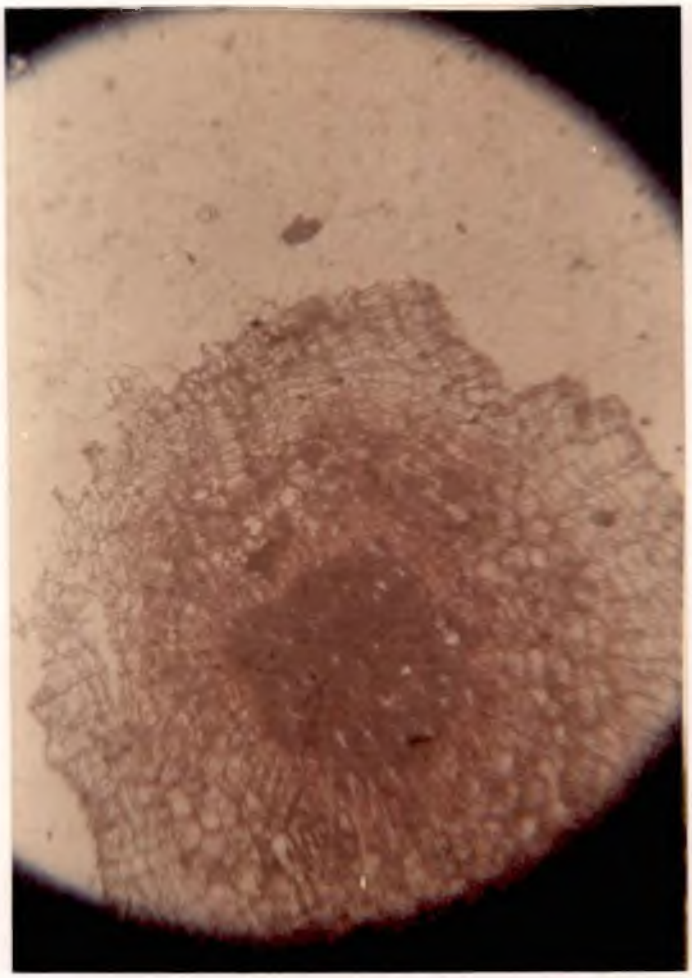


Plate 15. R. beddomei - C.S. of Root (X 50)

Plate 16. R. beddomei - C.S. of Stem (X 50)

Plate 17. R. beddomei - C.S. of Leaf (X 50)



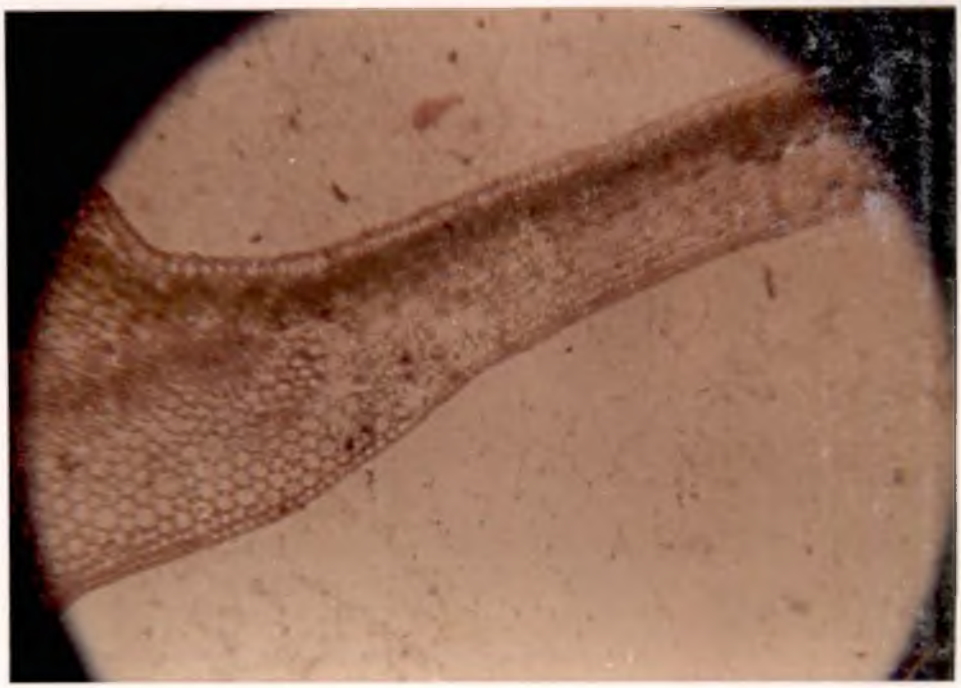
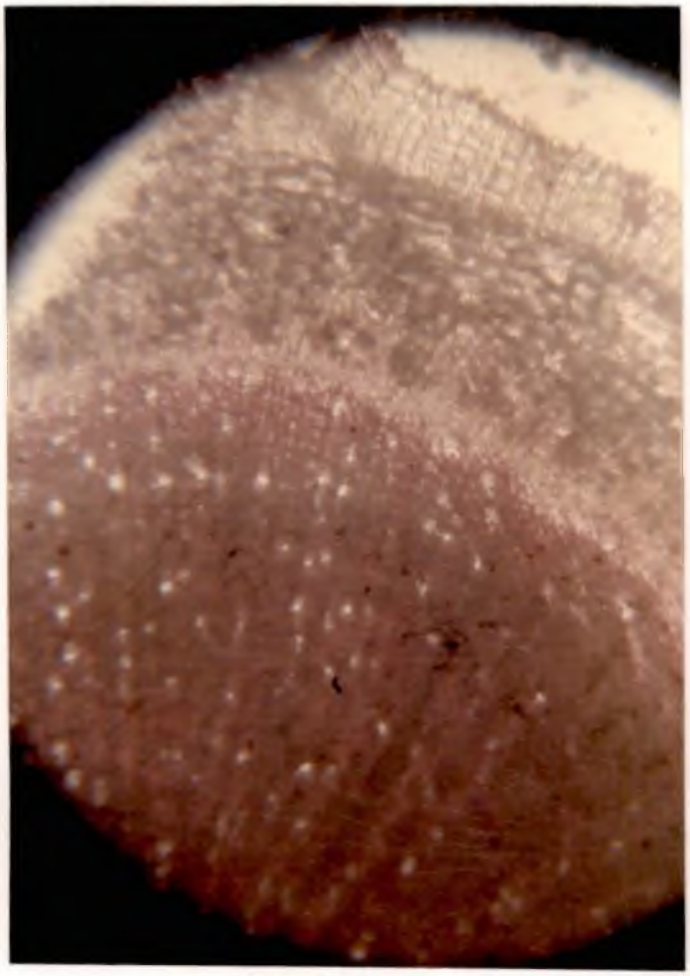
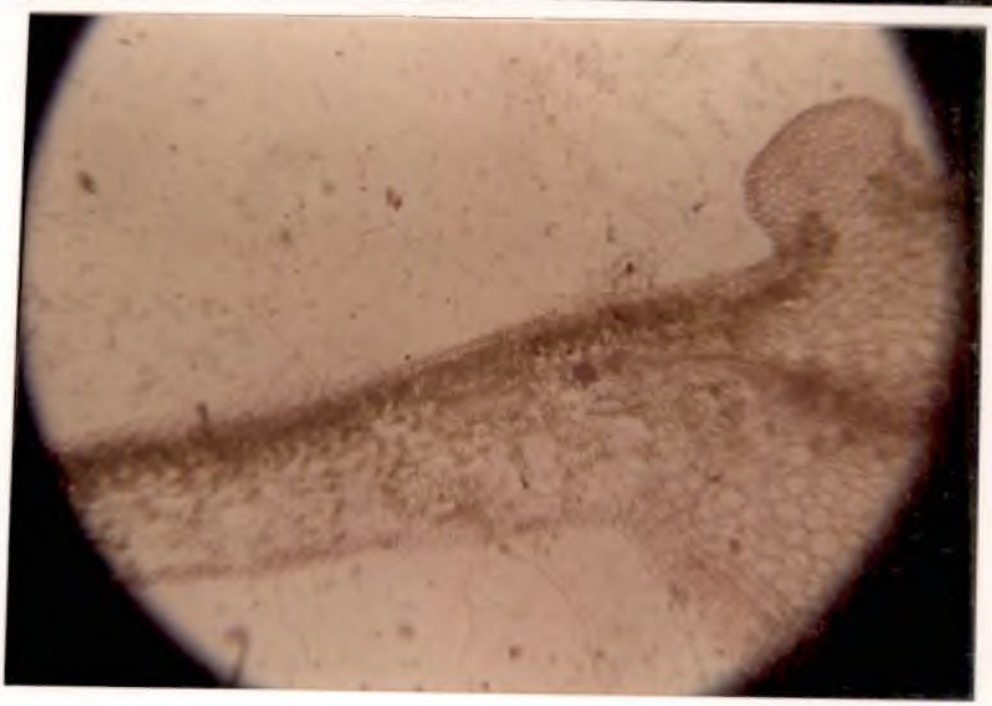


Plate 18. Accession E (Rauwolfia sp.) - C.S. of Root (X 50)

Plate 19. Accession E (Rauwolfia sp.) - C.S. of Stem (X 50)

Plate 20. Accession E (Rauwolfia sp.) - C.S. of Leaf (X 50)





Summary

SUMMARY

The study on the occurrence and distribution, variation of different morphological and anatomical characters and a preliminary comparative evaluation for the total alkaloids in Rauwolfia spp. was undertaken in the Department of Agricultural Botany, College of Horticulture, Kerala Agricultural University during 1991-93.

The salient results of the study are summarised below:

* Rauwolfia serpentina is widely distributed in Kerala. It prefers an open or partially shaded condition. The frequency of occurrence is low.

* R. tetraphylla is sparse in the forest lands. It occurs along the roadsides, waste lands and other places of human inhabitation. The distribution is wide and the frequency of occurrence is higher than that of R. serpentina.

* The two species R. densiflora and R. beddomei are in a state of almost near extinction in Kerala, while R. micrantha appears to have completely disappeared.

* Additional distinguishing features for the identification of different species of Rauwolfia in terms of morphology are suggested, among which the mean height, internodal length, shape and size of leaves, number of flowers per cyme, size and shape of sepals and corolla characters seems to be important.

* Wide variations in the viability, size and shape of pollen grains of different species of Rauwolfia are seen.

* There is high variability in the stomatal characteristics among the different species of Rauwolfia such as stomatal count, size of guard cells and length of stomata.

* Cross sections of leaf, stem and root show anatomical differences between the Rauwolfia spp. Presence of epidermal hairs and high chlorophyllous tissues is particular to R. tetraphylla.

* Chances for the existence of higher ploids of R. densiflora are likely. The total root alkaloid content varied widely in different species of Rauwolfia, the highest being in R. beddomei and lowest in R. tetraphylla.

* In Rauwolfia spp. the higher ploidy may have some role in the higher root alkaloid content, compared to their lower ploids.

* Total root alkaloid content in R. serpentina and R. tetraphylla varied with geographic locations, under Kerala conditions.

* The conditions suited for the higher root alkaloid production in R. serpentina may not be favourable to the root alkaloid production in R. tetraphylla.

* Chloroform extract and total chlorophyll content of the aerial parts varied among different species and ecotypes of Rauwolfia.

* Total crude alkaloid content in the roots and chloroform extract of the aerial parts, in Rauwolfia spp. are negatively correlated, with a correlation coefficient of -0.477.

* The regression equation

"Total crude alkaloids (roots) = 2.047 - 0.106 x chloroform extract of the aerial parts"

is formulated.

* Total crude alkaloid content of the roots and chlorophyll content of the aerial parts are negatively correlated, with a correlation coefficient of -0.697.

* The regression equation

"Total crude alkaloids (roots) = 2.304 - 1.434 x chlorophyll content of the aerial parts"

is formulated.

References

REFERENCES

Ahluwalia, K.S. 1963. Observations on cultivation and alkaloidal content of Rauwolfia serpentina (L.) Benth. ex Kurz in Saurashtra. Indian For. **89**:373-379

*Akram, M. and Ilahi, I. 1985. In vitro propagation of Rauwolfia serpentina through stem tissue. Pak. J. Sci. Ind. Res. **28**(6):412-416

*Akram, M., Ilahi, I. and Mirza, M.A. 1990.In vitro regeneration and yield transfer of Rauwolfia plants. Pak. J. Sci. Ind. Res. **33**(7):270-274

Ammal, J.E.K. 1962. Tetraploidy in Rauwolfia serpentina Benth. Curr. Sci. **31**(12):520-521

Amar, M.N. and Court, W.E. 1980. Leaf alkaloid of Rauwolfia vomitoria. Phytochem. **19**(8):1833-1836

Amar, M.A. and Court, W.E. 1981. Alkaloids of Rauwolfia nitida root bark. Phytochem. **20**(11):2569-2573

*Antipova, E.A., Nikolaeva, L.A. and Badzhelidze, A.S.H. 1988. Comparative characterestics of alkaloid content and composition of some Rauwolfia (L.) spices. Rastit. Resur. **24**(4): 575-578

Arnon, D.I. 1949. Plant Physiol. **24**:1

Babu, A. 1990. Flora of Malappuram district. Ph.D. thesis, University of Calicut, p.406-423

Badhuri, P.N. and Biswas, P.K. 1965. Increasing root yield of Rauwolfia serpentina by colchiploidy. Sci. Cult. **31**(4):197-199

Badhwar, R.L., Beri, R.M., Karira, G.V. and Ramaswamy, S. 1956. The wondor drug of India - Sarpagandha. Indian For. **81**(4): 258-268

Badhwar, R.L., Beri, R.M., Karira, G.V. and Ramaswamy, S. 1957. Rauwolfia serpentina - further studies on methods of propagation and their effect on root production. Indian Forest Leaflet. **158**:1-7

Badhwar, R.L., Beri, R.M., Karira, G.V. and Ramaswami, S. 1963. Rauwolfia serpentina - studies on optimum methods of propagation and their effect on production and alkaloidal content of roots. Indian For. **89**(11):729-735

Bajpai, M.P. and Sharma, V.N. 1956. Alkaloids of Rauwolfia serpentina. Indian J. Pharm. **18**:199

Banerjee, N. and Sharma, A.K. 1989. Chromosome constitution and alkaloid content in Rauwolfia (L.) (Apocyanaceae). Cytologia **54**(4):723-728

Bedi, Y.S. and Gill, B.S. 1982. An interchange heterozygote in Rauwolfia serpentina. Sci. Cult. **48**(4):152-154

*Belem, P.M.L., Rocha, A.F., Quetinleclervcq, J., Wantera, J.N. and Agnenot, L. 1988. Alkaloids of Rauwolfia sellowii from Amazonia. Fitoterapia **59**(5):429

Bhagat, N.R., Mital, S.P. and Hardar, M.V. 1980. Variation in Rauwolfia serpentina genetic resources in India. Euphytica **29**(3):747-750

Bhaskaran, U.P. 1964. Investigations on the effect of propagation materials and NPK on growth, yield and alkaloid content of Rauwolfia serpentina Benth. M.Sc. Thesis. Division of Agronomy, Agriculture College and Research Institute, Vellayani. p.1-75

Biswas, K. 1955. Cultivation of Rauwolfia serpentina in West Bengal. Indian J. Pharm. Sci. **18**(6):227-232

*Biswas, P.K. and Bhaduri, P.N. 1975. Induced tetraploidy in Rauwolfia serpentina Benth. and its propagation. Abstract Paper of National Symposium on Advanced Development, Production and Utilisation of Medicinal and Aromatic Plants, CIMPO, Lucknow.

Chadha, M.S. 1983. In vitro cultures of some medicinal plants for the formation of secondary metabolites. Proceedings of International Symposium on Medicinal and Aromatic Plants, CIMAP, Lucknow, India. p.170-180

Chandra, V. 1956. Studies on root development of Rauwolfia serpentina. J. Sci. Indus. Res. **14c**:36

Chatterjee, A. and Ray, A.B. 1962. Recent developments in the chemistry of indole alkaloids from Rauwolfia serpentina Benth. J. Sci. Indus. Res. **21(A)**:515-527

Chatterjee, S.K., Nandi, R.P., Bharathi, P., Yonjan, B. and Yonzon, M.K. 1988. Improvement studies on some alkaloid yielding medicinal plants. Acta Horticulturae. **188(A)**:39-46

*Chaturvedi, H.C., Sharma, M., Sharma, A.K. and Sane, P.V. 1990. Conservation of plant genetic resources through excised root culture. Conservation of Plant Genetic Resources through in vitro methods. Proceedings of International Workshop on tissue culture for conservation of Biodiversity and Plant Genetic resources, MNCPGR/CSC, Kaulalampur

*Cheng, C.K. and Liang, C. 1981. Callus culture of the three well known Chineese herbs and their medicinal content. Proceedings of Symposium on Plant Tissue Culture. London, U.K. p.469-479

Cook, T. 1905. The flora of the presidency of Bombay. Vol.2. (Reprint 1967), Botanical Survey of India, Culcutta, p.188-190

Cromwell, B.T. 1955. The alkaloids. Modern Methods of Plant Analysis Vol.IV, ed. K. Paech and M.V. Tracy, Springler Verlag, Berlin. p.367-374

CSIR. 1969. The wealth of India (Raw Materials) Vol.VIII. ed. Krishnamoorthy et al., Publication and Information Directorate, New Delhi. p.376-391

Dnyansagar, V.R. and Torne, S.G. 1967. Mitosis and Karyotype analysis of Rauwolfia serpentina. J. Biol. Sci. **10**(1-2):7-13

Dnyansagar, V.R. and Torne, S.G. 1969. Meiotic analysis in Rauwolfia serpentina (L.) Benth. ex. Kurz. Proceedings of National Institute of Science, India, Part B, Biological Sciences, **35**(5):368-376

Dutta, A.C. 1971. A Class Book of Botany. 14th Edn. Oxford University Press, London. p.27-137

Dutta, P.K. 1963. Viability of seed of Rauwolfia serpentina in storage. Sci. Cult. **29**(3):148-149

Dutta, P.K., Chaudhari, S.B. and Rao, P.R. 1962. Germination and chemical composition of Rauwolfia serpentina seeds. Indian J. Pharm. **24**(3):61-63

Dutta, P.K., Chopra, I.C. and Kapoor, L.D. 1963. Cultivation of Rauwolfia serpentina in India. Econ. Bot. **17**(4):243-251

*Endre, S., Suda, S., Takayama, H., Aimi, N., Shin-ichiro, S. and Stockigt, J. 1992. Isolation, identification and chemical synthesis of 6-alpha-hydroxyrau - macline - a novel alkaloid from cultivated Rauwolfia serpentina cells. Planta Medica. **58**(5):410-412

*Fuents, V., Rodriguez, N., Rodriguez, C. and Ranos, R. 1988. Screening of 51 medicinal species for salinity tolerance. Agrotecnia de cuba. **20**(1):1-6

Gamble, J.S. 1921. Flora of the presidency of Madras. Vol.II (Reprint 1986), Bishen Singh, Mahendra Pal Singh, Dehradun. p.806-808

Gauniyal, A.K., Kumar, A. and Virmani, O.P. 1988. Rauwolfia serpentina - A review. Curr. Res. Med. Arom. Pl. **10**(3): 113-137

*Gorodnyansskaya, L.M., Puchinina, T.N. and Vollosorich, A.G. 1984. Histochemical study of Rauwolfia serpentina Benth. Tissue culture. Rastit. Resur. **20**(2):218-224

Grill, E., Thumann, J., Winnacker, E.L. and Zenk, M.H. 1988. Induction of heavy metal binding phytochelatins by inoculation of cell cultures in standard media. Pl. Cell Rep. 7(6): 375-378

Guiller, J.T. and Chenieux, J.C. 1991. Somatic embryogenesis from leaf protoplasts of Rauwolfia vomitoria shoot cultures. Pl. Cell Rep. 10(2):102-105

*Gundlach, H., Muller, M.J., Kutchan, T.M. and Zenk, M.H. 1992. Jasmonic acid is a signal transducer in elicitor induced plant cell cultures. Proceedings of National Acadamy, USA. 89(6):2389-2393

Gupta, R. 1972. Raise Rauwolfia for export. Indian Farming 21(11): 9-12

Gupta, V. and Lamb, L.C. 1985. Developmental anatomy of the fruit in Rauwolfia serpentina. J. Sci. Res. Bhopal 7(2):103-106

Hamp, N. and Zenk, M.H. 1988. Homogeneous strictosidine synthase from cell suspension cultures of Rauwolfia serpentina. Phytochem. 27(12):3811-3815

*Hedayatullah, S. 1959. Culture and propagation of Rauwolfia serpentina Benth. in East Pakistan. Pak. J. Sci. Indus. Res. 2(2-3):116-122

Homji, V.M.M. 1965. Scope of Rauwolfia in Pondicherry. Indian Farming 15(4):14

Hooker, J.D. 1882. The flora of British India, Vol.II. Ash Ford Kent. p.632

*Huq, S., Milah, M.A.W. and Nada, M.K. 1986. Effect of Potassium Naphthenate on physiological and biochemical characteristics of Rauwolfia serpentina. Bangladesh J. Sci. Indus. Res. **21**(1-4):154-158

*Ilahi, I. and Akram, M. 1987. In vitro propagation of Rauvolfia serpentina plants through lateral bud culture. Pak. J. Agric. Res. **8**(2):204-210

*Ilahi, I. and Mehmood. 1987. Root callus cultures of Rauvolfia serpentina Benth. Pak. J. Sci. Ind. Res. **30**(3):224-229

*Ilahi, I., Akram, M. and Kvaur, L. 1988. In vitro studies on Rauwolfia for mass propagation and alkaloid synthesis. Pak. J. Sci. Indus. Res. **31**(2):114-117

*Kaukhova, I.E., Vollosovich, A.G. and Tsyganov, V.A. 1981. Selection of nutrient medium for deep tissue culture of Rauvolfia serpentina Benth. Rastit. Resur. **17**(2):217-224

*Kaukhova, I.E., Tsyganov, V.A. and Vollosovich, A.G. 1984. Surface culture of Rauvolfia serpentina Benth. tissue on liquid nutrient media. Rastit. Resur. **20**(3):412-415

Kaul, K.N. 1956. Observations on the natural occurance and cultural methods of Rauvolfia serpentina Benth. Indian J. Pharm. **18**:127-131

Kirthikar, K.R. and Basu, B.D. 1975. Indian Medicinal Plants. Vol.II, Bishen Singh Mahendra Pal Singh, New Delhi. p.1549-1552

*Krishnan, R., Chandra Vardara, M.V., Mohankumar, G.N. and Ramachandran, P.R. 1985. Effect of induced autotetraploidy on alkaloid content and root weight of Catharanthus roseus (L.). Herba. Hung. **24**(1):43-51

Kuriakkose, K.P. 1992. Bahurashtrakkuthaka, Mathrubhumi **35**(70):8

Maheswari, D.H., Naidu, K.C. and Girija, A.R. 1982. Report on seed germination of Rauvolfia Benth. Geobios **9**(4):191-192

Maheswari, S.K., Dahatonde, B.N., Yadav, S. and Gangrade, S.K. 1985. Intercropping of Rauwolfia serpentina for higher monetary returns. Indian J. agric. Sci. **55**(5):332-340

Maheswari, S.K., Sharma, O.P., Gangrade, S.K. and Thrivedi, K.C. 1991. Irrigation schedule for Sarpagandha (Rauvolfia serpentina) in shallow Black soil. Indian J. agric. Sci. **61**(3):169-171

Maheswari, S.K., Trivedi, K.C., Asthang, O.P. and Gangrade, S.K. 1984. Rauvolfia - a remunerative crop for Western Madhya Pradesh. Indian Hort. **28**(4):43-44

Maheswari, S.K., Yadav, S., Gangrade, S.K. and Trivedi, K.C. 1988. Effect of fertilizers on growth, root and alkaloid yield of Rauvolfia (R. serpentina). Indian J. agric. Sci. **58**(6):487-488

*Manilov, S.E., Komov, V.P., Kirillova, N.V., Strelkova, M.A., Samolin, Yu A., Samolin, L., Chernoborodova, L.S. and Nicolaeva, I.A. 1988. Chromatographic isolation of primary and secondary metabolites from plant cell cultures. J. Chromatography **440**:53-64

*Martinez, J.A., Gomez, C., Santana, T. and Velez, H. 1989a. Alkaloids from Rauwolfia cubana stem bark. Planta Medica. **55**(3):283-285

Martinez, J.A., Velez, H. and Santana, T. 1989b. An alkaloid from two Rauwolfia spp. Phytochem. **28**(3):961-962

Mathur, C.M. 1961. Trials with cultivation of plants of medicinal value in Rajasthan. Indian For. **87**(2):128

Mathur, A., Mathur, A.K., Kukreja, A.K., Ahuja, P.S. and Thyagi, B.R. 1987. Establishment and multiplication of colchi autotetraploids of Rauwolfia serpentina (L.) Benth. ex. Kurz. through tissue culture. Plant Cell Tissue Organ Culture **10**: 129-134

Misra, A. 1992. Nutritional requirements of some important medicinal and aromatic plants for higher productivity. Curr. Res. Med. Arom. Pl. **14**(2):121-132

Mitra, C.C. 1969. In vitro growth of excised roots of Rauvoltia serpentina in continuous culture. Indian J. Exp. Biol. **6**(4): 230-231

Monachino, J. 1954. Rauvolfia serpentina, it's history, botany and medical use. Econ. Bot. **8**(4):349-365

Nair, S.L. 1956. Experimental propagation and culture of Rauvolfia serpentina by seeds. Indian J. Pharm. **18**(4):125-126

Nandi, R.P. and Chatterjee, S.K. 1975. Effect of NPK on growth and alkaloid formation in Rauvolfia serpentina Benth. ex. Kurz. Geobios **2**(1):13-14

*Nikolaeva, L.A. and Vollosorich, A.G. 1972. Effect of auxins on the morphology and organogenesis of tissue culture of some medicinal plants and biosynthesis of tropane and indole alkaloids. Rastit. Resur. **8**(2):188-192

*Nikolaeva, L.A., Antipova, E.A., Kau, N.K., Gorodyanskaya, L.M. and Nikolova, I.G. 1990. Ajmaline containing Rauwolfia (L.) species. Rastit. Resur. **26**(2):219-225

*Nikolaeva, L.A., Shimolina, L.L. and Smirnova, I.I. 1988. Chemical composition in Tissue Culture of Rauwolfia serpentina Benth. Rastit. Resur. **24**(4):479-484

*Nikolaeva, L.A., Vollosovich, A.G. and Blinova, K.F. 1977. Dynamics of alkaloid accumulation and biomass in tissue culture suspension of Rauwolfia serpentina Benth. Rastit. Resur. **8**(1):75-78

*Nikolaeva, L.A., Vollosovich, A.G., Kaukhova, I.E. and Tsyganov, V.A. 1978. Growth of Rauwolfia serpentina tissues in suspension culture - I - factors influencing cell aggregation. Rastit. Resur. **14**(3):369-402

*Ohta, S. and Yatazawa, M. 1979. Growth and alkaloid production in callus tissue of Rauwolfia serpentina. Agric. Biol. Chem. **43**(11):2297-2303

Oomachan, M. and Masih, S.K. 1991. Ethnobotanical and conservational aspects of medicinal plants of Madhya Pradesh. Indian J. Pure appl. Biol. **6**(1):39-44

Parkashi, S.C. and Achari, B. 1968. Rauwolfia alkaloids in retrospect. J. Sci. Indus. Res. **27**(2):58-69

Raghavan, R.S. 1957. Chromosome numbers in Indian medicinal plants. Proceedings of National Academy of Science. **45B**:294-298

Rajkhova, S. 1964. Cultivation methods of R. serpentina and Root yield. Indian For. **90**(2):125-126

Roja, P.C., Benjamin, B.D., Hebble, M.R., Patankar, A.V. and Sipahimalani, A.T. 1990. The effect of plant growth regulators and nutrient conditions on growth and alkaloid production in Multiple Shoot Cultures of Rauwolfia serpentina. Phytotherapy Res. **4**(2):49-52

Roja, P.C., Sipahimalani, A.T., Heble, M.R. and Chadha, M.S. 1987. Multiple shoot cultures of Rauvolfia serpentina - growth and alkaloid production. J. Natural Products. **50**(5): 872-875

*Ruyter, C.M., Akram, M., Ilahi, I. and Stockigt, J. 1991. Investigations of the alkaloid content of Rauwolfia serpentina roots from regenerated plants. Planta Medica. **57**(4):328-330

*Ruyter, C.M., Schubel, H. and Stockigt, J. 1988. Novel glucoalkaloids from Rauwolfia cell cultures - Acetylrauflucine and related glucosides. Zeitschrift. Naturfer. **73**(7-8):478-484

Sahu, B.N. 1969. Studies on Rauvolfia serpentina. I propagation and yield of roots. Prakruthi Utkal. Univ. J. Sci. **6**(2):45-50

Sahu, B.N. 1970. Intercropping Rauvolfia serpentina Benth. ex. Kurz. Indian J. Agron. **15**(3):229-235

Sahu, B.N. 1979. Rauvolfias: Botany and Agronomy Vol.I. Today and Tomorrow Printers and Publishers, New Delhi. pp.360

Sahu, B.N. 1983. Rauvolfias - Chemistry and Pharmacology Vol.II. Today and Tomarrow Printers and Publishers, New Delhi pp.595

Santapau, H. 1956. The botanical aspect of Rauwolfia serpentina Benth. Indian J. Pharm. **18**(4):117-118

Sarin, Y.K. 1982. Cultivation and Utilization Rauvolfia serpentina in India. Cultivation and Utilization of Medicinal Plants. ed. C.K.Atal and B.M.Kapur, RRL, CSIR, Jammu-Thavi. p.289-294

*Schmidt, D., Ruyter, C.M. and Stockigt, J. 1990. Entire enzymatic pathway to ajmaline and raucaffricine in Rauwolfia (Rauvolfia) serpentina cell cultures. Planta Medica **56**(6):601-602

Schubel, H. and Stockigt, J. 1984. RLCC - isolation of Raucaffricine from its most efficient source - cell suspension cultures of Rauwolfia serpentina Benth. Pl. Cell Rep. **3**:72-74

Schutte, H.R. 1991. Secondary plant substances monoterpene indole alkaloids. Progress in Bot. **52**:84-96

Sethi, K.L., Kazim, M., Kidwar, M.A. and Mital, S.P. 1991. Variation of chemobotanical charactors in the indegenous collection of Rauvolfia serpentina. Indian J. Genet. Pl. Breed. **51**(1): 134-138

Sharma, N. and Chandel, K.P.S. 1992. Low temperature storage of Rauvolfia serpentina Benth. ex. Kurz. Pl. Cell Rep. **11**(4): 200-203

*Shchizelskii, O.A., Vollosovich, A.G. and Yashchenko, V.K. 1974. Effect of Copper and Magnesium on biosynthesis of alkaloids on the isolated tissue culture of Rauwolfia serpentina Benth. Rastit. Resur. **10**(4):563-567

*Shimolina, L.L., Astakhova, T.V., Nikolaeva, L.A. and Minima, S.A. 1984. Quantitative estimation of total alkaloids in Tissue Culture of Rauvolfia serpentina Benth. Rastit. Resur. **20**(1):137-141

Singh, J. 1964. Trials on cultivation of some medicinal and aromatic plants in Punjab. Indian For. **90**(8):500-510.

Singh, I.S. and Singh, C.P. 1987. Response of excised roots of Rauvolfia serpentina Benth. to growth regulatory substances. Acta Horticulturae **208**:141-155

Sobti, S.N., Abrol, B.K., Handa, K.L. and Chopra, I.C. 1959. Cultivation of Rauvolfia serpentina in Jammu and Kashmir. Indian For. **85**:233-236

Sobti, S.N., Handa, K.L. and Chopra, I.C. 1957. Propagation of Rauvolfia serpentina in Jammu and Kashmir. J. Sci. Indus. Res. **16A**(6):268-269

*Solovyan, V.T., Kostenyuk, I.A. and Kunakh, V.A. 1987. Genome changes in Rauwolfia serpentina Benth. cells cultivated in vitro. Genetika. **23**(7):1200-1208

Srivasthava, A.K. 1978. Rauvolfia serpentina and its cultivation in India. CIMAP Farm Bull. **10**:1-17

Sudhadevi, P.K. 1992. Ethnobotany of medicinal plants used by Tribes of Kerala. Ph.D. Thesis. College of Horticulture, Kerala Agricultural University, Vellanikkara, p.103-172

Sulochana, C.B. 1959. Indian species of Rauvolfia serpentina. J. Indian Bot. Soc. **18**(4):575-594

Tapadar, R.N.N. and Sen, N.K. 1960. Cytotaxonomical studies on the economic plants of the family Apocyanaceae. Cytologia **12**:306-307

Tapadar, R.N.N. 1963. Studies in induced tetraploids of the family Apocyanaceae. 1. Rauwolfia serpentina Benth. Cytologia **28**(3):229-241

Tapadar, R.N.N. 1964. Cytotaxonomic studies in Apocyanaceae and delineation of the different evolutionary tendencies operating within the family. Cytologia **17**:103-108

Vasishta, P.C. 1974. Taxonomy of Angiosperms. R. Chand and Co., Delhi. p.57-112

Verma, N. 1969. Scope for the cultivation of medicinal and aromatic plants in the province of Bihar. Indian oil soap J. **34**(9): 184-186

Verma, N. 1970. Opportunities for the large scale cultivation of aromatic and medicinal plants in the province of Uttar Pradesh (India). Flavour India. **1**(5):332-334

*Vollosovich, A.G. and Butenko, R.G. 1968. Culture of Rauwolfia serpentina tissue as a producer of alkaloids. Kult Izoliro-vannykh Organov Tkanei Kletok Rast Tr Vsu Kanf. Ist. p.253-257

*Vollosovich, A.G., Nikolaeva, L.A., Pozdnyakova, N.N. and Puchinina, T.N. 1977. A method for quantitative determinat-ion of the indoline alkaloid group in tissue cultures of Rauvolfia serpentina Benth. Rastit. Resur. **13**(1):127-132

*Vollosovich, A.G., Puchinina, T.N. and Listunova, N.A. 1982. Opti-misation of macrosalt composition for Rauvolfia serpentina tissue culture. Rastit. Resur. **18**(2):239-243

* Vollosovich, A.G. and Tsarenko, N.Y. 1968. Quantitative content of serpentine alkaloid in stem tissue culture of Rauwolfia serpentina. Tr. Leningrad Khim Farm Inst. **26**:218-221

* Vollosovich, N.E. and Vollosovich, A.G. 1982. The use of industrial waste for Rauvolfia serpentina tissue cultivation. Rastit. Resur. **18**(3):353-357

Witham, F.H., Blades, D.F. and Delvin, R.M. 1971. Experiments in Plant Physiology. Van Nastrand, New York. p.245

Yamamoto, O. and Yamada, Y. 1986. Production of reserpine and its optimisation in cultured Rauwolfia serpentina Benth. cells. Pl. Cell Rep. 5:50-53

* Not seen in original

COLLECTION, CATALOGUING AND EVALUATION OF *Rauwolfia spp.*

BY

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ABSTRACT OF A THESIS

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ABSTRACT

A study on 'Collection, cataloguing and evaluation of Rauwolfia spp' was conducted in the Department of Agricultural Botany, College of Horticulture, Vellanikkara during 1991-93, with the objectives of understanding the distribution pattern of various species of Rauwolfia in Kerala, detailed descriptive study of the morphological and anatomical characters of the different species of Rauwolfia and a preliminary comparative evaluation study for the total alkaloids in roots and the chlorophyll content of aerial parts.

Survey of 10 geographic locations of Kerala from North to South was conducted and four species of Rauwolfia were collected. Nine ecotypes of R. serpentina and four ecotypes of R. tetraphylla were also collected.

Different species were compared based on 60 morphological characters, 15 anatomical characters and three characters of pollen grains. Evaluation for total root alkaloids was done using chloroform as solvent and determination of total chlorophyll content was done using acetone as solvent.

The study on distribution aspects showed that R. serpentina was widely distributed in Kerala but the frequency of occurrence was low, while R. tetraphylla was widely distributed in non-forest areas only, with a higher frequency of occurrence. R. densiflora

and R. beddomei are in a state of near extinction while R. micrantha was almost disappeared from Kerala.

Morphological and anatomical characters and the morphology and viability of pollen grains showed wide variability among different species of Rauwolfia. Characters in addition to that available in the literature, for identifying the four species of Rauwolfia are suggested. It is seen that chances are there for the occurrence of higher ploids of the same species having higher alkaloid content, in Rauwolfia.

Total alkaloid content of roots, chloroform extract and total chlorophyll content of aerial parts varied with different species and ecotypes of Rauwolfia. The conditions for the higher root alkaloid production in R. serpentina may not be favourable for the alkaloid production in R. tetraphylla.

The chloroform extract and total chlorophyll content of aerial parts were negatively correlated to the total root alkaloid content in all the species of Rauwolfia.

The relationship between these was found to be

"Total root alkaloid content = 2.047 - 0.016 x chloroform extract of aerial parts

= 2.304 - 1.434 x total chlorophyll content of aerial parts"

This relationship can be effectively utilized in the estimation of root alkaloids, in Rauwolfia spp., even at the early stages of growth, without uprooting the plants.