Analysis of growth, flowering and quality in 'Koduveli', *Plumbago* spp.

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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DECLARATION

I hereby declare that the thesis entitled **'Analysis of growth, flowering and quality in 'Koduveli', *Plumbago* spp.'** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title of any other University or Society.

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JALAJA S. MENON

Dedicated to my
Family

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LIST OF ABBREVIATIONS

CGR	Crop Growth Rate
CSIR	Centre for Scientific and Industrial Research
HDPE	High Density Poly Ethylene
LAI	Leaf Area Index
LAR	Leaf Area Ratio
LDPE	Low Density Poly Ethylene
LGR	Linear Growth Rate
LWR	Leaf Weight Ratio
MAP	Months After Planting
MAS	Months After Storage
NAR	Net Assimilation Rate
Rf	Retention factor
RGR	Relative Growth Rate
TDM	Total dry Matter
TLC	Thin Layer Chromatography

Introduction

INTRODUCTION

Herbal medicines have occupied an important position in India and other countries of the world. After having tapped the immense advantage of synthetic chemistry, the situation has now changed and the idea of use of nature and natural products arose. More than 60 per cent of Indian population still depend on ayurveda for the treatment of common diseases (Nair *et al.*,1992). Plant and plant products are the main sources for various ayurvedic preparations. Nearly 2000 plant species are used in ancient and modern system of medicines (Chatterjee and Nandi, 1983).

The demand for traditional drug plants in India and abroad is ever increasing. In fact, among the developing countries, India occupies the foremost position in the export of raw drugs. Shortage in the availability of crude drugs of good quality is projected as the major limitation for the manufacture of medicines to meet the increasing demands. In the case of wild collections, the source, stage of growth, time of collection, period of storage etc. of the drug, which are vital factors influencing the effectiveness of the medicines are not known. In order to get a homogenous and reproducible quality of herbal medicines, commercially grown plants harvested at suggested period has to be used. So, to cope up with the increasing demand and to provide good quality raw material at required level, there is an urgent need to scale up production by undertaking commercial cultivation.

In India, two species of *Plumbago* are being utilised. *Plumbago rosea*, rose coloured leadwort or *Chethikoduveli* or *Citraka* is being utilised in ayurvedic preparations of South Indians. *Plumbago zeylanica,* white flowered leadwort or *Vellakoduveli* is being utilised in ayurvedic preparations of North Indians. The freshly harvested plumpy roots after curing and drying are used for ayurvedic preparations. The roots are digestive stimulants. It is pungent, astringent, diuretic, germicidal, vescicant and abortifacient. It overcomes flatulence, oedema, piles, coughs, worms, diseases due to *vata* and *kapha* predominance and haemorrhoidal anal inflammation. The drug also cures enlargement of abdomen, anaemia, diabetes, leucoderma, leprosy, diarrhoea, dyspepsia and elephantiasis (Sivarajan and Balachandran, 1994). Root is the officinal part and it enters into the composition of preparations like *Citrakasavam, Citrakachurnam, Dasamularistam, Gulgulutiktakam, Yogarajachurnam* etc.

P. zeylanica is also used in veterinary medicines against stomach troubles (Sikawar, 1994).

The annual demand of plumbago in Kerala is to the tune of 57 tonnes (Ramanathan, 1993). As in any other medicinal plant, the problems in popularising this crop are lack of awareness, insufficient land availability and lack of technical know-how especially crop management and post harvest handling. To standardise the agro-techniques, it is essential to have a thorough understanding of the basic nature of the plant, its adaptation to different seasons, changes in quality, post harvest handling practices etc.

In order to undertake crop improvement programmes, a basic knowledge of the pattern of flowering, floral biology and breeding behaviour is essential. Studies on this line are very few in this crop.

The active principle in plumbago is referred to as "plumbagin" (2-methyl-5-hydroxy-1, 4-naphthoquinone). It is obtained as golden yellow needle shaped crystals (Chopra *et al.*, 1958). It is known to impart antifungal (Ito *et al.*, 1995), antimicrobial (Gonclaves *et al.*, 1972), abortifacient (Goel *et al.*, 1987), antifertility (Chowdhury *et al.*, 1982), anticoagulant (Santhakumari *et al.*, 1978), antiviral (Singh *et al.*, 1983) and insecticidal (Rao and Gujar, 1995) properties. It is also effective in treatment of liver disorder (Gujar, 1990) and common warts (Pillai *et al.*, 1981).

In ayurvedic preparations *P. rosea* is recommended as an excellent remedy as digestive (*Deepanam*) and stomachic (*Pachanam*). For these preparations, *P. rosea* roots are used only after adequate curing in lime water and drying (Pandey, 1960). Plumbagin is highly caustic and causes blisters on skin. With higher doses, plumbagin causes paralysis leading ultimately to death (CSIR, 1969). When this is the fact, importance of plumbagin in the above ayurvedic preparations is doubtful. It is presumed that plumbagin may be undergoing several biochemical changes during curing and drying which helps to impart stomachic and digestive properties. Sasidharan (1996) suggested the rationale behind processing of plumbago roots as removal of toxic phytosterol. The exact nature of changes is still unknown.

Under the above background, the present study was undertaken at College of Horticulture, Vellanikkara for the period from 1995 to 1998 with the objectives of analysing growth, physiological growth attributes, biochemical changes occurring during different growth phases, anatomical characters, reproductive behaviour and post harvest changes in quality of the two species of *Plumbago*.

Review of literature

REVIEW OF LITERATURE

Plumbago is generally known as *Citraka* in ayurveda. The two species recognised in Kerala are red flowered, *Plumbago rosea* and white flowered, *Plumbago zeylanica. P. zeylanica* occurs as wild species and *P. rosea* has never been recorded as wild. Roots of *P. rosea* are widely used in ayurvedic preparations in Kerala and roots of *P. zeylanica* are accepted by North Indians.

Plumbago has been domesticated only recently and as such, studies are limited. Therefore, an attempt has been made to review available literature on growth, quality, flowering and post harvest handling of the plants coming under the family Plumbaginaceae and some other related medicinal plants.

2.1. Growth, biomass production and yield as influenced by stages of harvest

At the Kerala Agricultural University, studies in *Plumbago rosea* have been initiated in 1990, when the need for taking up cultivation of crop was badly felt. Subha (1990) studied the effect of different spacing levels and type of propagules for realising maximum yield of root of plumbago. The results of her study revealed that the optimum spacing was 50 cm x 15 cm. Among the different types of cuttings tried, it was found that semi - hard wood cuttings were the best. The study also indicated the suitability of raising *Plumbago rosea* as an annual crop in Kerala. Subsequently, studies were undertaken by Menon (1994) in the same species to find out the best method of planting. Among the four methods of planting compared viz., ridge and furrow, flat bed, mound and pit followed by mound, it was found that the methods had no significant influence on biometric and root characters. The rooting percentage of two noded cuttings could be significantly improved by treating with IBA 500 ppm for 60 seconds. The results suggested that plumbago performs well under the shade of coconut as well and thus can be recommended as an intercrop in coconut gardens.

It is to be noted that the above studies have been restricted to a period of one year after planting. Under natural conditions, the plant continues to survive in the field for longer periods and thereby the optimum stage of harvest needs to be found out to realise the maximum returns.

The yield and quality of roots of plumbago vary according to the locality, growth and age of the plant, conditions of soil and season of year. The older the plant, greater is the active principle in roots (CSIR, 1969).

A critical evaluation of the results obtained in other root yielding medicinal plant species clearly shows a distinct correlation between morphological characters and yield at different stages of harvest. The general trend is that the root yield increases with age. In ayurvedic system, it is taken as a general principle that roots should be harvested in winter or summer (Gupta, 1901)

In *Glycyrrhiza glabra,* Shah *et al.* (1976) found that dry root recovery was considerably low when the plant was harvested twice at 15 and 27 MAP (3843 kg ha^{-1}). Their studies indicated that a single harvest at 27 MAP resulted in the highest yield of dry roots of 4545 kg ha^{-1}. Parikh (1993) reported that the rhizomes of this crop could be harvested in three to four years and it was recommended to harvest the crop in autumn after rains. However, Legha *et al.* (1995) suggested 2 ½ to 3 years for getting higher yields.

Studies conducted in *Costus speciosus* (Sarin *et al.*, 1977) showed significant influence of stage of harvest on yield. The maximum yield of green rhizome could be obtained at 6 MAP. Joseph (1983) studied the effect of harvest intervals on the yield and quality constituents of *Costus speciosus.* The results revealed that increase in crop duration significantly increased some of the rhizome and finger characters. Though the yield of green rhizome and diosgenin contents were higher at 6 MAP, dry matter content was low at this stage. Yields of dry rhizome and diosgenin were higher at 9 MAP due to high dry matter content. Quadratic response function showed 8 months to be the optimum stage of harvest for obtaining maximum yield of diosgenin per unit area.

Nybe (1978) obtained maximum yield of green ginger at 180 DAP. However, maximum dry ginger yield was obtained during the period between 210 – 225 DAP due to better dry recovery.

Karnick (1979) reported that the best season for harvesting vetiver coincided with the rainy season.

Pareek *et al.* (1981) were of the opinion that in *Catharanthus roseus* harvesting at 200 DAP gave better yields of leaves, stem and root. However, Krishnan (1995) suggested that the crop should be harvested at 6 MAP for getting maximum root yield and optimum alkaloid content.

Rajagopalan (1983) analysed the influence of harvest time on the growth and yield of *Kaempferia galanga* and reported that the crop harvested after six months was significantly superior to other treatments with respect to fresh and dry yields. It was also indicated that harvest interval did not significantly influence the volatile oil yield.

According to Soldati and Tanaka (1984), in *Panax ginseng* the major development in root weight occurred between fourth and fifth year of planting and they concluded that the best time for harvesting the crop was at the end of summer season of fifth year.

Granda *et al.* (1986) studied the foliage and root growth of *Rauvolfia tetraphylla* at bimonthly intervals from 8 to 36 MAP. The results indicated that foliage growth increased markedly during rainy season, and decreased during dry season. It was also indicated that root growth was less affected by seasonal changes. Alkaloid content was the highest in the leaves between March and May (up to 2 per cent) and lowest in July. Root alkaloid content was the highest in January to March (up to 4 per cent) and lowest between May and November. It was indicated that despite lower alkaloid concentration, roots harvested in July yielded 20 per cent more alkaloid than roots harvested in January due to higher total root weight in July. The alkaloid yields were the highest in plants harvested at 24 MAP.

Nayar(1992) studied the growth pattern of Jeevanti (*Holostemma annulare*) and he found that the plant produced maximum yield after 17 months of planting. The harvest time was fixed based on qualitative parameters also, when the roots contained 10 per cent protein as against 5.5 per cent protein contained in the market sample. In Jeevanti, Samuel *et al.* (1993) showed that yield of dry roots after 11 months of planting was 1450 kg ha^{-1}.

Meera (1994) studied the effect of different stages of harvest on growth and yield of Jeevanti. She found that the age of plant significantly influenced all biometric characters viz. vine length, branches/vine, diameter of vine and internodal length. These

characters showed a progressive increase with the duration of crop. The number of leaves, total leaf area/plant, fresh and dry weight of stem and leaf showed an increasing trend up to 12 months and there after declined. The yield parameters were significantly influenced by different stages of harvest. It was found that crop duration brought about a progressive increase in all the root characters and fresh and dry root yield ha^{-1}. The maximum root weight was realised at 18 months stage (4.14 t ha^{-1} of fresh yield and 1.56 t ha^{-1} of dry yield). The dry recovery and harvest index were also maximum at this stage.

Nigam and Kandalkar (1995) recommended to harvest *Wiithania somnifera* 150 to 170 days after sowing for getting higher root yield.

In *Piper longum,* Viswanathan (1995) observed that the maximum yield of 1000 kg ha^{-1} was obtained in third year of planting. But Ayisha (1997) found that the peak bearing stage in *P. longum* was at 17 months after planting after which there was a drastic reduction in yield.

Raj (1997) studied the growth pattern of *Baliospermum solanifolium* and *Sida rhombfolia* ssp. *retusa* under domestic environment. The results indicated that in respect of *B. solanifolium*, there was about 62 per cent increase in all growth parameters as compared to its wild environment. The total dry matter production was as high as 420.6 g and dry root yield 290.9 g per plant with a root shoot ratio of 1 : 0.45 and harvest index of 0.69 at 9 MAP. In *S. rhombifolia* ssp. *retusa,* plants grew vigorously with 93 per cent increase in plant height and total leaf area as compared to its wild environment. The biological yield recorded was 400.6 g and economic yield 55.4 g giving a root shoot ratio of 1: 6.23 and harvest index of 0.14 at 9 MAP.

Shina (1998) studied the influence of stages of harvest on growth and yield of *Hemidesmus indicus.* The results indicated that plant height, number of branches, leaf area and leaf area index were significantly higher during the seventh month. The rate of leaf production was found to be unaffected by different stages of harvest. The number of roots were found to increase with age and maximum value of 3.2 was obtained at eighth month. Harvesting at 8 months after planting resulted in maximum fresh root weight of 2.8 g and dry root yield of 0.94 g per plant. Harvest index decreased with age of plant

and maximum value was observed at 5 months after planting and minimum at 8 months after planting.

2.1.1. Active principles in roots of Plumbago

Plumbagin

The roots of *P. rosea* and *P. zeylanica* contain plumbagin, a naturally occurring naphthoquinone. (2 methyl, 5 hydroxy, 1, 4 naphthoquinone) (CSIR, 1969). Naturally occuring quinones have been found to have antiviral, antibacterial, antifungal and insecticidal properties. It is also used as purgative.

The basic quinone structure is that of an unsaturated cyclic diketone derived from a monocyclic or polycyclic aromatic hydrocarbon. Plumbagin is basically a naphthalene derivative, 1,4 naphthoquinone with methyl and hydroxy substituents. 1,4 naphthoquinones are most widespread and important as pigments (Britton, 1983). The characteristic chemical reaction of quinone is reversible reduction to the corresponding phenol. Many natural quinones have additional phenolic hydroxy substituents and are thus slightly acidic, consequently ionising and forming salts with alkaline solution. This property is especially pronounced in 2 hydroxy – 1,4 naphthoquinone which are vinylogous carboxylic acids. All natural quinones are solids and crystallise readily. Most are readily soluble in organic solvents, though glycosides and some carboxylic acids will dissolve in water. Those quinones that are also phenols or carboxylic acids are soluble in alkaline aqueous solution. The main absorption band of 1,4-naphthoquinone are 245, 257 and 335 nm. Hydroxy derivaties of quinone are capable of ionising under basic conditions and this results in considerable bathochromic shifts. The 1,4-naphthoquinones are widely distributed in nature and some of them are juglone (*Juglans regia*) and lawsone (*Lawsonia alba*) apart from plumbagin (*Plumbago* spp.) (Britton, 1983).

Other components

The roots are reported to contain other related compounds apart from plumbagin (up to 1.0 per cent). Dinda and Chel (1992) obtained from the petrol extract of aerial part of *P. indica*, a new naphthoquinone, 6- hydroxy plumbagin apart from four known compounds, plumbagin, sitosterol, stigmasterol and campesterol.

From the phenolic fraction of ethyl acetate extract of roots of *P.rosea,* Dinda *et al.* (1995) isolated a new binaphthoquinone, roseanone apart from droserone, elliptinone and zeylanone. The roots of *P. zeylanica* showed the presence of stigmasterol acetate, lupeol acetate, friedelinol, lupeol, lupanone, sitosterone, and stigmasterol, nonyl nonanate, nonyl and methyl dodec 7 enoate and hexzyl 2, 5, dihydroxy 6 methoxy benzoate. The compound 2,3,dimethyl 5 hydroxy 6 acetyl chromene was isolated for the first time from natural source (Gupta *et al.* 1995).

The neutral fraction of hot light petroleum extract of *P. zeylanica* root contains sitosterol apart from plumbagin and droserone (Kamal *et al.*, 1983; Gunaherath *et al.*, 1983). Kamal *et al.* (1984) isolated a quinone, plumbazylanone, an orange crystalline solid from the alkali soluble fraction of hot light petroleum extract of roots of *P. zeylanica.* It produces a purple solution in aqueous Na OH. It is identified as a trimer of plumbagin with an additional methyl group. The aerial part of *P. zeylanica* also contains amino acid (Dinda and Saha, 1987).

A dihydroflavonol, plumbaginol was isolated from the aerial parts of *Plumbago indica* (Dinda *et al.*, 1994). *P. auriculata* also contains flavonoids apart from plumbagin.

From the related species *Drosera peltata,* plumbagin, droserone and isoshinaole were isolated from phenolic fraction of light petroleum. Apart from plumbagin, they also contain flavonols (Nair *et al.*, 1990). *Drosera natalensis* and *D. capensis* also contain plumbagin, but the content was low when compared to *P. auriculata* (Crouch *et al.*, 1990).

The family plumbaginaceae was reported to contain sitosterol, stigmasterol, sitosterol glycosides and flavonoids like Kamepferol, luteolin etc. (Bashir *et al.* 1994). Schcherbanvoskii (1981) pointed out that in Crimnea, *Ceratostigma* spp. of family Plumbaginaceae is widely used for plumbagin production. The aerial part and roots of *C. plumbaginoids* and *C. wimottianes* were utilized.

2.1.2 Quality attributes as influenced by stages of harvest and season

Yield and chemical composition depend on pedoclimatic conditions and on the ontogenic stage of the plant. Certain pharmacopoeias specify the time and stage of collection of certain important drugs as they should be collected when the active

principle will be the high and they will have a good appearance when dried. Storage tissues like root, rhizomes, bulbs etc. are better to be collected at the end of active assimilating period (Balbaa, 1983). Mizrahi (1988) emphasised the necessity of harvesting medicinal crop species at optimum harvest stage in the context of synthesis of secondary compound at certain stages of maturity at specialised cells.

It has long been established that yield and quality of roots of *P. indica* vary according to the locality, growth and age of plant, condition of soil and season of the year. The older the plant the greater is the active principle in roots (CSIR, 1969).

Subha (1990) compared the effect of different levels of spacing and planting material on yield and quality of *P. rosea*. The results indicated that the plumbagin content in the species varied from 0.16 to 0.17 per cent in roots. Different spacing levels did not influence the plumbagin content of roots in the plant.

Studies conducted by Menon (1994) to compare the methods of planting and influence of shade on growth, yield and plumbagin content of *P. rosea* indicated that planting method had no profound influence on plumbagin content of roots. There was a slight difference in crude plumbagin under open and shaded conditions. A maximum of 0.86 per cent was noticed under shaded condition, while plants under full sunlight recorded 0.74 per cent. However there was no variation in purified plumbagin content between open and shade (0.32 per cent).

Villavicencio and Escandon (1994) reported that in *Plumbago pulchella* the highest concentration of plumbagin on fresh weight basis (0.95 – 1.79 per cent) was seen in leaves.

Kitanov and Pashankov (1994) studied the effect of harvest date on plumbagin concentration in stem, leaves, flowers, fruits and roots of *P. europea*. Based on the results of HPLC, UV, IR and NMR analysis, the highest plumbagin concentration of 3.06 per cent was present in flowers during the main period of anthesis (September). Young stem and leaves (combined) contained only 0.04 – 0.08 per cent while the older stem, leaves and flowers contained 1.35 – 1.65 per cent plumbagin. In contrast to the aerial part young roots contained higher amount of the pigment than older perennial roots (2.07 and 1.53 per cent respectively).

In *Costus speciosus* , Sarin *et al.* (1977) obtained the highest diosgenin content with the onset of flowering in July. Gupta *et al.* (1981) also observed that the diosgenin content increased from dormant stage of the plant to a maximum at the stage of flower bud emergence. The content again declined till the plant became dormant again. On the contrary, Joseph (1983) recorded maximum diosgenin content at 6 MAP (November) and minimum at 12 MAP (May) in *Costus speciosus*. The reproductive stage of development revealed higher diosgenin content in *C. speciosus* whereas diosgenin or solosodin content was high at post reproductive stage of development in *Dioscorea sp.* and *Solanum sp.* (Chatterjee and Nandi, 1983).

In studies conducted by Mandal *et al.* (1983), it was found that total alkaloid and ajmalicine increased progressively up to 210 DAP. In respect of ajmalicine and serpentine proportions there was no change during different intervals of harvest. The serpentine content continued to increase and reached the highest at 330 days. They opined that these stages coincided with autumn period when low temperature resulted in shedding of leaves and consequently higher translocation of secondary metabolites towards roots. Sen and Datta (1986) pointed out that in *Catharanthus roseus* the highest quantity of ajmalicine was obtained during summer and the lowest in winter. Total alkaloid was the highest in winter and lowest in summer. They also found that in leaves of *Ervatamia coronaria* the highest accumulation of coronarids was in rainy season while that of total alkaloids in summer. In *Rauvolfia serpentina* also accumulation of alkaloid (total and reserpine) followed an almost similar trend of rise and fall.

Hegde *et al.* (1981) observed a progressive increase in diosgenin content in *Dioscorea deltoidae* with increase in harvesting stage up to 32 MAP.

In *Physochlaiana orientalis* proportion of alkaloids differed according to the organ and phase (Aslanov, 1983). During fruiting, the maximum total alkaloids and hyoscyamine occurred in the whole plant. The maximum scopalamine occurred in the leaves and roots during fruiting and in stem during budding.

Singh *et al.* (1983) speculated that maximum pyrethrin in chrysanthemum was at the start of anthesis.

Pandita *et al.* (1983) reported a variation in the content of alkaloids in the leaves of *Adhatoda vesica* at different seasons.

Balbaa (1983) established that the chemical constituents vary in amount and value throughout the year. Rhubarb registered anthrol in winter, which were converted by oxidation to anthroquinone in warmer weather. The colchicum corm is almost free of bitterness and almost devoid of its active constituent, colchicine in autumn and it is full of starch. It is bitter and more active in spring and early summer when it is collected for medicinal use. Similarly *Hyoscyamus* contained less alkaloid in winter than in summer. He also noted that conium fruits contained high percentage of conine when fruits are mature and unripe.

Increased ginsenoside with increase in maturity was observed in *Panax ginseng* (Soldati and Tanaka, 1984). But the active principles in *Balanites roxburgii* varied according to season (Ghanim *et al.*, 1984).

In *Adonis amurensis* the total cardenolide content was the highest during maximum flowering and at the beginning of fruiting (Skurzoa and Shnyakina, 1985).

Indrayanto *et al.* (1985) noticed that solasodine content of fruits of *Solanum wrightii* reached a maximum of 2.57 per cent (dry weight) in fruits of 4 to 4.99 cm diameter and it was recommended that the fruits should be harvested at this stage.

Banerjee and Datta (1991) observed that in *Andrographis paniculata*, the accumulation of andrographolide was phase specific. They also found that in *Azadirachta indica* nimbidine content decreased with age.

It is also proved that the active ingredients in plant parts are influenced by season than stage of harvest. Seasonal effect on secondary metabolite was compound specific. Every individual compound has its specific favourite season (Banerjee and Datta, 1991).

Mario *et al.* (1993) pointed out that artemisinin accumulation in *Artemisia annua* significantly increased during fourth, fifth and sixth harvest and there after it declined.

The American podophyllum contained 4.50 per cent podophyllum resin where as Indian species contained 7-14 per cent, varying according to season of collection and locality. In certain cases, as much as 20.00 per cent resin has been recorded. The highest per cent of resin was in May, when the plant was in bloom. This resin also had

double the amount of podophyllotoxin which is the active constituent used in the manufacture of anticancer drug (Thakur, 1993).

In peppermint total dry matter and oil yields were high at full flowering (Marotti, *et al.* 1993) and ageing diminished biomass yield, plant height, leaf size and produced oil with higher menthofuran and menthyl acetate content (Piccaglia *et al.*, 1993). Studies by Leela and Angadi (1994) revealed that essential oil content and menthol content were the highest and menthone content was the minimum when harvested in July.

Samuel *et al.* (1993) studied the influence of harvesting stage ranging from 8 to 11 months on chemical constituents of *Holostemma annulare*. They reported that the carbohydrate content showed a decreasing trend as the age of the plant increased and it was the lowest (56.00 per cent) in 11 months old crop. The protein and alkaloid contents were higher in 11 months old crop as compared to 8 months and 9 months old crop.

Meera (1994) studied the influence of harvesting intervals on quality of *Holostemma annulare*. The results indicated that the different harvesting intervals significantly influenced number of free amino acids. The total amino acid content was found to increase with increase in age of plant. The content was maximum (0.014 per cent) at 18 months after planting. The stage of harvest exhibited a significant influence on soluble carbohydrates. The maximum content was obtained at 18 months old root (7.72 per cent) and minimum from 9 months old root (2.36 per cent).

Shina (1998) found that harvesting *Hemidesmus indicus* at 8 months after planting resulted in higher essential oil content of 0.3 per cent, in the roots. The per hectare and per plant oil yield was the highest at 8 months after planting. Seven and eight months old crop resulted in higher amount of water-soluble extract of roots (6.11 per cent). The highest value of alcohol soluble extract was obtained in 8 months old crop (3.16 per cent).

Apart from season and stage of maturity, time of collection also plays an important role in the content of active principle of medicinal plants. According to Balbaa (1983) the concentration of the desired glycosides was higher in *Digitalis* sp. leaves collected in the afternoon than in the leaves collected in morning. This was due to the fact that the active glycosides undergo hydrolysis to physiologically less active

aglycone during the night and recombines with sugars during day time. It was also pointed out that the alkaloid content of solanaceous leaves was high in morning than in afternoon.

However, harvesting stage did not exert significant influence on active principles in Ipecac (Banerjee, 1974) and *Sida* spp. (Shankar, 1998).

2.1.3 Anatomy of plant parts

Iyer and Kolammal (1960) described the structure of roots of plumbago. The Description is given below.

The transverse section of the roots of *P. rosea* is nearly circular. The cork is narrow with five rows of thin walled cubical to rectangular cells. The cell walls are light yellow to yellowish brown. Peripheral cortex cells have dark yellow contents. Greater part of cortex consists of thin walled rounded, polygonal tangentially elongate cells with well-defined intercellular spaces. The cells are devoid of starch grains. In some cells yellow or reddish yellow content is seen. There is no clear demarcation between cortex and bast. Bast cells contain yellow contents. Phloem is small combined with narrow xylem vessels in radial rows. Medullary rays are 10 to 12 in number. There is no pith in centre. Primary xylem is tetrarch. Mechanical elements are present in bast.

The transverse section of roots of *P. zeylanica* are found to be circular, cork is thin, some of which containing dark brown contents. Phellogen is in a single row. Some of these contain dark yellow contents. Cortex is wide and uniform in size. Cells are packed with starch grain. In inner part of cortex, there are small fibre cells and medullary rays are few in number. Primary xylem is tetrarch.

2.2 Studies on flowering, fruit set and seed germination

Plumbaginaceae is the only family of the order plumbaginales, (Trease and Evans, 1985). It contains 19 genera and 775 species. The members of the family are mainly herbs and shrubs. Genera include *Plumbago, Ceratostigma, Limonium* etc. According to the above workers the genus *Plumbago* contains 12 species. The three common species found in Kerala include *P. rosea* (*Chethikoduveli* – red flower type), *P. zeylanica* (*Vellakoduveli* – white flower type) and *P. capensis* syn. *P. auriculata*

(*Neelakoduveli* – blue flower type). According to CSIR (1969) *P. zeylanica* is more wide spread and common than *P. indica* and is possibly indigenous to South East Asia. The chromosome number of *P. zeylanica* is 2n = 24 while it is 2n = 12 in *P. indica* and other species. Scott (1992) described a new cultivar of *P. auriculata* cult. Monott. These have larger and wider blue petals and plants are dense with compact growth.

2.2.1 Floral biology

Iyer and Kolammal (1960) described the floral biology of *P. zeylanica* and *P. rosea*. *Plumbago zeylanica* flowers are pure white, sub-sessile with bract and bracteoles born on simple or branched terminal and axillary spikes half to one foot long. The rachis of the inflorescence is covered with very short glutinous hairs or glands. The bracts are considerably larger and about ten times larger than bracteoles. They are also covered with sticky glandular hairs. Fruits are enclosed within persistent calyx. It is one seeded membraneous capsule.

In *P. rosea*, flowers are beautiful scarlet or bright red coloured about one and a half inches long, in long loose twiggy spikes which are terminal as well as axillary from upper leaves. The spikes continue to elongate even after flowering reaching a length of one to two feet. Rachis of spikes is glabrous or smooth. Bracts and bracteoles are nearly equal and ovate.

Russell (1982) conducted ultra structural studies on megagametophyte of *P. zeylanica* which lacks synergids and suggested that the egg apparatus is highly specialised consisting of a single modified egg cell with synergid function. Gao *et al.* (1992) indicated that the degenerated synergids prevent male cytoplasm from being transmitted to the egg and central cell.

2.2.2 Pollen studies

In order to initiate crop improvement programmes, it is essential to have knowledge on the pollen morphology, viability and germination. However, studies on pollen morphology, production, viability and germination have not yet been undertaken in *Plumbago* spp.

Stanley and Linskens (1974) suggested various methods of testing the viability of pollen grains, including germination and non-germination assays.

Stain test

Stains, which give colour to viable pollen are often used as indices of viability. Zirkle (1937) described the method of mounting pollen grains in acetocarmine. The pollen grains that stained well and well shaped were taken as fertile and unstained shrivelled ones as non viable or sterile.

Sherly (1994) studied the pollen fertility of male and bisexual flowers of *Garcinia cambogia* using this technique and she recorded 71.8 per cent fertile pollen in male flowers and 27.03 per cent in bisexual flowers. The studies indicated the acceptability of acetocarmine stain test. The pollen fertility under *in vitro* condition was 66.91 per cent and 23.47 per cent respectively.

In vitro pollen germination

Sugar solutions are generally used as media for *in vitro* pollen germination. Sugar is reported to control the osmotic concentration during germination of pollen (Brink, 1924, O'Kelley, 1955, Vasil, 1958). Apart from osmotic role, the externally applied sugars in the medium serve as nutrient material for growing tubes. Brink (1924) observed that when pollen was cultured in sugar or sugar and agar medium, the pollen tubes were longer than those found in nature. The optimum sugar concentration for pollen germination was found to vary with the crop. In nutmeg, Nazeem (1979) reported better germination of pollen grains in 4 % sucrose and Bavappa and Banda (1981) reported the optimum concentration of sucrose to be 5 per cent. A higher germination percentage of 96 per cent was obtained in *Calotropis* when pollinia were cultured in Brewbaker and Kwack's (Brewbaker and Kwack,1963) medium. Manju (1997) found that irrespective of season of flowering, germination and pollen tube growth of *Holostemma annulare* continued to be higher in Brewbacker and Kwack's medium. A significantly better pollen germination and pollen tube growth was recorded in ME_3 medium (Leduc *et al.*, 1990) in ginger (Valsala, 1994).

Schumucker (1932) observed stimulant action of boric acid on pollen germination and tube growth. In many plants 1 to 10 ppm of boric acid in artificial

medium stimulated pollen germination and pollen tube growth. Munzer (1960) found that 0.01 to 0.1 per cent boric acid has a stimulating effect on pollen germination and tube growth in more than 60 angiosperm species.

In vivo pollen germination

The reasons for lack of pollen germination and seed set have been investigated in ginger, which lacks seed set under natural conditions, by Hooker (1892), East (1940), Fryxell, (1957), Ramachandran (1969), Pillai *et al.* (1978) and Sathybhama (1988).

East (1940) and Fryxell,(1957) suspected that the failure to set seed may be due to the existence of self incompatibility. According to Ramachandran (1969) and Ratnambal (1979) the lack of seed set in ginger was due to chromosomal aberrations. Pillai *et al.* (1978) suspected three possible reasons for the absence of seed set namely 1) defects in micro and mega sporogenesis, 2) lack of suitable pollinating agents and 3) failure of pollen germination on stigma or due to incompatibility. Jayachandran and Vijayagopal (1979) reported that in the event of incompatibility, inhibitory action may not be located on stigma surface. Usha (1983) opined that incompatibility reaction may not be the factor causing failure of seed set in ginger as she failed to get seed by bud pollination or with stigma and style removal.

Stott (1972) suggested to examine of pollen tube growth on stigmatic surface as a direct assessment of compatibility. According to Mock and Loescher (1973), in wide crosses pollen tube may grow along the entire length of style and penetrate the ovule but fertilization may not occur.

Kho and Baer (1968) suggested the technique of artificially pollinating the stigma with the desired pollen and examining the extent of pollen germination using fluorescence microscopy to establish whether incompatibility system is operating. Kho *et al.* (1980) studied *in vivo* pollen tube growth in interspecific hybrids of *Cucumis.*

Sathyabhama (1988) used the technique of fluorescence microscopy for studying the pollen-pistil interaction in ginger. It was found that the stigma of ginger flower was highly spiny in nature, which were closely arranged on the stigmatic surface. The pollen grains applied on stigma were either sticking to spine tips or were damaged by spines. Because of the closeness of the spines it was quite difficult for the pollen grains to gain

contact with the stigmatic surfaces. After two hours of pollination, the stigma showed fertile and sterile pollen grains on the stigmatic surfaces of all pollinated pistils. But the pollen grains did not germinate. However, examination of pistils at 4 hrs of pollination revealed 10 per cent germination, pollen tubes of which later coiled and did not reach the ovule. The pistils after 8 and 12 hrs of pollination did not reveal pollen germination.

2.2.3 Pollination and fruit set

Frankel and Galun (1977) reported that there exists heteromorphic incompatibility in *Plumbago, Ceratostigma* and *Limonium* species of the family Plumbaginaceae. Dulberger (1975) pointed out that structural stigma dimorphism may be probably involved in incompatability. In *P. europea* and *P. capensis* papillae of the long styled and short styled morphs differ in the way the cuticle is attached to the cellulose layer. The intermorph pollination is incompatible and inhibition of the pollen occur at the stigma surface.

CSIR (1969) did not report fruit set in *P. rosea.* It never produced fruits under Vellanikkara conditions also. However, Iyer and Kolammal (1960) and Sivarajan and Balachandran (1994) described the fruits of *Plumbago spp.* The fruits are enclosed in persistent calyx in one seeded membraneous capsule.

Ortega *et al.* (1995) reported that the members of the related family Droseraceae are predominantly self-pollinated.

2.2.4 Seed germination

As *Plumbago* is a crop of recent domestication and as it is propagated easily through cuttings, studies on germination have not been attempted and as such there are no reports on seed germination of this species. However, the experience is that seeds after harvesting fail to germinate under natural conditions. The failure of seed to germinate under natural conditions can be due to environmental factors or genetic factors.

A number of plant families contain hard seed coats which are impermeable to water and such seeds can be germinated by giving scarification using sand or acid or by rupturing the seed coat by mechanical means. The dormancy can also be due to the

presence of germination inhibitors (Evenari, 1949). In some cases, where inhibitors are present germination can be improved by leaching with water or by removing the seed cover or both (Norton, 1980). Germination inhibitors are reported to be widespread in seeds of tropical species (Nikolaeva, 1977). Dormancy in *Iris* seed is due to a water and ether soluble germination inhibitor in the endosperm, which can be leached from the seed with water or avoided by embryo excision (Arditti and Pray,1969).

Almeida *et al.* (1991) reported the results of their studies in *Canna indica* seeds. It was indicated that in treatments involving ruptured integuments, the seeds started to germinate within 12 to 24 hours and showed 95 to 100 per cent germination. Intact seed took 60 days to start germination. However, the final germination per cent was similar in all the cases.

Carpenter *et al.* (1993) studied the seed germination of needle palm (*Rhapidophyllum hystrix*) which usually take six months to two years to germinate under normal conditions. The results of this study indicated that high synchrony and rate of germination was achieved after removing the sclerotesta and embryocap that imposed physical dormancy. After scarification, recently harvested seeds or seeds stored for 12 months at 5^0C and at 100 per cent RH recorded 96 and 98 per cent germination respectively.

Pandyal *et al.* (1994) carried out germination tests of *Ammomum subulatum* cv. Ramsey seeds. The results showed that the highest germination of 68 per cent was achieved for seeds rubbed with sand paper for one minute.

2.3 Post harvest handling

The preparation of crude drug for market from harvested medicinal plants involves cleaning or garbling to remove soil particles and other extraneous matter. It involves curing in some cases, careful drying and good package. The faulty storage conditions itself is the difficulty in consistently capturing markets in developed countries. In humid areas, the material stored may acquire a moisture content of over 12 per cent (Wijesekera, 1993).

Plumbago species, the roots of which are used in many ayurvedic preparations, are usually used both in dry and fresh form. In the market, the roots are available in the

fresh form. However, in ayurvedic pharmaceuticals the roots are used in the dry or cured state.

Sivarajan and Balachandran (1994) suggested that the drug could be used only after adequate curing and purification. The freshly harvested roots are immersed in lime solution after being crushed. The deeply purple coloured solution is changed every day and the process will continue till the solution become colourless. They are then washed in fresh water, drained and dried and kept in godowns for use (Pandey, 1960). It is also reported that fresh root give much higher yield of plumbagin than roots stored for long period (CSIR, 1969).

Sasidharan (1996) suggested that curing which involved repeated washing of wet root in lime water reduced the toxicity of *P. rosea* root. He speculated that toxic principle phytosterol got dissolved in lime water.

It is reported that at times, severe scarcity is being experienced in procuring the roots in sufficient quantities. Therefore, the pharmaceuticals collect the material as and when they became available and store them after curing and drying in godowns under ambient conditions. However, effect of storing the roots for varying periods on the quality has not been investigated.

The effect of different containers on storage of dry fruits and vegetables have been studied in detail. However, studies are limited in medicinal plants. Drying itself is an effective way of preserving plant tissues (Cracker, 1995). The fundamental function of drying as a method of preservation is to inhibit the growth of micro organisms through the reduction of moisture level. The moisture content usually lies between 5 to10 per cent. But they are extremely hygroscopic and are to be packed as soon as drying is completed and special precautions must be taken against moisture absorption.

Adsule and Anand (1977) reported that among different types of plastic containers LDPE and HDPE tended to loose SO_2 most rapidly followed by polyvinyl chloride and glass irrespective of the product and storage temperature. Mahadeviah *et al.* (1977) reported that for long term storage of 8 – 10 months and to withstand physical and environmental hazards, LDPE 400 gauge were quite suitable in offering desired protection for pulses of unit packs of 500 gauge. However, for short term storage of

about 3 to 4 months 200 gauge of LDPE film was quite adequate under all conditions of storage.

Thompson and Schrader (1949) reported that during storage, changes do occur including loss of SO_2, hydrolysis of starch and increase in sugars.

Singh (1983) reported the results of his studies on the effect of different packaging materials on storage life of stored dry fruits. His results indicated that material packed in craft paper bag, butter paper bag, polyethylene bag, polypropylene bag and open storage conditions were quite unsatisfactory as there was serious quality deterioration after 50 days of storage.

Veenakumari (1992) found that 600 gauge LDPE and 100 gauge polypropylene packages were efficient up to three months of storage of dehydrated bitter gourd. When the cost of packaging was considered, polypropylene 100 gauge was found to be the cheapest package for dehydrated bitter gourd. For household use, she proposed the use of transparent plastic jars with screw type lids. Unpacked samples kept as control developed mould growth and were mainly due to *Pencillium* spp.

Kuriakose (1995) found that dehydrated products of *Solanum torvum, S. nigrum* and *Nelumbo nucifera* with an initial moisture content of 7 ± 1 per cent could be stored up to six months without deterioration in colour, texture and consumer acceptability. The moisture uptake was not significantly different between four packages under study namely, polyethylene 80, 100, 150 and 200 gauges. In economic terms polypropylene 80 gauge was found to be the cheapest.

Materials and methods

MATERIALS AND METHODS

Plumbago rosea and *P. zeylanica* are the two medicinally important species of the genus *Plumbago*. The cultivation of the crop has been commenced in recent years as the availability from the forest has become limited. Before extending the cultivation to larger areas the hortotechniques of the crop need to be standardised and for this, it is essential to have a thorough knowledge on the growth, flowering and quality attributes. Thus, to gather information on the basic aspects of growth, flowering, quality and post harvest handling methods, the present investigations were carried out at the College of Horticulture, Kerala Agricultural University, Vellanikkara during the period from 1996 to 1998. Three separate experiments conducted to fulfil the objectives were, studies on (1) vegetative growth, yield, biochemical and anatomical characters of the two species (2) reproductive behaviour and (3) post harvest storage of roots of *Plumbago rosea* .

3.1 Analysis of growth, yield quality parameters in *Plumbago* spp.

The experiment was laid out in split plot design with seven stages of harvest as two main plots and species as the sub plots. The two species studied were *Plumbago rosea* (*Chethikoduveli*) and *P. zeylanica* (*Vellakoduveli* or *Thumbakoduveli*). The seven stages of harvest were, 6, 8, 10, 12, 14, 16 and 18 months after planting (MAP).

The planting material was collected from 'Valakkavu' of Thrissur district. Two noded semi-hard woodcuttings were planted at the rate of four numbers in a polybag containing potting mixture. The rooted cuttings were three months old when planted in the field.

The main plots of 3.5 x 3.0 m^2 were taken and divided into two subplots of 3.5 m x 1.05 m. The rooted cuttings were planted at a spacing of 70 cm x 15 cm in ridges of 50 cm. height. At the time of planting, a basal dressing of cow dung @ 8 t ha^{-1} was given. Top dressing was done after 2 months of planting @ 25: 25: 25 kg NPK ha^{-1}. After 10 months of planting, the second dose of cow dung was applied @ 8 t ha^{-1}. Earthing up and weeding were carried out as per the package of practices recommendations of the Kerala Agricultural University (Kerala Agricultural University, 1993). Destructive sampling was done at bimonthly intervals and the following observations were recorded.

3.1.1 Biometric characters

Ten plants each per replication were uprooted at 6, 8, 10, 12, 14, 16 and 18 MAP. The plants were washed free of soil and the following observations were recorded.

Plant height

Height of the plant up to the base of inflorescence was noted and expressed in centimetres.

Internodal length

Internodal length between second and third leaf from tip was measured and expressed in centimetres.

Total leaf area

The length of fully opened leaf from top was recorded. The distance between the point of attachment and tip of the leaf blade was measured as the length of leaf. The width of leaf was measured at the point of maximum width.

A correction factor was worked out using the relationship between length, width and mean area of leaf recorded graphically.

Correction Factor (Index) = EA / (L x W)

EA – Area of leaf determined by graphical method

L – Maximum length of leaf

W – Maximum width of leaf

Fifty such leaves were taken to record the leaf area graphically. The actual leaf area in each treatment was obtained by multiplying the correction factor with length and width.

Length of root

Length of the longest root was recorded in centimetres.

Girth of root

Girth of the thickest root was recorded in centimetres.

Number of roots

Total number of roots emerging from base of stem was recorded as number of roots per plant.

Fresh weight of roots

The roots were washed free of soil, water was drained off completely and fresh weight of roots was recorded and expressed as grams per plant.

Dry weight of roots

The roots were air-dried for one day, packed in labelled paper bags and oven dried at 50 – 60^{0}C for three days. The dry weight of root was recorded in an electronic balance and recorded in grams per plant.

Fresh weight of stem

All leaves were separated and fresh weight of stem was recorded in grams per plant.

Dry weight of stem

The shoot portions were air-dried at room temperature for one day, packed in labelled paper bags and oven-dried at 50 – 60^{0}C for three days. The dry weight was observed in an electronic balance and recorded in grams per plant.

Fresh weight of leaves

Weight of separated leaves was recorded using top loading balance and expressed in grams per plant.

Dry weight of leaves

Leaves were dried in an electric oven at 50 – 60^{0}C for three days and weight was recorded in grams per plant.

3.1.2 Physiological characters

The data on leaf area, leaf dry weight, total plant dry weight and dry weight of shoot and root were utilised for calculating the following physiological parameters. The other growth parameters were calculated using the formulae suggested by Watson (1952), Friend *et al.* (1962) and Radford (1967).

Total Dry Matter (TDM)

It was measured as the dry weight produced per plant at each harvesting stage.
TDM = Dry weight of root + dry weight of shoot + dry weight of leaf
Total dry matter production per hectare was also worked out.

Root : Shoot ratio

Root : Shoot ratio was calculated by dividing the dry weight of root by dry weight of shoot of individual plants and the mean values were worked out.

Harvest Index (HI)

The harvest index at each stage of harvest was calculated from the dry weight of root and total dry matter production.

Leaf Weight Ratio (LWR)

Leaf dry weight (LW) was divided by plant dry weight (W) per unit area to arrive at leaf weight ratio and expressed in g $g^{-1}m^{-2}$.

LWR = LW/ W g $g^{-1}m^{-2}$

Leaf Area Ratio (LAR)

Leaf area (A) was divided by plant dry weight (W) to obtain leaf area ratio.

LAR = A/W m^2leaf area g^{-1}

Leaf Area Index (LAI)

LAI = Leaf area (cm^2) / Land area (cm^2)

It was measured in terms of total leaf area (m^2) per square meter of land area.

Crop Growth Rate (CGR)

It represents dry weight gained per unit area of crop in unit time.

$$CGR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \text{ g day}^{-1}\text{m}^{-2}$$

where, W_1 and W_2 are plant dry weights at time t_1 and t_2.

Net Assimilation Rate (NAR)

Dry weight gained in time $t_2 - t_1$ was divided by average leaf area during $t_2 - t_1$ to arrive at NAR.

$$NAR = \frac{W_2 - W_1}{(t_2 - t_1)} \times \frac{\text{In. } A_2 - \text{In. } A_1}{A_2 - A_1} \text{ g m}^{-2}\text{leaf area day}^{-1}$$

where, W_1 and W_2 denote dry weight of plant at time t_1 and t_2 (g), A_1 and A_2, leaf area at time t_1 and t_2 (in days) and In. A_1 and In. A_2 increase in leaf area.

Relative Growth Rate (RGR)

It represents increase in dry weight in time $t_2 - t_1$, over dry weight at time t_1.

$$RGR = \frac{(\text{In. } W_2 - \text{In. } W_1)}{(t_2 - t_1)} \text{ g g}^{-1}\text{day}^{-1}$$

where, ln. W_2 and In. W_1 are increasing plant dry weight in time t_2 and t_1

3.1.3 Linear Growth Rate (LGR)

Linear growth rate of the two species was studied by fitting linear models using the formula $Y = a + bt$, where 'a' and 'b' are constants and 't' is the time in bimonthly interval.

3.1.4 Response function

Response function relating fresh root weight / plant and total dry matter production / plant with different characters were fitted repeatedly using step-wise regression technique (Martgannery, 1991).

3.1.5 Biochemical characters

Plumbagin content

The variation in the quality of roots at different stages of harvest was evaluated at two months interval after planting. The roots were collected from three replications, pooled and a composite sample was prepared for analysis.

a. Preparation of crude extract

The roots were washed and air-dried for one day, after which they were oven-dried at 50-60°C for three days and powdered. Thirty grams of powdered sample was mixed with 100 ml acetone and kept it as such for 48 hours under darkness. Filtrate was collected and repeatedly extracted with acetone till the extract became colour less.

The acetone extract was pooled and concentrated by evaporation to a volume of 50 ml. It was then transferred to a separating funnel and equal quantity of ethyl acetate and water was added and shaken well. The epiphase ethyl acetate fraction was collected and process was repeated until the ethyl acetate fraction became colourless. Ethyl acetate fraction was pooled and concentrated under vacuum to a volume of 50-60 ml and then air dried at room temperature. The weight of crude extract was recorded.

b. Purification and quantification of plumbagin

The crude plumbagin extract was dissolved in 3ml of Hexane : Ethyl acetate (5:1) solvent system and fed to a silica gel (120 mesh) column of 30 m x 1.6 cm size and eluted with the same solvent system @ 5 ml / 5 minutes. The orange red band was identified as plumbagin. This was collected and evaporated to dryness at room temperature. The weight of golden yellow needle shaped crystals was recorded as weight of plumbagin .

The crystals were subjected to spectral analysis and spectral characters were recorded.

Curing of roots

Added 100 ml of 0.5 per cent $Ca(OH)_2$ to 50 g of fresh macerated roots. The milky solution suddenly turned purple. *P. rosea* gave deep purple colour and *P. zeylanica*

produced light purple colour. The clear supernatant liquid was collected after 24 hours of curing. Repeated treatment of the root sample was carried out using lime water for complete transfer of plumbagin. The complete extraction of plumbagin was ascertained by fractionation with ethyl acetate.

Plumbagin content in the root samples after lime treatment was estimated.

Effect of different solvents on removal of plumbagin from roots

The roots of the two species was immersed in water, ethyl acetate, acetone and 0.5% $Ca(OH)_2$ till the extraction of plumbagin was completed. The clear supernatant liquid was used for estimation of plumbagin content.

Characterisation by Thin Layer Chromatography (TLC)

The crude extracts were separated by Thin layer Chromatography (TLC) by using silica gel G and solvent system Hexane : Ethyl acetate (5:1). Silica gel G of M/S Merk having the binder calcium sulphate was used. A slurry of silica gel was prepared by mixing silica gel with distilled water in 1:2 (w/v) ratio and spread on glass plates of 20 x 20 cm with the help of the applicator which was adjusted to a thickness of 5µm. The plates were dried at room temperature and activated at 120^0C for 30 minutes in thermoregulated hot air oven.

Hexane: Ethyl acetate (5:1) was the solvent system used for getting clear separation. Chromatographic chamber (30 cm x 20 cm x 25 cm) was saturated with solvent system. The chromatographic run was carried out at room temperature.

Ethyl acetate extracts of both the species were spotted on the same plate and run was carried out for 30 to 40 minutes. The plates were dried at room temperature and Rf values were recorded. Photographs were taken both under visible and ultra violet light.

Chlorophyll content

First fully opened leaf from top was used for chlorophyll analysis. Chlorophyll content was estimated as per the method suggested by Sadasivam and Manikam (1992). A sample of 0.5g of finely cut and well-mixed representative sample of leaf was ground to a fine pulp using 80 per cent chilled acetone. It was centrifuged at 5000 rpm for 5 min. The

supernatant liquid was transferred to a 50 ml amber- coloured volumetric flask. 80 per cent chilled acetone was added to the residue, mixed well and centrifuged. Supernatant liquid was again collected.

This procedure was repeated until the acetone became colourless. The supernatant liquid collected by centrifuging was made up to 50 ml and the absorbance of the solution was read at 645 and 663 nm against 80 per cent acetone as blank.

The chlorophyll content in both the species was calculated from the following relationship.

Chlorophyll a (mg/g) = $12.7(A_{663}) - [(2.69(A_{645}) \times V)/ (1000 \times W)]$

Chlorophyll b (mg/g) = $22.9(A_{645}) - [(4.68 (A_{663}) \times V)/ (1000 \times W)]$

Total chlorophyll (mg/g) = $20.2 (A_{645}) + [(8.02 (A_{663}) \times V)/ (1000 \times W)]$

A. - absorbance at specific wave length

V - final volume of chlorophyll extract (50ml)

W - fresh weight of tissue extracted (0.5g)

The chlorophyll a, chlorophyll b and total chlorophyll in the leaves of both the species were estimated at 8, 12, 16 and 18 MAP. Ratio of chlorophyll a: chlorophyll b ratio was also calculated.

Protein

The protein content of leaves and roots of the two species of *Plumbago* were estimated at four stages of growth i.e. at 8, 12, 16 and 18 MAP using Lowry method (Whitaker,1972)

Malate dehydrogenase Activity (MDH)

The MDH activity of leaves of *P. rosea* and *P. zeylanica* was found out using the method suggested by Sadasivam and Manikam (1992). However, some modifications had to be effected with respect to the concentration of enzyme and substrate, oxalo acetic acid.

Fresh tender leaves of both the species were collected from field in an ice bucket. One gram of the leaf sample was macerated in a chilled mortar by using an extraction buffer of pH 7 (50 mM Tris HCl, 50 mM $MgCl_2$, 5 mM 2 mercaptoethanol, 1 mM EDTA) and made upto 30 ml and centrifuged at 10,000 rpm at 5^0C. Supernatant liquid was

collected for activity studies. Two ml of the supernatant liquid was diluted to 10 ml with the same extraction buffer and 0.4 ml was used for enzyme activity study. The concentration of the substrate oxalo acetic acid used was 20 per cent as against 10 per cent suggested by Sadasivam and Manikam (1992).

A test solution for reading the absorbance was prepared using 1 ml oxalo acetic acid and 0.5 ml $MgCl_2$, 0.4 ml enzyme extract and 2.6 ml Tris HCl. A blank was prepared by adding 3.1 ml Tris HCl, 1 ml oxalo acetic acid 0.5ml MgCl2 and 0.4 ml enzyme extract. The absorbance of blank was read at 340 nm and that of test solution was then recorded.

Protein content was determined using Lowry method(Whitaker,1972). Five ml of 10 per cent TCA was added to 5 ml enzyme extract for precipitating protein. It was centrifuged and the supernatant liquid was decanted. To the residue 3 ml 0.1 N NaOH was added for dissolving the protein. 0.8 ml of the protein solution was diluted to 1 ml using distilled water. Five ml. reagent C (alkaline copper solution) was added and allowed to stand for 10 minutes. Finally added 0.5 ml reagent D (Folin – ciocalteau reagent), mixed well and kept at room temperature in dark for 30 minutes. The blue colour developed was then read at 660 nm. A standard graph was drawn using bovine serium albumin and the protein content was calculated.

Specific activity was calculated at 30°C for 2 minutes by using the following formula

$$\text{Specific activity} = \frac{\text{Enzyme activity of x ml extract}}{\text{Protein content of x ml extract}}$$

Phospho enolpyruvate carboxylase (PEPC) activity

Phospho enolpyruvate carboxylase activity was estimated as per the method suggested by Sadasivam and Manikam (1992). Modifications were effected with respect to concentration of enzyme and temperature. The procedure for estimation was same as that followed for MDH activity except that the enzyme extraction was carried out at room temperature, as low temperature recorded loss of enzyme activity. The effect of concentration of substrate on enzyme activity was also studied at different levels of substrate concentration.

Specific activity of phospho enol pyruvate carboxylase was estimated as described in malate dehydrogenase activity.

3.1.6 Number of stomates and Diffusive resistance

Number of stomates on the lower epidermis of leaf was recorded under high power (40x) of compound microscope. Observations on number of stomates were recorded from tip, base and middle portions of the leaf. Epidermal peelings were taken from lower surface of the leaf with the help of 'Quickfix' (adhesive), mounted on slides and number of stomates per field was noted.

Stomatal conductance was measured using steady state porometer during May 1997. Observations on diffusive resistance, transpiration and leaf temperature were recorded at 8.00 a.m. and 2.00 p.m. and diffusive resistance was worked out.

3.1.7 Anatomical studies

Root

The cross sections of root tip portions were taken for anatomical studies. Sections were stained for five minutes in one per cent aqueous saffranine solution and washed with distilled water for removing excess stain. The sections were observed under microscope and photographs were taken.

Stem

Hand transverse sections from second internode of vegetative branches of both the species were taken and were stained as described earlier and observations recorded.

Leaf

The leaves in the third node of vegetative branches were selected for taking transverse sections. Sections were stained and observed as in 3.1.6.1

3.2 Analysis of reproductive behaviour in Koduveli (*Plumbago* spp.)

Plumbago rosea and *P. zeylanica* were raised in plots of size 3.5 m x 1.05 m during July 1996. The plants were grown under uniform growing conditions under the shade of 20 year-old coconut plantation. Thirty plants were tagged for taking observations on flowering attributes. Observations on number of flowers per inflorescence, days taken for

completion of flowering per inflorescence, floral characters, time of anthesis, pollen characters, pollination and seed germination were recorded.

3.2.1 Inflorescence characters

Days for opening of first flower in an inflorescence

Twenty-five inflorescence buds were labelled in each of the species immediately after visual emergence of reproductive bud. Days taken for opening of first flower were noted. . The inflorescences were watched daily for the opening of the flower.

Number of flowers per inflorescence

Twenty-five inflorescences labelled for the above observation were utilised for recording this character. After completion of flowering, inflorescences were collected and the number of flowers per inflorescence was recorded by counting the persistent bracts.

Days for the completion of flowering per inflorescence

The inflorescences labelled for studying the number of days required for opening of first flower were observed till the completion of flowering. The day of completion of flowering was noted and days for completion of flowering worked out.

Length of inflorescence

After the completion of flowering, the length of inflorescence was measured and expressed in centimetres.

3.2. 2 Flower characters

Fully opened flowers of the two species were collected. Longitudinal sections were taken and observed under dissection microscope. Diagrams on longitudinal section of flower, cross section of ovary and floral diagrams were prepared.

Twenty-five flowers each were collected from both the species and observations on length of flower, length of sepal, length of corolla tube, width of corolla and length of style were recorded and expressed in cm. The observations were repeated after one week and mean was worked out.

3.2.3 Time of anthesis

Twenty-five mature flower buds, which would open on the day were tagged in both species during early morning hours. The flower buds were observed at half hourly intervals starting from 6.30 a.m. The observations were continued for three days.

3.2.4 Pollen studies

The characters like pollen morphology, number of pollen grains per anther, pollen fertility and pollen germination were recorded.

Pollen morphology

Anthers from freshly dehisced flowers were collected in the morning and pollen grains were extracted from it on clean slides and stained using acetocarmine. The slides were examined under microscope and diameter of 20 normal sized, well-shaped and well stained pollen grains were recorded at random from each slide using an ocular micrometer and expressed in μm.

Number of pollen grains produced per anther

The number of pollen grains per anther was estimated by using haemocytometer as suggested by Rao and Khader (1962). Flower buds of both the species were collected separately in the early morning before flower opening. One hundred non-dehisced anthers were carefully gathered. They were put in a small vial, separately, containing 2.5 ml of water with a drop of Teepol. After dehiscence the contents were then stirred thoroughly in order to obtain an even dispersion of pollen grain in each vial.

A drop of suspension drawn by a pasture pipette was transferred to each of the two counting chambers of a Spencer Bright Line Haemocytometer. Each chamber has an area of 9 mm^2 ruled into smaller divisions. Each of the four corner square millimeter area is ruled into 16 areas while the five, square millimeter areas are ruled into smaller divisions. The counting chambers are 0.1 mm in depth so that the volume over 1 mm^2 is 0.1 mm^3. On this basis the number of pollen grains per anther can be derived as follows.

The contents of 100 anthers were suspended in 2.5 ml of the solution. Thus the contents of each anther is suspended in 0.025 ml of the solution or 25 mm^3.

If X = Number of pollen grains per anther

N = Average number of pollen grains counted per corner square

N : X = 0.1 : 25

X = 250 N

The pollen grains in each of the four corner squares of each counting chamber were counted using low power (10 x) objective of miscroscope.

Pollen fertility

Fully opened flowers were collected and pollen grains were extracted from anthers on clean slides and stained using acetocarmine method (Zirkle, 1937). The slides were observed under microscope after 10 minutes. The number of well-stained and non-stained pollen grains was counted from 10 fields from microscopic slides in each species. The experiment was repeated. The mean pollen fertility was worked out and expressed in per cent.

In vitro pollen germination

Pollen grains were collected from freshly dehisced anthers from both the species and were kept for germination in different media as detailed below.

1. Sucrose 1%
2. Sucrose 1% + agar 1%
3. Sucrose 1% + agar 0.5%
4. Sucrose 5%
5. Sucrose 5% + agar 1%
6. Sucrose 5% + agar 0.5 %
7. Sucrose 10 %
8. Sucrose 10% + agar 1%
9. Sucrose 10% + agar 0.5 %
10. Sucrose 20%
11. Sucrose 20% + agar 1%
12. Sucrose 20% + agar 0.5%
13. Tap water
14. Tap water + Boron 25 ppm

15. Tap water + Sucrose 1% + Boron 25 ppm
16. Tap water + Boron 50 ppm
17. Tap water + Sucrose 1% + Boron 50 ppm
18. ME_3 medium (Leduc *et al.*, 1990)
19. Bruebaker's and Kwack's medium (Bruebaker and Kwack, 1963)

The slides were kept in a dessicator up to 48 hours and observations were recorded at 24-hour interval.

In vivo pollen germination

The flowers of both *P. rosea* and *P. zeylanica* were artificially pollinated. The pollinated flowers were collected after 24 hours and fixed in FAA solution (Formalin 6 parts : Absolute alcohol 10 parts : Glacial Acetic Acid 1 part). After 24 hours, the gynoecium was separated and transferred to small vials containing 1N NaOH and retained for 3 hours. The softened gynoecium was then transferred very carefully using a brush to another vial containing 0.1% aniline blue in 0.1N K_2HPO_4. The stained gynoecium was then mounted in the same stain on a microscopic slide and observed under UV microscope (Leitz Dialux 20 epifluorescence microscope). The fluorescence of pollen grains and pollen tubes on the stigmatic surface of different crosses was observed and photographs were taken.

3.2.5 Pollination studies

While *P. zeylanica* sets fruit, *P. rosea* rarely sets fruit. In order to assess the exact mode of pollination in the two species, the following experiments were designed.

Open pollination

Twenty-five flower buds of *P. zeylanica* were selected and tagged. These were later examined for fruit set and calyx retention up to four days of blooming. The percentage of fruit set on open pollination was worked out.

Self pollination

Twenty-five flower buds of *P. zeylanica* were selected and covered with butter paper covers, one day prior to anthesis. Fruit set was observed after four days and extent of self- pollination was recorded.

Natural cross pollination

Twenty-five flower buds of *P. zeylanica* were emasculated one day prior to anthesis and left for natural cross pollination. Fruit set was observed after four days and percentage of natural cross pollination was worked out.

Artificial pollination

Twenty five flower buds of *P. zeylanica* were selected, emasculated and covered with butter paper covers one day prior to anthesis. On the next day, flowers were pollinated using pollen grains of *P. rosea*. Fruit set was observed after four days and per cent worked out.

Since *P. rosea* does not produce fruits under Vellanikkara conditions, studies on mode of pollination had to be restricted to artificial pollination alone. For studying the extent of fruit set by artificial pollination, 25 flowers were pollinated using pollen grains of same flower, pollen grains of another plant of *P. rosea* and pollen grains of *P. zeylanica*. The fruit set was observed after four days.

3.2.6 Seed germination

As seeds are not produced in *P. rosea* under Vellanikkara conditions, the study was restricted to *P. zeylanica* only. Seeds were collected during February 1997 and sown immediately after collection and also after storage for three months. The seeds were kept for germination after imposing the following treatments.

1. Fresh seeds kept on moist filter papers in petridishes.
2. Fresh seeds soaked in water for 24 hours and kept on moist filter paper in petridishes.
3. Fresh seeds after cutting the micropylar end and kept in moist filter paper in petridishes.

4. Fresh seeds after cutting the chalazal end of the seed and kept on moistened filter paper in pretridishes.
5. Fresh seeds after scarification using sand and kept on moistened filter paper in petridishes.
6. Stored seeds (3 months) kept on moist filter paper in glass petridishes.
7. Stored seeds (3 months) after cutting the chalazal end of the seed and kept on moistened filter paper in petridishes.
8. Fresh seeds after cutting the micropylar end and kept under laboratory conditions in mud pots filled with sand.
9. Stored seeds after cutting the micropylar end, kept under laboratory condition in mud pots filled with sand.

Fifty seeds were tested under each treatment. The number of seeds germinated was counted in each of the treatment and the percentage was worked out.

Survival of seedlings

The seedlings obtained in the above study were transplanted in small mud pots filled with potting mixture. The seedlings were covered with polythene cover in order to retain humidity. The number of leaves produced by the seedlings after 15 days of planting was recorded. The hardened seedlings were then field planted after one month and rate of survival was recorded for three months.

3.3 Post harvest storage of roots of *Plumbago rosea*

The study has to be restricted to *P.rosea* alone as *P. zeylanica* is not recommended for commercial cultivation in Kerala. Fresh roots of *P. rosea* were collected from Marottichal in Thrissur district and oven-dried. The dried samples were cut into small pieces, oven-dried and 30 g of roots were packed in 5 different containers for 18 months. The different containers used are furnished below.

1. Polypropylene bag –100 gauge
2. Polyethylene bags – 100 gauge
3. Polythene lined gunny bags
4. Black plastic bottles
5. Paper plates (control)

Samples were taken at 2, 4, 6, 8, 10, 12, 14, 16 and 18 months of storage and observations were recorded on the following parameters.

3.3.1 Moisture content of sample

The moisture content in the fresh roots of *P. rosea* was estimated by immiscible solvent distillation method as suggested by Ranganna (1977). The air-dried roots and oven- dried roots were also analysed for moisture content using the same method. Later weight of samples at 2, 4, 6, 8, 10, 12, 14, 16 and 18 months after storage were recorded

3.3.2 Fungal and insect contamination

The samples were observed for fungal and insect contamination after 2, 4, 6, 8, 10, 12, 14, 16, and 18 months of storage. The fungi infecting the samples were also identified.

3.3.3 Plumbagin content

The method described earlier for analysis of plumbagin was employed here also. The samples were analysed for plumbagin content after 2, 4, 6, 8, 10, 12, 14, 16, and 18 months of storage.

3.4 Weather data

The weather data pertaining to the period of study were collected from the Department of Agrometeorology, College of Horticulture, Vellanikkara and furnished in Appendix 1

3.5 Statistical analysis

The entire data generated were subjected to statistical analysis using the method suggested by Panse and Sukhatme (1985).

Results

RESULTS

The two species of *Plumbago* recognised in ayurvedic preparations are *P. rosea* and *P. zeylanica.* These crops have been domesticated recently and preliminary investigations indicated the feasibility of growing this crop as intercrop in coconut plantations. In order to standardise the hortotechniques and arrive at package of practice recommendations, a through knowledge of the crop physiology is essential. To undertake crop improvement programmes, it is necessary to know the flowering pattern, fruit set and seed formation. Post harvest handling techniques are to be standardised before recommending the crop for large-scale cultivation. With the above objectives, the present investigations were carried out at College of Horticulture, Kerala Agricultural University, Vellanikkara. The results obtained are described below.

4.1 Analysis of biometrical, physiological and biochemical characters in *Plumbago* spp.

4.1.1 Biometric characters

The data on influence of stages of growth on plant height, internodal length and leaf area have been furnished in Table 1.

Plant height

The plant height was significantly higher in *P. zeylanica* (120.46 cm). The interaction between the species and stages of harvest was significant at 1per cent level (Appendix 2).

The plant height in P. *zeylanica* steadily increased from 88.48 cm at 6 MAP to 148.47 cm at 14 MAP and thereafter there was no significant difference in this character. Height at 16 MAP was on par with that at 14 and 18 MAP. In *P. rosea,* plant height showed a steady increase from 38.07 cm at 6 MAP to 69.8 cm at 16 MAP. There after no significant difference was noticed. The heights at 16 MAP and 18 MAP were on par. It is evident that the plant height of both the species increased up to 16 MAP and there after there was no appreciable increase in plant height (Fig. 1).

Table 1. Influence of stages of harvest on plant height, length of internode and total leaf area in two species of *Plumbago*

Stages of harvest	Plant height (cm)		Internodal length (cm)		Total leaf area (m^2 $plant^{-1}$)	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
6 MAP	38.07	88.48	5.20	4.73	0.02	0.12
8 MAP	45.23	93.70	7.03	6.43	0.06	0.13
10 MAP	43.33	86.40	6.10	5.73	0.03	0.13
12 MAP	56.00	123.67	6.53	3.97	0.05	0.67
14 MAP	58.93	148.47	4.87	5.00	0.10	0.27
16 MAP	69.87	157.20	5.03	3.93	0.17	0.51
18 MAP	68.07	145.30	4.83	3.47	0.14	0.14
Mean	54.21	120.46**	5.66**	4.75	0.08	0.28**
Stages of harvest						
SE m ±	5.489		0.372		0.004	
CD	16.65		1.13		0.01	
Stages of harvest *vs* Species						
SE m ±	7.681		0.359		0.004	
CD	23.29		1.07		0.01	

MAP: Months After Planting
** Significant at 1 % level

Length of internodes

In contrast to plant height, internodal length was significantly higher in *P. rosea*. The mean value for internodal length of *P. zeylanica* was 4.75 cm as compared to 5.66 cm in *P. rosea*. The data on interaction between stages of harvest and species indicated significant difference (Appendix 2). The internodal length was the highest at 8 MAP in both the species. The data also indicated that the length of internodes showed smaller peaks at 8, 12 and 16 MAP in *P. rosea*. In the case of *P. zeylanica*, no definite pattern in internodal length was noticed.

Total leaf area per plant

Leaf area, which contributes the major assimilatory surface of the plant, was measured at different stages of growth and the data are furnished in Table 1 and Fig. 2. Total leaf area per plant was significantly higher in *P. zeylanica* (0.28 m^2 per plant) as compared to 0.08m^2 per plant in *P. rosea*. The interaction between stages of harvest and species was also significant (Appendix 2). In *P. rosea* the total leaf area per plant increased from 0.02 m^2 per plant at 6 MAP to 0.06 m^2 per plant at 8 MAP. Thereafter there was a

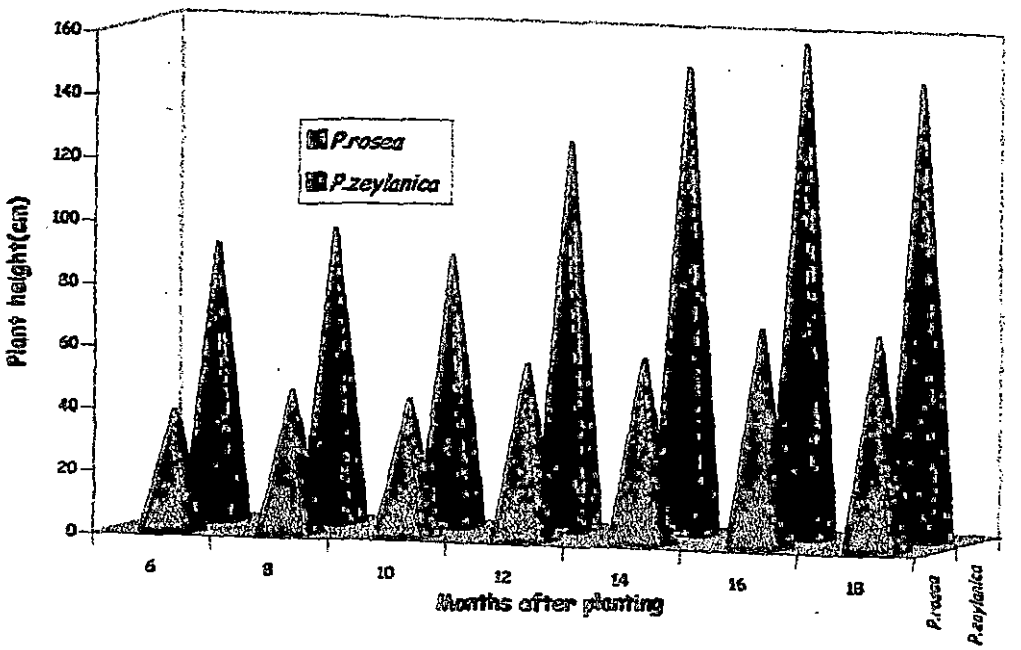


Fig.1. Plant height at different stages of growth in Plumbago spp.

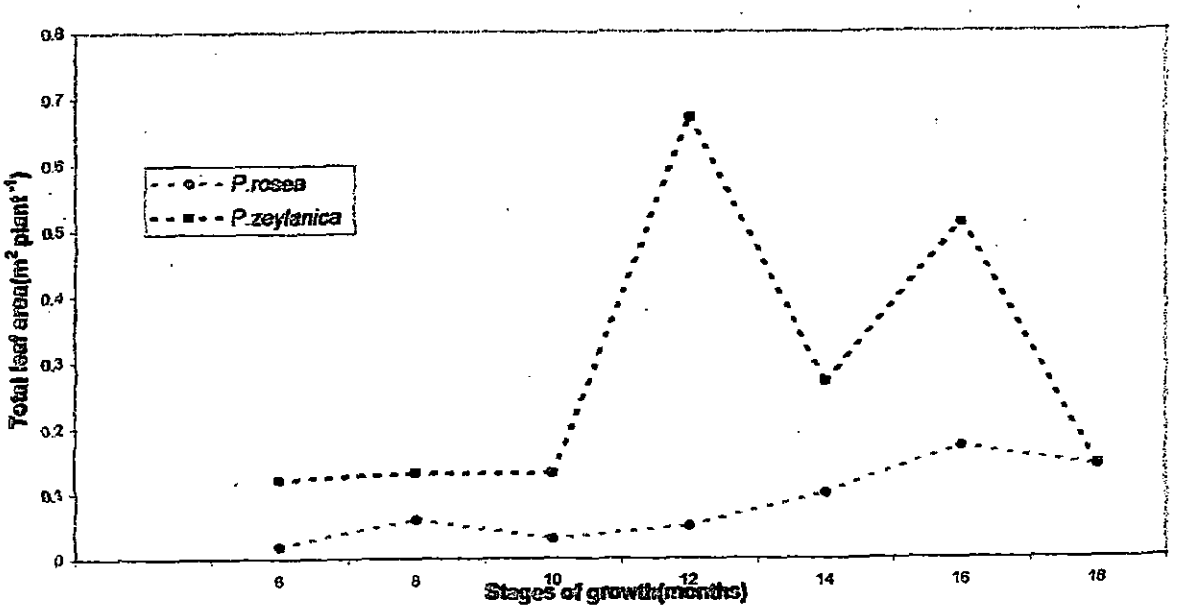


Fig.2. Total leaf area per plant in Plumbago species at different stages of growth

decline in leaf area to 0.03m^2 per plant at 10 MAP, which coincided with peak summer. With the onset of south-west monsoon, there was progressive increase in leaf area up to 16 MAP when it recorded the maximum area of 0.17 m^2 per plant. The leaf area declined thereafter.

In respect of *P. zeylanica*, the leaf area increased from 0.12m^2 at 6 MAP to 0.13m^2 at 8 MAP. After one year of planting the leaf area increased appreciably to 0.67m^2 per plant which was the peak leafy stage. At 16 MAP, the leaf area was 0.51m^2 per plant.

Length of roots

P. zeylanica had longer roots (49.19 cm) as compared to *P. rosea* (40.33 cm) (Table 2). The interaction between stages of harvest and species was not significant (Appendix 3). This indicated that both the species recorded similar pattern of growth in respect of root length at different stages of harvest. The root length of both the species showed a steady increase and significantly higher values were observed at 18 MAP (57.07 cm and 64.37 cm) respectively by *P. rosea* and *P. zeylanica*) (Fig. 3).

Table 2. Effect of different stages of harvest on the root parameters of two species of *Plumbago*

Stages of harvest	Root length (cm)		Root girth (cm)		Root number (no. pl^{-1})	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
6 MAP	29.07	37.63	1.97	1.10	7.37	13.2
8 MAP	30.93	40.76	1.97	1.13	7.87	16.73
10 MAP	35.33	44.2	2.23	1.33	11.67	15.93
12 MAP	40.77	47.13	2.53	1.57	12.13	15.3
14 MAP	41.4	56.1	2.7	1.83	12.8	18.7
16 MAP	47.7	54.17	3.03	1.90	17.53	20.5
18 MAP	57.07	64.37	3.13	2.00	14.47	25.6
Mean	40.33	49.19**	2.51**	1.55	11.98	17.99**
Stages of harvest						
SE m ±	2.456		0.069		0.822	
CD	0.21		2.49		7.45	
Stages of harvest *vs* Species						
SE m ±	3.184		0.119		1.77	
CD	NS		NS		NS	

MAP: Months After Planting
** Significant at 1 % level

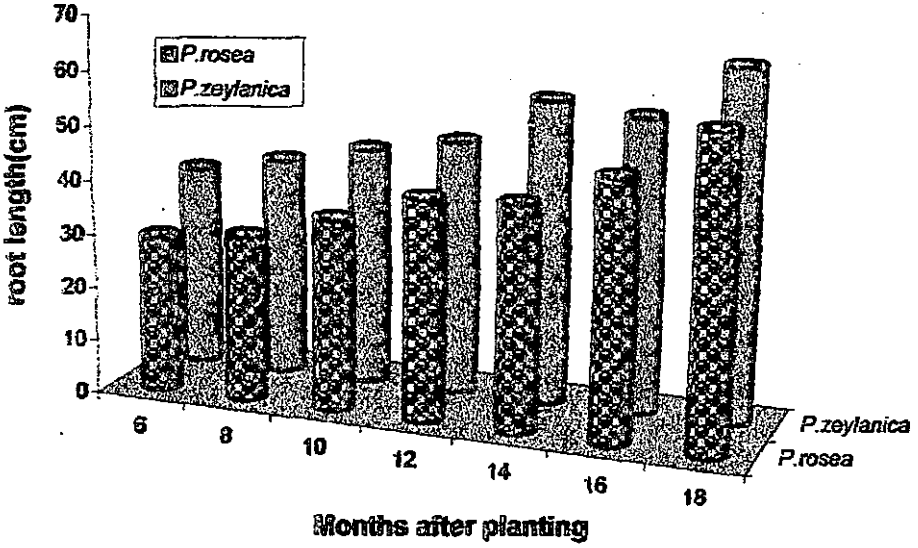


Fig.3. Root length at different stages of harvest in *Plumbago* spp.

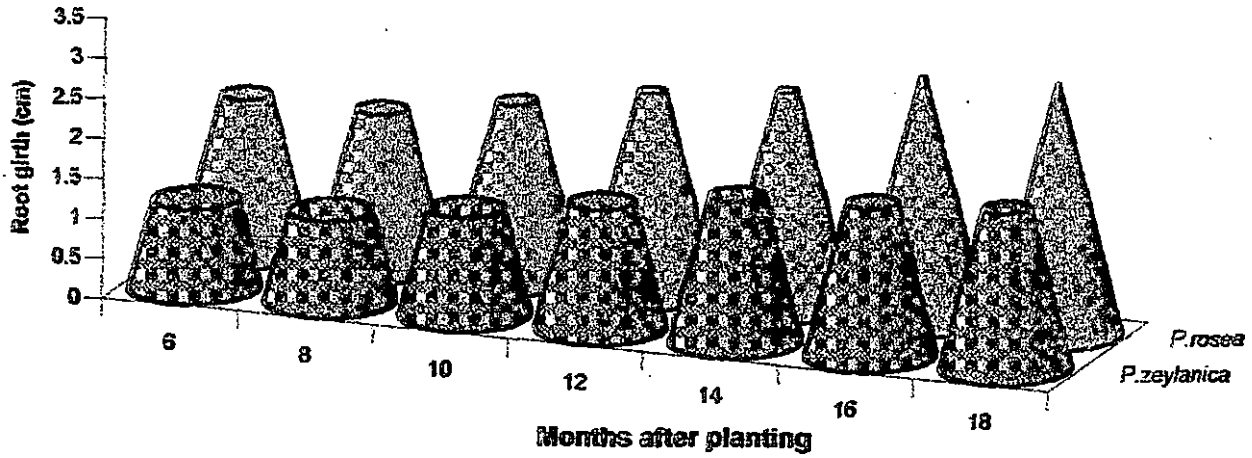


Fig.4. Root girth at different stages of harvest in *Plumbago* spp.

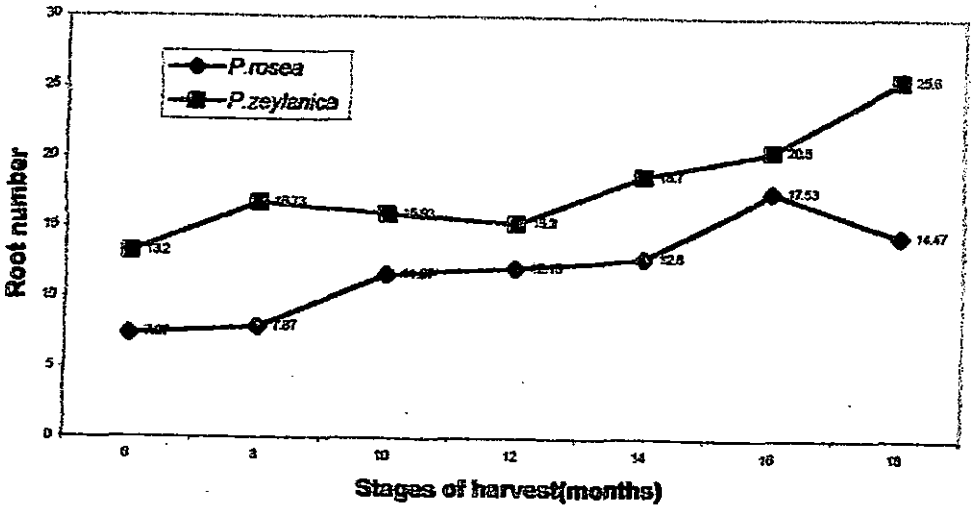


Fig. 5. Root number at different stages of growth in Plumbago spp.

Girth of roots

The data furnished in Table 2 showed that the species differed significantly in girth of roots. A significantly higher girth was noticed in *P. rosea* (2.51 cm) compared to in *P. zeylanica* (1.55 cm). The interaction between the species and stages of harvest was not significant (Appendix 3). The girth of roots increased with increase in age of crop in both species and the maximum value was noticed at 18 MAP (3.13 cm and 2.00 cm respectively by *P. rosea* and *P. zeylanica*) (Fig. 4). However, the root girth at 16 and 18 map were on par.

Number of roots

The results shown in Table 2 indicated that rate of root production was significantly higher in *P. zeylanica* (17.99) as compared to *P. rosea* (11.98) (Table 2). The interaction between stages of harvest and species was not significant (Appendix 3). In both the species the number of roots increased steadily up to 18 MAP (14.47 and 25.60 by *P. rosea* and *P. zeylanica* respectively) (Fig. 5). However, these were statistically on par with that observed at 16 MAP in both the species.

Fresh weight of roots per plant and per hectare

The data on the fresh root yield as influenced by stages of harvest in *Plumbago* spp.is given in Table 3 (Plate 1 and 2). There was no significant difference between the two species at 1 percent level. However, *P. rosea* recorded a higher fresh weight of root of 55.53 g per plant as compared to *P. zeylanica* (51.55 g pl^{-1}). The fresh weight of root progressively increased with age (Fig. 6). Interaction between the species and the stage of harvest was also not significant (Appendix 4). However, *P. rosea* recorded 7.78 per cent higher yield than *P. zeylanica* at 18 months after planting. The fresh root yield per hectare also showed similar pattern (Table 3).

Dry weight of roots per plant and per hectare

The results furnished in Table 3 showed that there was significant difference in dry weight of roots of both the species. *P. zeylanica* recorded the higher value of 25.08 g per plant as against 12.29 g per plant in *P. rosea.* The percentage increase in yield over *P. rosea* was 83.5. It was observed that retaining the crop in the field up to 18 months increased the dry root yield by 2.27 times in *P. rosea* and 2.01 times in *P. zeylanica* as

compared to harvesting after 12 months. The interaction between the species and stages of harvest was significant (Appendix 4). The results furnished in Fig. 7 showed that dry weight of root of *P. zeylanica* was the maximum during 18 MAP (41.73 g pl^{-1}). In *P. rosea* also maximum dry weight was observed at 18 MAP (22.73 g pl^{-1}). This was on par with the dry weight of root at 16 MAP (17.7 g pl^{-1}). Similar results were also noticed in terms of per hectare yield (Table 3).

Table 3. Influence of different stages of harvest on fresh and dry weight of root in two species of *Plumbago*

Stages of harvest	Fresh weight of root (g $plant^{-1}$)		Dry weight of root (g $plant^{-1}$)		Fresh weight of root (t ha^{-1})		Dry weight of root (t ha^{-1})	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
6 MAP	21.63	26.56	5.27	9.70	2.06	2.52	0.50	0.92
8 MAP	32.43	29.23	8.13	17.87	3.08	2.78	0.77	1.69
10 MAP	36.03	39.63	8.43	21.90	3.42	3.77	0.80	2.08
12 MAP	60.70	50.53	10.00	20.83	5.77	4.80	0.95	1.98
14 MAP	69.87	66.77	13.80	29.83	6.64	6.34	1.31	2.83
16 MAP	79.43	70.83	17.70	33.67	7.55	6.73	1.68	3.19
18 MAP	88.63	77.26	22.73	41.73	8.42	7.34	2.16	3.97
Mean	55.53	51.55	12.29	25.08**	5.28	4.89	1.17	2.38**
Stages of harvest								
SE m ±	1.842		0.949		0.1673		0.087	
CD	5.59		2.88		0.51		0.294	
Stages of harvest *vs* Species								
SE m ±	3.561		1.828		0.338		0.174	
CD	NS		5.55		NS		0.527	

MAP: Months After Planting
** Significant at 5 % level

Fresh weight of stem

Fresh weight of stem was significantly higher in *P. zeylanica* (65.84 g pl^{-1}) as compared to. 14.99 g per plant in *P. rosea* (Table 4). The interaction between different stages of harvest and species was also significant (Appendix 5). The values showed a progressive increase with advance in stages of harvest in both the species. The fresh weight of shoot at 18 MAP was on par with that at 14 and 16 MAP.

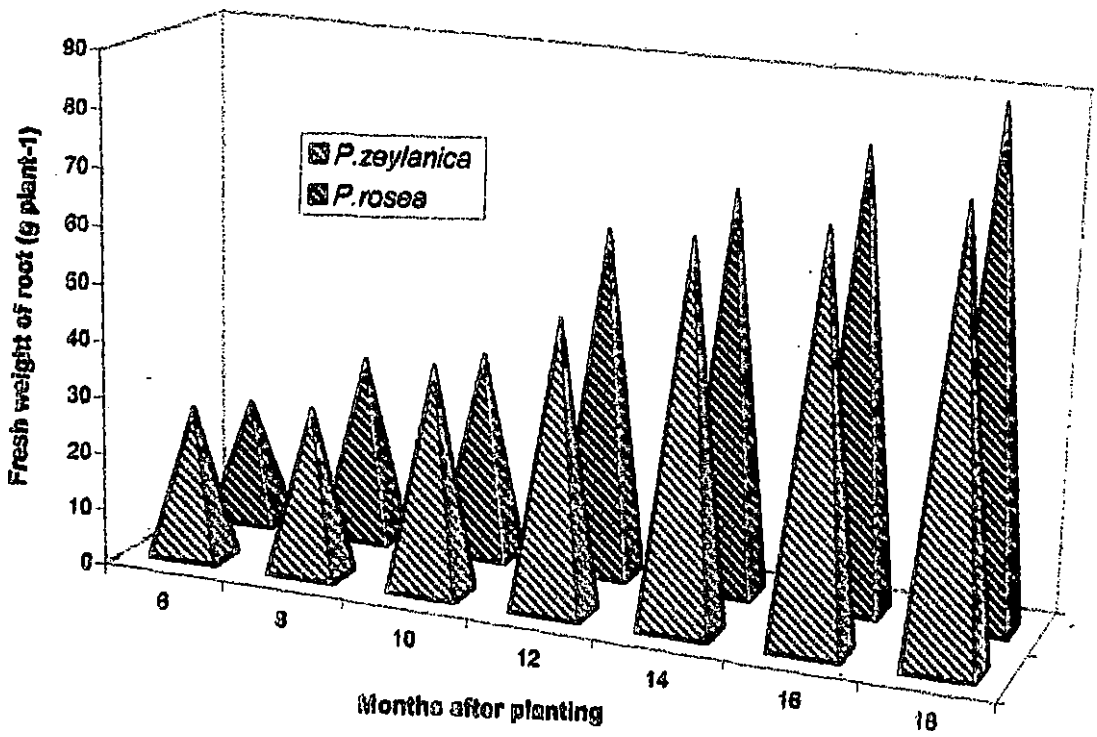


Fig.6. Fresh weight of roots at different stages of harvest in Plumbago spp.

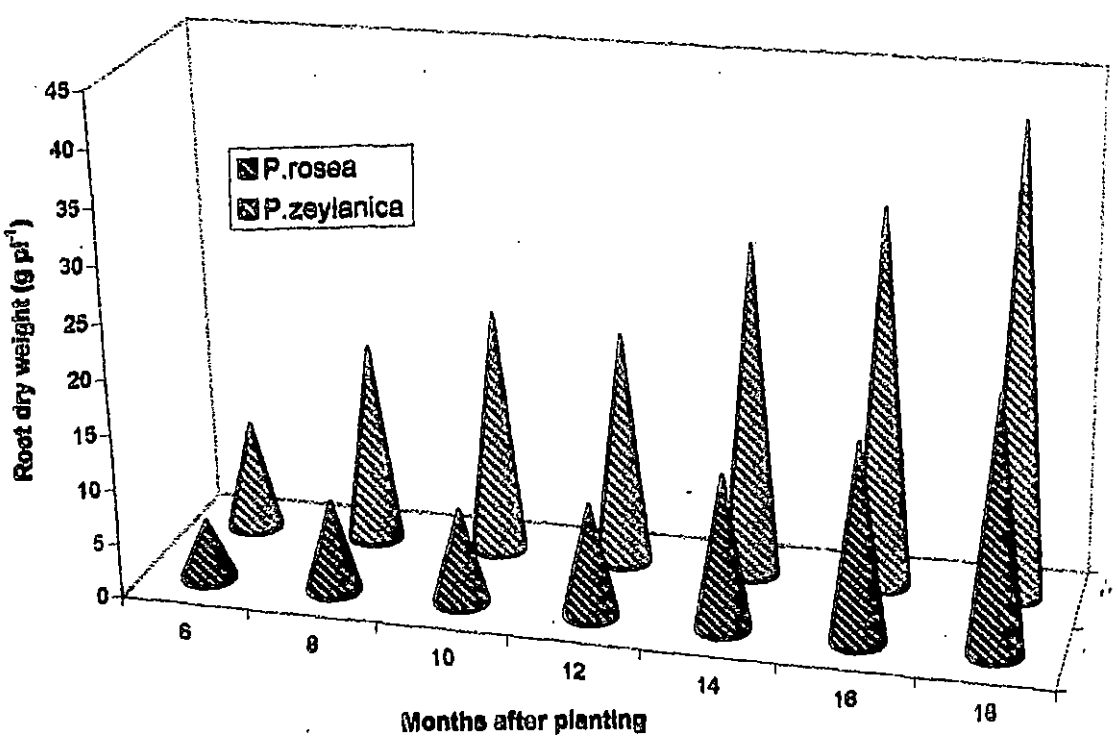


Fig.7. Dry weight of roots at different stages of harvest in *Plumbago* spp.

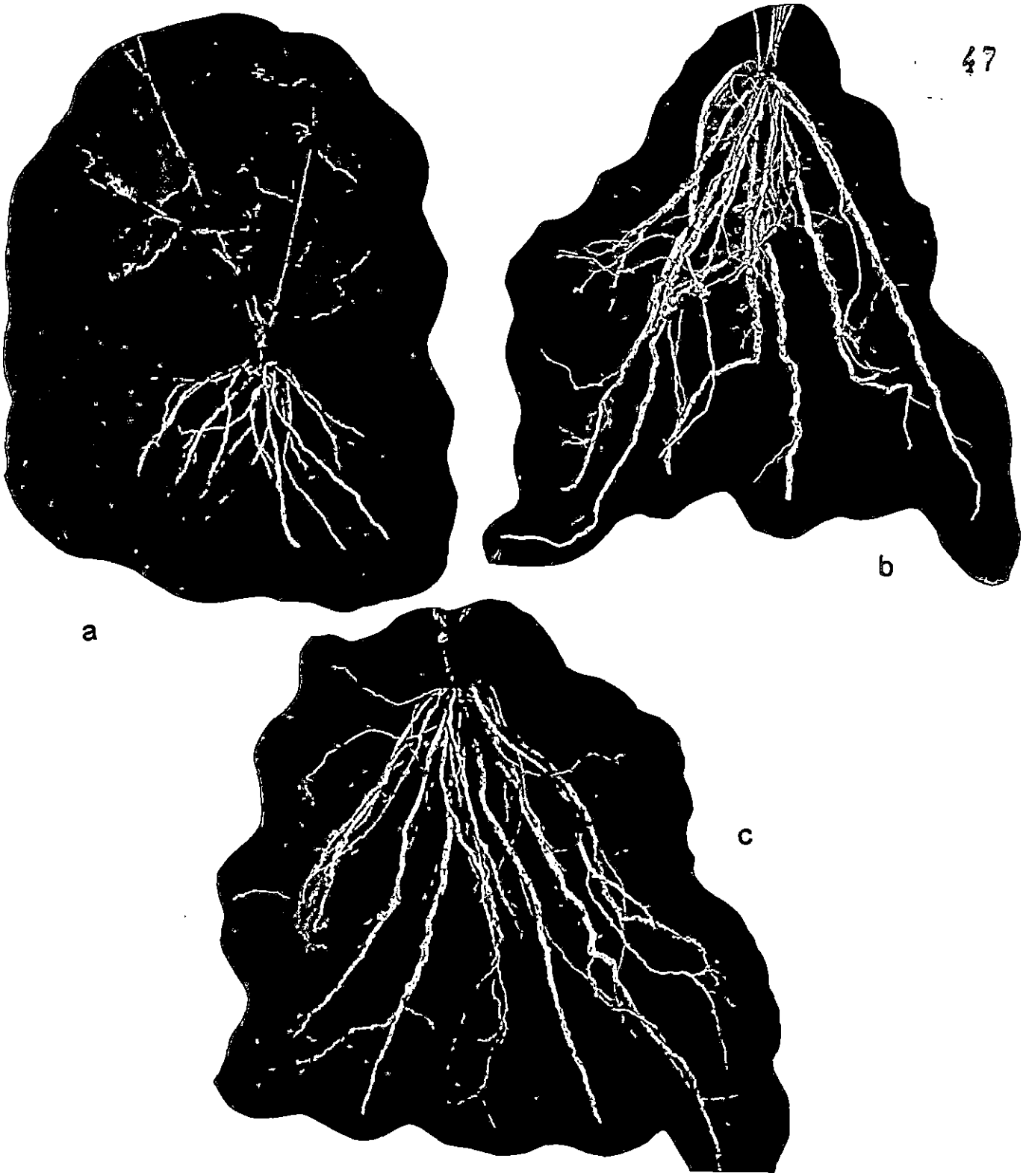


Plate 1 Roots of *Plumbago rosea* at different stages of growth

1a at 8 months after planting
1b at 12 months after planting
1c at 18 months after planting

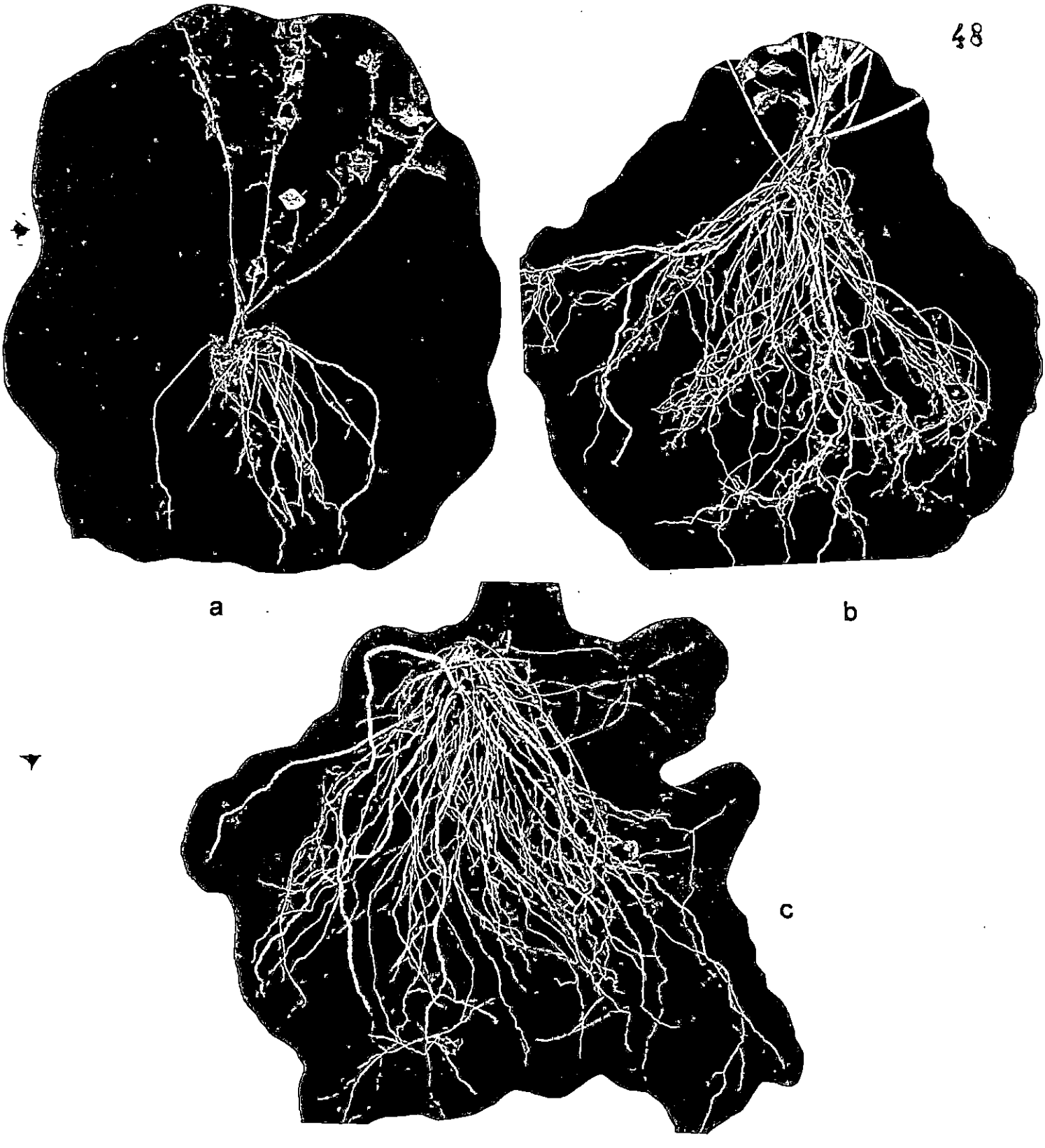


Plate 2 Roots of *Plumbago zeylanica* at different stages of growth

2a at 8 months after planting
2b at 12 months after planting
2c at 18 months after planting

Table 4. Influence of stages of harvest on fresh and dry weight of stem and leaf in two species of *Plumbago*

Stages of harvest	Fresh weight of stem (g plant^{-1})		Dry weight of stem (g plant^{-1})		Fresh weight of leaf (g plant^{-1})		Dry weight of leaf (g plant^{-1})	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
6 MAP	3.77	22.50	1.87	9.77	3.06	15.83	1.43	2.93
8 MAP	6.20	26.10	2.50	14.27	4.90	12.50	2.07	6.47
10 MAP	7.10	34.27	2.87	18.73	2.93	8.57	1.17	5.47
12 MAP	10.70	79.90	3.33	26.93	6.67	64.10	1.90	15.10
14 MAP	18.63	91.43	5.87	42.40	13.87	30.00	3.60	9.20
16 MAP	24.37	98.50	7.63	45.40	16.83	33.00	4.90	11.13
18 MAP	34.20	108.17	12.13	56.26	12.50	11.50	5.03	5.17
Mean	14.99	65.84**	5.17	30.54**	8.68	25.12**	2.87	7.92**
Stages of harvest								
SE m ±	4.528		S		2.32		0.598	
CD	13.73		6.07		1.735		1.814	
Stages of harvest *vs* Species								
SE m ±	7.332		S		2.835		0.890	
CD	22.24		8.94		8.59		2.69	

MAP: Months After Planting
** Significant at 1 % level

Dry weight of stem

Dry weight of stem was also significantly higher in *P. zeylanica* (30.54 g pl^{-1}). The dry weight recorded by *P. rosea* was only 5.17 g per plant (Table 4). The interaction was also significant (Appendix 5). As in the case of fresh weight, the increase in dry weight was steady up to 18 MAP and significantly higher values were observed at 18 MAP in both the species (12.13 g pl^{-1}in *P. rosea* and 56.27 g pl^{-1}in *P. zeylanica*).

Fresh weight of leaf

The data furnished in Table 4 suggested that fresh weight of leaf was significantly higher in *P. zeylanica* (25.12 g pl^{-1}). The value recorded by *P. rosea* was only 8.68 g per plant. The interaction between the species and stages of harvest was also significant (Appendix 5). The fresh weight of leaf was significantly high at 12 MAP in *P. zeylanica* (64.1 g pl^{-1}) and thereafter the fresh weight tended to decline. In *P. rosea*, there was a progressive increase up to 14 MAP. The fresh weights at 14,16 and 18 MAP were on par.

Dry weight of leaf

As in the case of fresh weight, dry weight of leaf was significantly higher in *P. zeylanica* (7.92 g pl^{-1}) as compared to *P. rosea* (2.87 g pl^{-1}) (Table 4). The interaction was also significant (Appendix 5). Significantly higher dry weight of leaf was observed at 12 MAP in *P. zeylanica* (15.1 g pl^{-1}). In *P. rosea,* there was a steady increase in dry weight of leaf up to 14 MAP and there after there was no significant difference.

4.1.2 Physiological characters

Total dry matter per plant and per hectare

Total dry matter, which included the dry matter of various parts viz., leaves, stem and root showed a progressive increase with advance in the age of plant in both the species. *P. zeylanica* recorded higher total dry matter of 63.54 g per plant as against 20.88 g per plant in *P. rosea* (Table 5). The interaction between stages of harvest and species was significant at 1% level (Appendix 6). The total dry matter was significantly high at 18 MAP in both the species (39.9 g pl^{-1}in *P. rosea* and 103.17 g pl^{-1} in *P. zeylanica*) (Fig. 8). Similar results were obtained in respect of total dry matter per hectare (Table 5, Fig. 9).

Table 5. Total dry matter, root: shoot ratio and harvest index in two species of *Plumbago* as influenced by different stages of growth

Stages of harvest	Total dry matter (g $plant^{-1}$)		Total dry matter (t ha^{-1})		Root: shoot ratio		Harvest index	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
6 MAP	8.57	22.40	0.81	2.13	1.59	0.78	0.61	0.44
8 MAP	12.70	38.60	1.21	3.67	1.87	0.87	0.65	0.47
10 MAP	12.47	46.10	1.18	4.38	2.10	0.91	0.68	0.47
12 MAP	19.03	62.87	1.81	5.97	1.92	0.51	0.58	0.33
14 MAP	23.27	81.43	2.21	7.74	1.45	0.59	0.59	0.37
16 MAP	30.23	90.20	2.87	8.57	1.43	0.60	0.59	0.38
18 MAP	39.90	103.17	3.79	9.80	1.33	0.91	0.57	0.41
Mean	20.88	63.54**	1.98	6.04**	1.67**	0.71	0.61**	0.41
Stages of harvest								
SE m ±	1.914		0.1676		0.091		0.025	
CD	5.81		0.51		0.28		0.08	
Stages of harvest *vs* Species								
SE m ±	3.932		0.374		0.119		0.045	
CD	11.93		1.13		NS		NS	

MAP: Months After Planting ** Significant at 1 % level

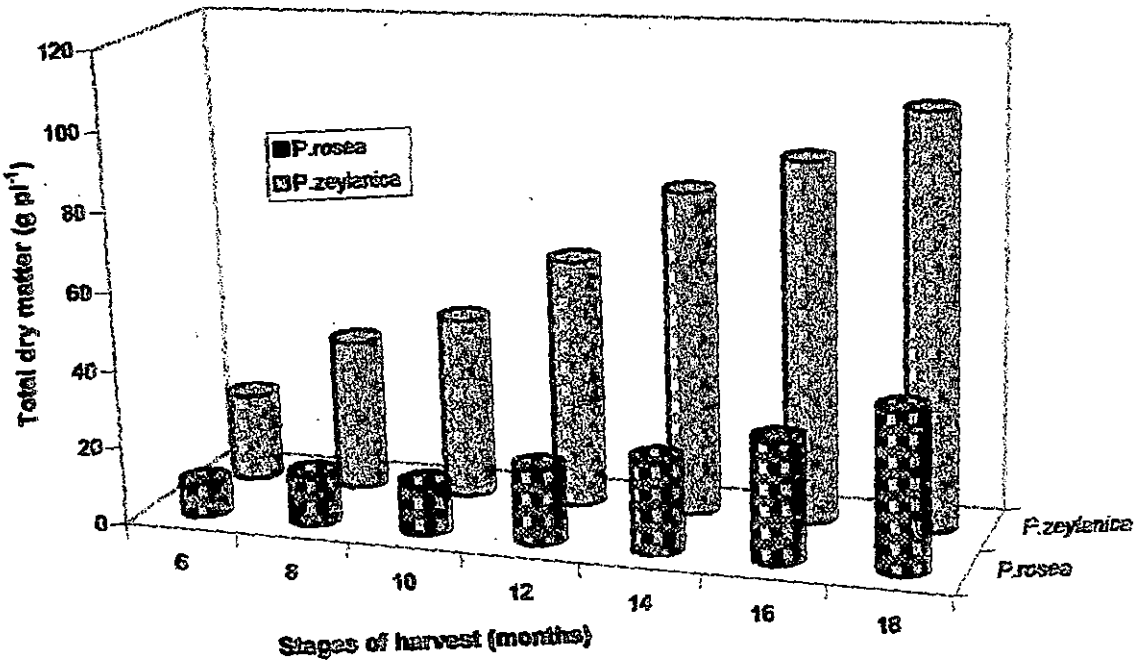


Fig. 8. Total dry matter per plant in *Plumbago* spp. at different stages of growth

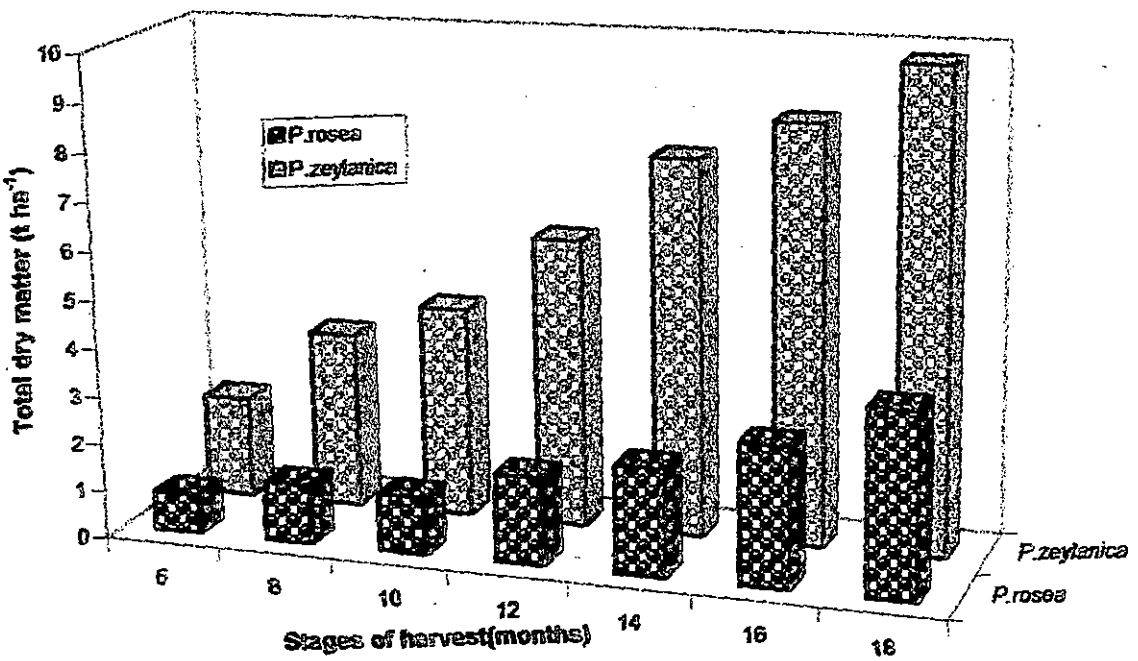


Fig 9. Total dry matter per hectare in *Plumbago* spp. at different stages of growth

Root : shoot ratio

The data furnished in Table 5 showed that the species differed significantly. A higher root: shoot ratio of 1.67 was recorded in *P. rosea* as compared to 0.71 in *P. zeylanica.* The interaction between the species and stages of harvest was not significant (Appendix 6). Both the species behaved in a similar manner throughout the various stages of harvest and higher root: shoot ratios were observed at 10 MAP (2.10 and 0.91 in *P. rosea and P. zeylanica* respectively). Thereafter there was a decline in root : shoot ratio.

Harvest Index

The harvest index was significantly higher in *P. rosea* (0.61) (Table 5). The interaction between the stages of harvest and species was not significant (Appendix 6). The highest harvest index was noticed at 10 MAP in both the species (0.68 in *P. rosea* and 0.47 on *P. zeylanica*). The values showed a decline at 12 MAP and increase was marginal in the two species there after.

Leaf Weight Ratio (LWR)

Leaf Weight Ratio (LWR) indicates the proportion of total dry matter contributed by the leaf surface. The data on the effect of stages of growth on the LWR in *Plumbago* spp. has been presented in Table 6. It showed that the LWR did not vary between species The interaction between stages of harvest and species was significant (Appendix 7). The leaf weight ratio did not show a definite pattern during various stages of harvest in both species. In *P. rosea,* at 10 MAP, leaves contributed only nine per cent of the total dry weight. At 8, 14 and 16 MAP, it was 16 per cent. In respect of *P. zeylanica,* the contribution by the dry leaf weight was only 5 per cent at 18 MAP, while it was 24 per cent at 12 MAP.

Leaf Area Ratio (LAR)

Leaf area ratio measured as the ratio of assimilatory surface to plant dry weight or the leaf area required to produce one gram of dry matter was estimated at different stages and the data are presented in Table 6. Leaf area ratio was significantly higher in

P. zeylanica (0.005). The interaction was also significant (Appendix 7). The highest leaf area ratio was noticed at 12 MAP in *P. zeylanica* (0.011). In *P. rosea* significantly higher value of 0.006 was observed at 16 MAP. In *P. zeylanica* leaf area ratio recorded at 16 MAP and 6 MAP were on par.

Table 6. Influence of different stages of harvest on leaf area index, leaf weight ratio and leaf area ratio in two species of *Plumbago*

Stages of harvest	Leaf area index		Leaf weight ratio		Leaf area ratio	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
6 MAP	0.22	1.16	0.16	0.13	0.003	0.006
8 MAP	0.52	1.24	0.16	0.17	0.004	0.003
10 MAP	0.32	1.19	0.09	0.12	0.003	0.003
12 MAP	0.44	6.36	0.12	0.24	0.003	0.011
14 MAP	0.98	2.58	0.16	0.11	0.004	0.003
16 MAP	1.58	4.86	0.16	0.12	0.006	0.005
18 MAP	1.35	1.33	0.13	0.05	0.004	0.001
Mean	0.77	2.67**	0.14	0.13	0.004	0.005**
Stages of harvest						
SE m ±	0.0372		0.0156		0.0002	
CD	0.113		0.05		0.0006	
Stages of harvest *vs* Species						
SE m ±	0.043		0.024		0.005	
CD	0.130		0.07		0.0015	

MAP: Months After Planting
** Significant at 1 % level

Leaf Area Index (LAI)

Leaf area index represents the total assimilatory surface per unit area of land surface. How leaf area index varied with age for the two species is shown in Table 6. The results showed that *P. zeylanica* recorded a significantly higher overall mean leaf area index of 2.67 as against 0.72 in *P. rosea.* The interaction was significant (Appendix 7). In *P. zeylanica,* significantly higher value of 6.36 was observed at 12 MAP. During other stages, the data did not follow any definite pattern. But in *P. rosea* the highest value was recorded at 16 MAP (1.58). The LAI was the lowest at 6 MAP in *P. rosea* (0.22).

Crop Growth Rate (CGR)

It is the increase in dry weight in unit area of the crop in unit time. The crop growth rates during different stages of growth in two species are summarised in Table 7. Crop growth rate was significantly higher in *P. zeylanica* (2.138 g per day per m^2 land area)

as compared to *P. rosea* (0.829 g per day per m^2 land area). The data on CGR at different growth intervals in the two species showed wide fluctuation and it did not differ significantly. The CGR was the highest at 18 MAP in *Plumbago rosea* (1.533 g per day per m^2 land area). At 10 MAP it showed a negative value of 0.037 g per day per m^2 land area. Crop growth rate of *P. zeylanica* was the highest at 14 MAP (2.950 g per day per m^2 land area). In this species, the lowest value was recorded at 10 MAP(1.190 g per day per m^2 land area).

Table 7. Influence of different stages of harvest on crop growth rate, net assimilation rate and relative growth rate in two species of *Plumbago*

Stages of harvest	Crop growth rate ($g\ day^{-1}m^{-2}$ land area)		Net assimilation rate ($g\ day^{-1}m^{-2}$ land area)		Relative growth rate ($g\ day^{-1}m^{-2}$ land area)	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
10 MAP	-0.037	1.190	-0.016	0.187	-0.073	-0.201
12 MAP	1.040	2.663	0.364	0.277	0.114	0.154
14 MAP	0.672	2.950	0.049	0.733	-0.039	0.030
16 MAP	1.106	1.394	0.012	0.390	0.046	-0.163
18 MAP	1.533	2.063	-0.013	0.357	0.045	0.070
Mean	0.829	2.138**	0.061	0.389**	0.019	-0.022
Stages of harvest						
SE m ±	0.365		0.202		0.075	
CD	NS		NS		NS	
Stages of harvest *vs* Species						
SE m ±	0.438		0.205		0.082	
CD	NS		NS		NS	

MAP: Months After Planting
** Significant at 1 % level

Net Assimilation Rate (NAR)

Net assimilation rate (NAR) is the increase in plant weight per unit area of assimilating surface per unit time was calculated during the different stages of growth. The data are presented in Table 7. The results showed that there was significant difference between the species. *P. zeylanica* recorded a higher net assimilation rate of 0.389 g per m^2 leaf area per day as against 0.061 g per m^2 leaf area per day recorded by *P. rosea*.

The results showed that at different stages of harvest, the NAR did not vary significantly. However, it was found that the NAR was the highest (0.364 g per m^2 leaf area per day) in *P. rosea* at 12 MAP. In the case of *P. zeylanica*, the highest value of

0.733 g per m^2 leaf area per day was recorded at 14 MAP. The two species did not exhibit a definite pattern in NAR during different harvest intervals. Negative values were obtained in *P. rosea* at 10 and 8 MAP.

Relative Growth of Rate (RGR)

Relative growth rate (RGR) is an index of rate of increase in biomass per unit of existing biomass. The two species did not differ significantly during various stages of growth in respect of this character (Table 7). The results showed that RGR was 0.073 g per g per day in *P. rosea* at 10 MAP, which rose to 0.114 g per g per day at 12 MAP and thereafter it decreased. In *P. zeylanica*, the highest value of 0.154 g per g per day was recorded at 12 MAP and negative values were recorded at 10 and 16 MAP.

4.1.3 Growth rate

Linear growth rate of two species was calculated using the method of least square and data are furnished in Table 8. The observed and expected values of different parameters are furnished in Fig. 10 and Fig. 11 after fitting linear model equation (Appendix 8).

The two species exhibited linear pattern of growth with respect to most of the characters studies up to 18 MAP. The rate of increase in fresh weight of root, fresh weight of leaf, length and girth of root and internodal length were higher in *P. rosea*. *P. zeylanica* recorded higher rate of growth in all other growth parameters.

Table 8. Linear growth rate (LGR) in two species of *Plumbago*

Character	Linear growth rate (%)	
	P. rosea	*P. zeylanica*
Fresh weight of root	11.740	9.370
Fresh weight of shoot	4.970	16.390
Fresh weight of leaf	2.250	1.790
Dry weight of root	2.750	4.870
Dry weight of shoot	1.570	8.050
Dry weight of leaf	0.680	0.710
Total dry matter	4.990	13.600
Plant height	5.530	12.840
Internodal length	-0.226	-0.340
Total leaf area	0.023	0.034
Girth of root	0.218	0.170
Number of root	1.490	1.690
Length of root	4.410	4.250

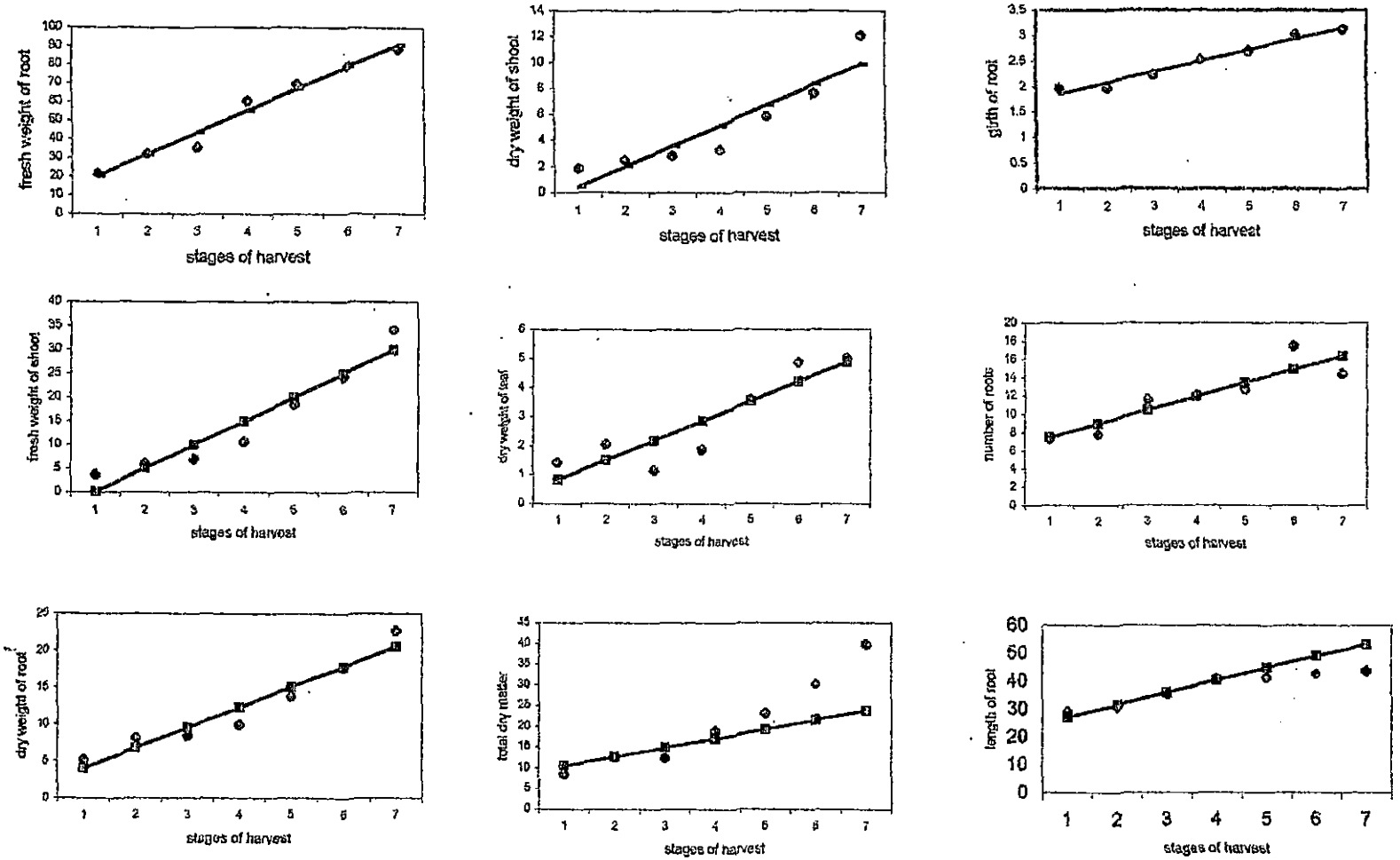


Fig. 10 Observed and expected growth parameters in *P. rosea*

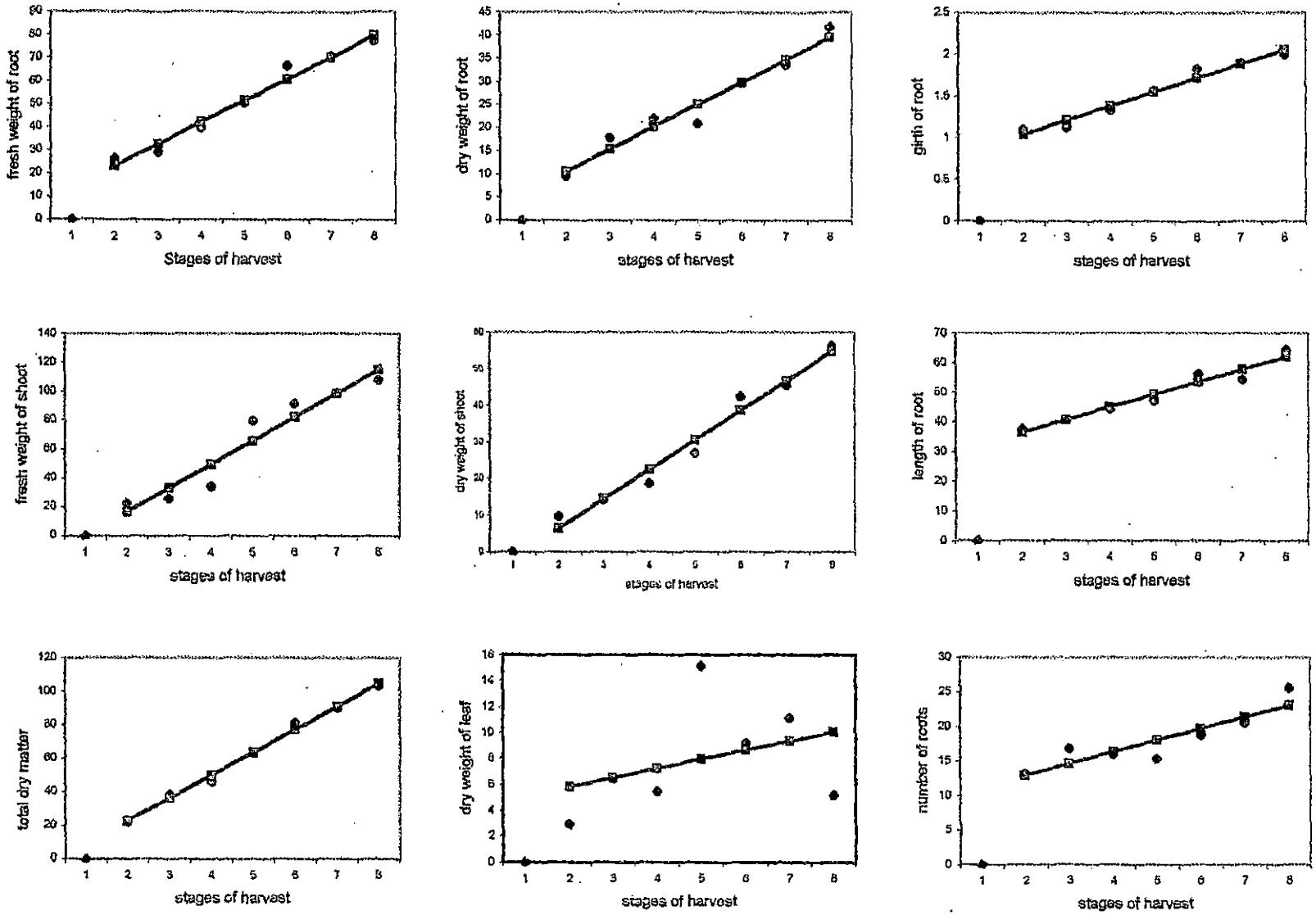


Fig. 11 Observed and expected growth parameters in *P. zeylanica*

4.1.4 Response function

Fresh weight of roots

In order to find out the characters that influence the fresh weight of roots, step wise regression analysis was carried out and the results are given in Appendix 9. The results revealed that fresh weight of roots per plant was directly influenced by fresh weight of leaf and root length in *Plumbago rosea* and root girth in *P. zeylanica.* The prediction equations derived are furnished below:

Fresh weight of root (g plant^{-1}) = $1.994 X_1 + 1.472 X_2 - 21.13$ for *P. rosea.*

$R^2 = 0.94$

Fresh weight of root (g plant^{-1}) = $51.522 X_3 - 28.43$ for *P. zeylanica.*

$R^2 = 0.91$

where,

X_1 = fresh weight of leaf (g pl^{-1})

X_2 = length of root (cm.)

X_3 = girth of root (cm.)

Total dry matter per plant

In order to find out the important biometric characters that influence the total dry matter, step down regression analysis was conducted. The results are furnished in Appendix 10. The data indicated that the character was directly influenced by fresh weight of shoot, length of root and plant height in *P. rosea.* The result in respect of *P. zeylanica* showed the direct influence of fresh weight of shoot, fresh weight of root and number of roots on total dry matter per plant. The prediction equations derived are given below:

Total dry matter (g plant^{-1}) = $0.465 X_4 + 0.375 X_2 + 0.181 X_7 - 11.04$ for *P. rosea.*

$R^2 = 0.92$

Total dry matter (g plant^{-1}) = $0.373 X_4 + 0.595 X_5 + 0.964 X_6 - 9.02$ for *P. zeylanica.*

$R^2 = 0.98$

where,

X_4 = fresh weight of shoot (g pl^{-1})

X_5 = fresh weight of root (g pl^{-1})

X_6 = number of root

X_7 = Plant height (cm.)

Path coefficient analysis

The direct and indirect effect of most dependable yield parameter on total dry matter production was calculated. The results suggested that in *P. rosea* (Table 9) fresh weight of shoot had a direct effect on total dry matter production (0.4663). The effect of root length (0.3424) and plant height (0.2453) were indirect, through their influence on the weight of shoot (0.3882 and 0.3228 respectively).

Table 9. Path coefficient analysis in *P. rosea*

Characters	Direct and indirect effect on total dry matter production			Correlation coefficient
	Fresh weight of shoot	Root length	Plant height	
Fresh weight of shoot	**0.4663**	0.285	0.1698	0.9211
Root length	0.3882	**0.3424**	0.1519	0.8825
Plant height	0.3228	0.2121	**0.2453**	0.7802

Residual path value : 0.0769

In the case of *P. zeylanica* (Table 10), the fresh weight of shoot had higher direct effect on the total dry matter (0.489). The effects of fresh weight of root (0.4136) and root number (0.1604) were indirect, through their influence on fresh weight of shoot (0.4348 and 0.2903 respectively).

Table 10. Path coefficient analysis in *P.zeylanica*

Characters	Direct and indirect effect on total dry matter production			Correlation coefficient
	Fresh weight of roots	Fresh weight of shoots	Root number	
Fresh weight of roots	**0.4136**	0.4348	0.1041	0.9325
Fresh weight of shoots	0.3672	**0.4897**	0.0951	0.9520
Root number	0.2683	0.2903	**0.1604**	0.7100

Residual path value : 0.0246

4.1.5 Biochemical characters

Plumbagin

The crude and purified plumbagin crystals extracted from the roots are depicted in Plate 3. The total quantity of acetone extract and purified plumbagin recovered from the roots of both the species are furnished in Table 11 and Fig. 12. The data revealed that the quantity of crude extract and plumbagin recovered were higher in *P. rosea*. However, *P. rosea* showed fluctuations in quantity of acetone- extractibles at different stages of harvest. The highest value of 4.67 per cent was recorded at 16 MAP, which coincided with flowering of this species. The variations in respect of *P. zeylanica*, was only slight, ranging from 0.87 per cent at 16 MAP to 1.85 per cent at 8 MAP.

Table 11. Crude extract and plumbagin in the roots of *Plumbago* spp. at different stages of harvest (per cent on dry weight basis)

Stages of harvest	*P. rosea*		*P. zeylanica*	
	Crude extract (%)	Plumbagin (%)	Crude extract (%)	Plumbagin (%)
6 MAP	2.44	1.12	1.36	0.19
8 MAP	4.61	0.82	1.85	0.23
10 MAP	3.63	1.17	1.63	0.33
12 MAP	4.42	1.14	1.09	0.25
14 MAP	3.12	0.69	1.00	0.22
16 MAP	4.67	1.40	0.87	0.27
18 MAP	3.57	0.72	1.6	0.26

In *P. rosea*, the highest plumbagin recovery was obtained at 16 MAP, which coincided with flowering season (1.402 %). At all other stages this character showed wide variation. In *P. zeylanica*, plumbagin content remained almost same with a slightly higher value at 10 MAP (0.327 %) which coincided with the rainy season.

Changes during curing

The data pertaining to dry recovery, plumbagin levels and reduction of plumbagin at 12 and 18 MAP are furnished in Table 12.

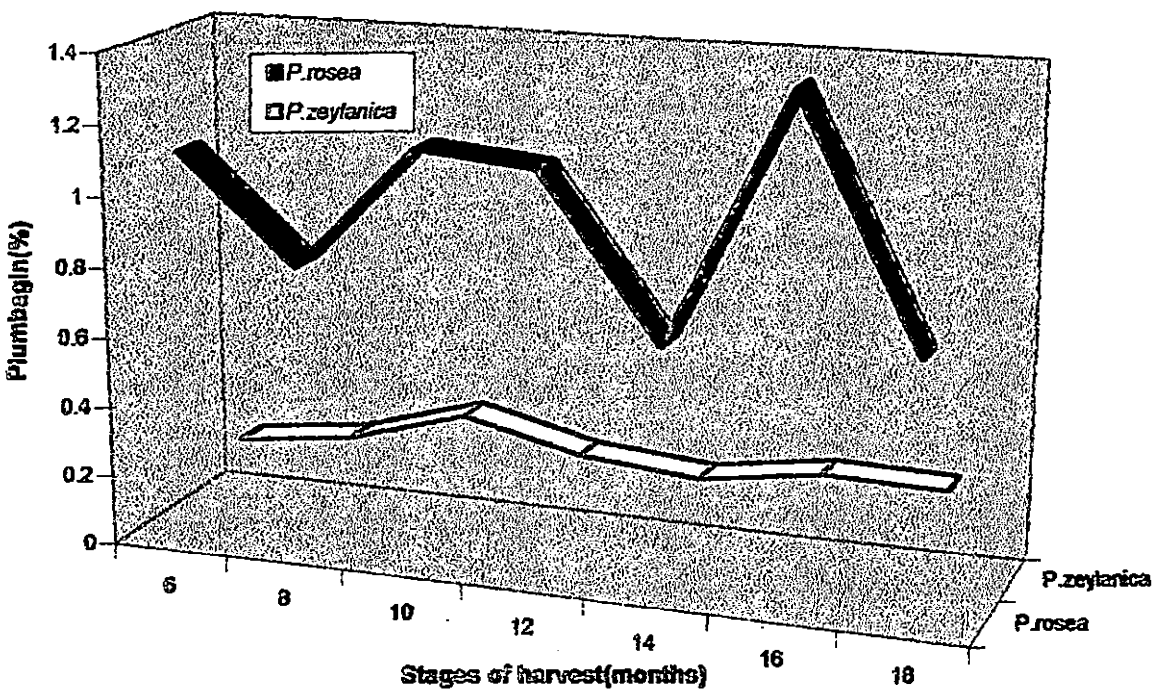
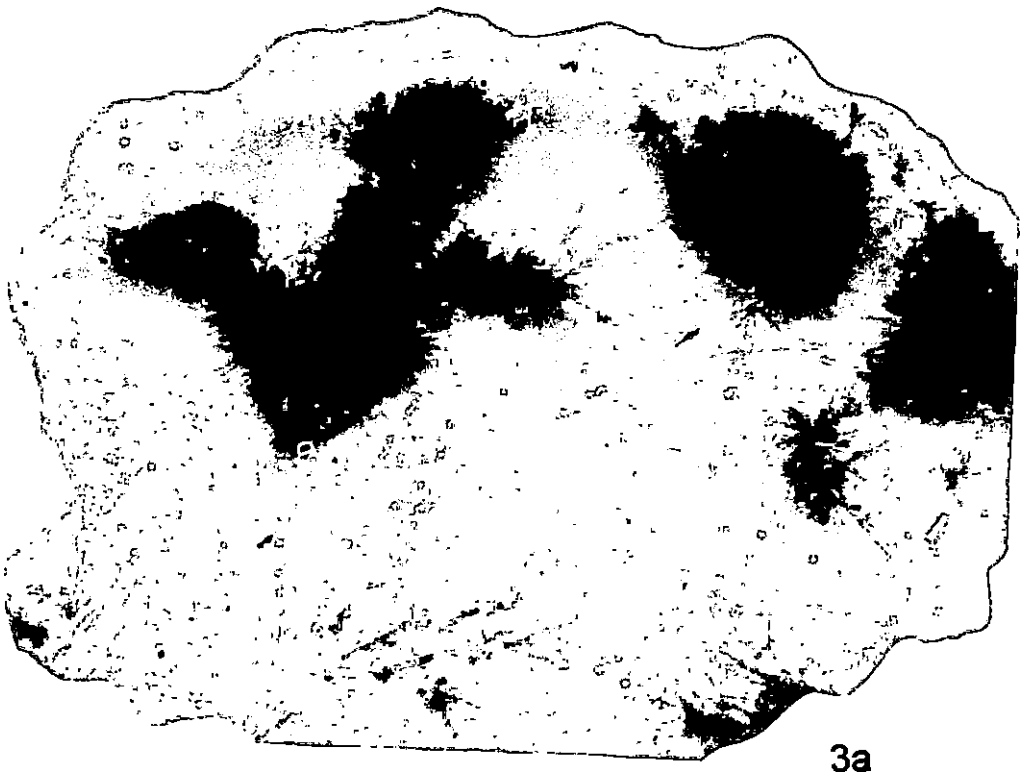


Fig.12. Plumbagin content in the roots of *Plumbago* spp. at different stages of harvest



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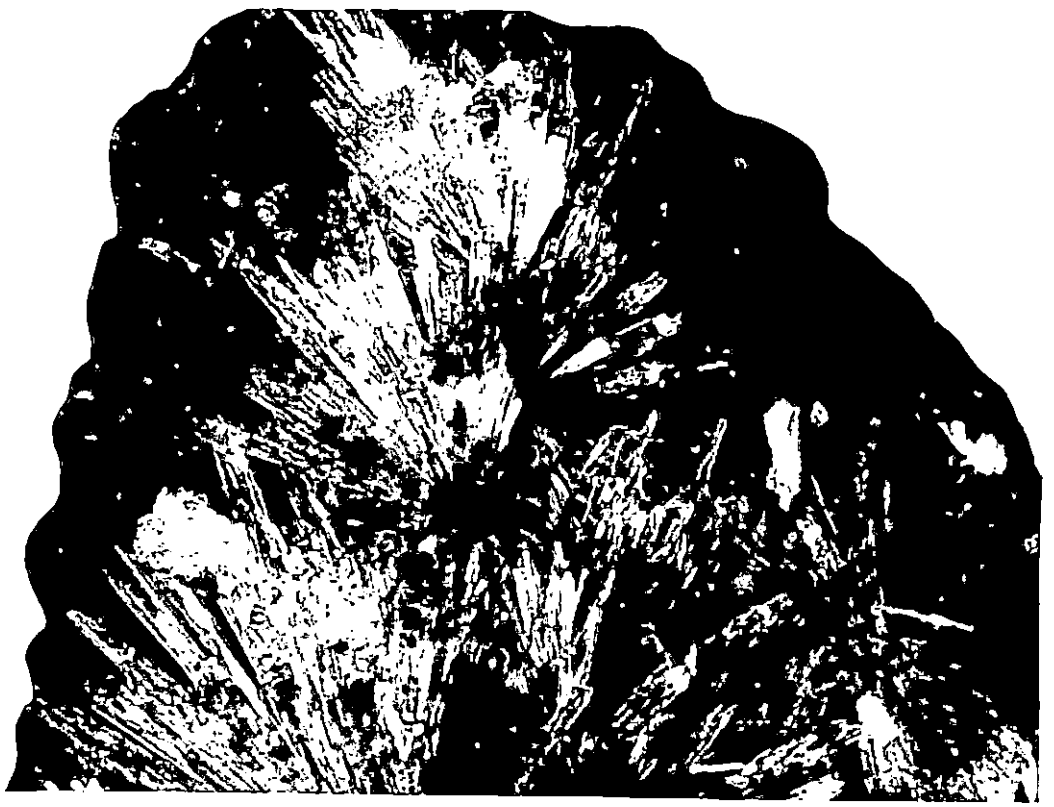


3b

Plate 3 Plumbagin

3a Crystals of plumbagin

3b Yellow needle shaped crystals as visible under stereomicroscope

Table 12. Dry recovery and plumbagin content in fresh, dry and cured roots of *Plumbago* spp.

Species / treatment	12 MAP			18 MAP		
	Dry recovery (%)	Plumbagin (%)	Loss of plumbagin (%)	Dry recovery (%)	Plumbagin (%)	Loss of plumbagin (%)
P. rosea	15.3			25.3		
Fresh roots		0.46			0.57	
Dry roots		1.14	62		0.72	68.33
Cured roots		0.03	93.47		0.07	87.71
P. zeylanica	40.5			54.1		
Fresh roots		0.46			0.36	
Dry roots		0.25	79.38		0.26	60.97
Cured roots		0.03	93.47		0.01	97.22

The fresh roots of *P. rosea* recorded a plumbagin content of 0.46 per cent at 12 MAP and 0.57 per cent at 18 MAP. In respect of dry recovery at 12 MAP, *P. rosea* recorded a lower value of 15.3 per cent as against 25.1 per cent at 18 MAP. The data indicated that on drying, the roots recorded a loss in plumbagin to the extent of 62 per cent at 12 MAP and 68.33 per cent at 18 MAP (Fig. 13). Curing the roots in lime solution (5 %), recorded a further reduction to 93.47 per cent plumbagin at 12 MAP and 87.71 per cent at 18 MAP (Fig. 15).

In the case of *P. zeylanica*, the fresh roots recorded a plumbagin content of 0.46 per cent at 12 MAP and 0.36 per cent at 18 MAP. Dry recovery was high when compared to *P. rosea* (40.50 % at 12 MAP, 54.10 % at 18 MAP). It was observed that the loss of plumbagin content in *P. zeylanica* at 12 MAP and 18 MAP were 93.47 and 97.22 per cent (Fig. 16) respectively on curing. Drying resulted in reduction of plumbagin content to the extent of 79.38 per cent at 12 MAP and 60.97 per cent at 18 MAP (Fig. 14).

Efficiency of different solvents on extraction of plumbagin

The results are furnished in Table 13. Among the different solvents tried, ethyl acetate gave the highest recovery of plumbagin (0.49 %) in *P. zeylanica*. In *P. rosea* acetone gave the highest recovery per cent of 0.49 from fresh roots. The data showed that tap water and lime solution (5 %) could recover only negligible quantities of plumbagin (Plate 4).

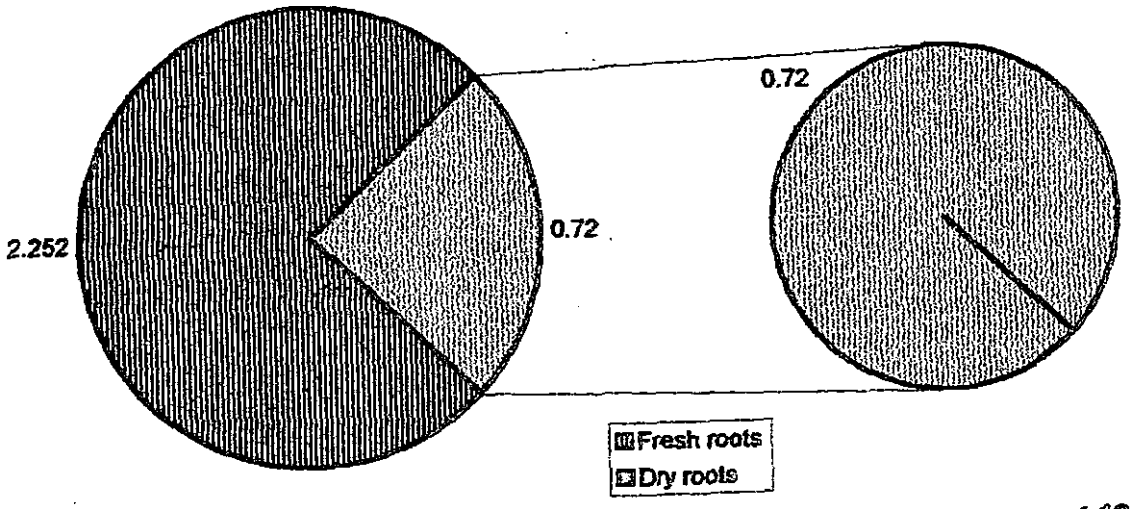


Fig.13. Plumbagin content in fresh and dry roots of *Plumbago rosea* at 18 MAP

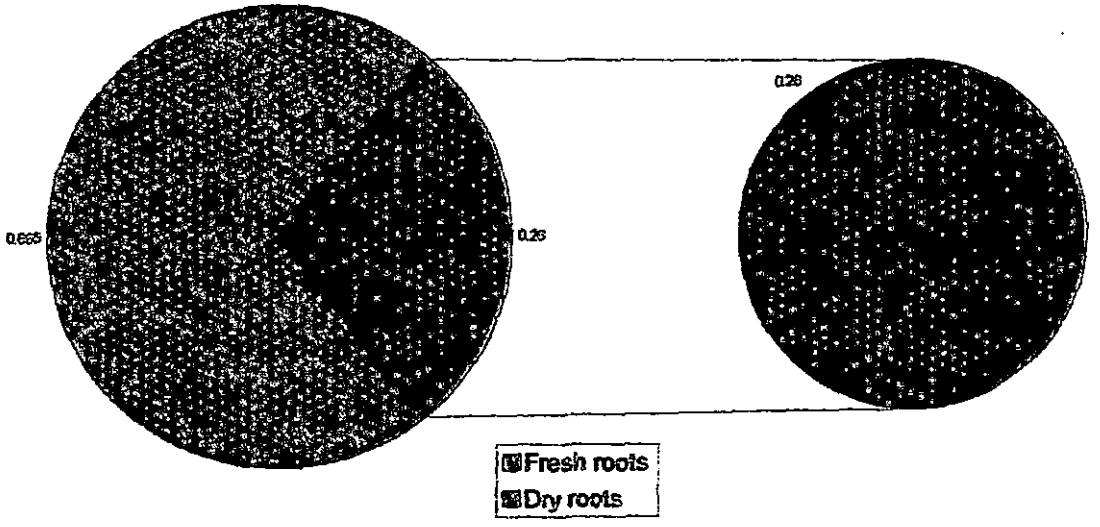


Fig.14. Plumbagin content in fresh and dry roots of *P. zeylanica* at 18 MAP

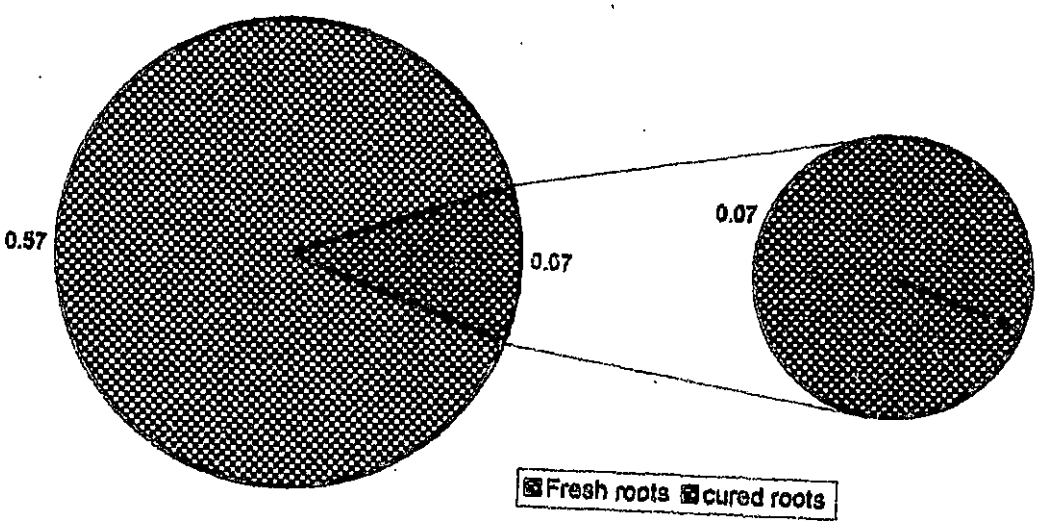


Fig.15.Loss of plumbagin on curing in *P.rosea* at 18 MAP

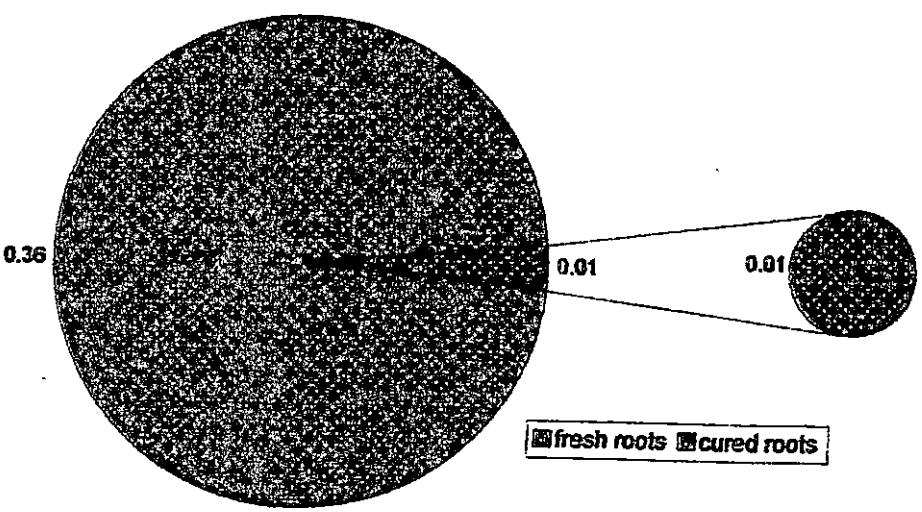


Fig.16. Loss of plumbagin (%) on curing in *P.zeylanica* at 18 MAP.

P. rosea *P. zeylanica*

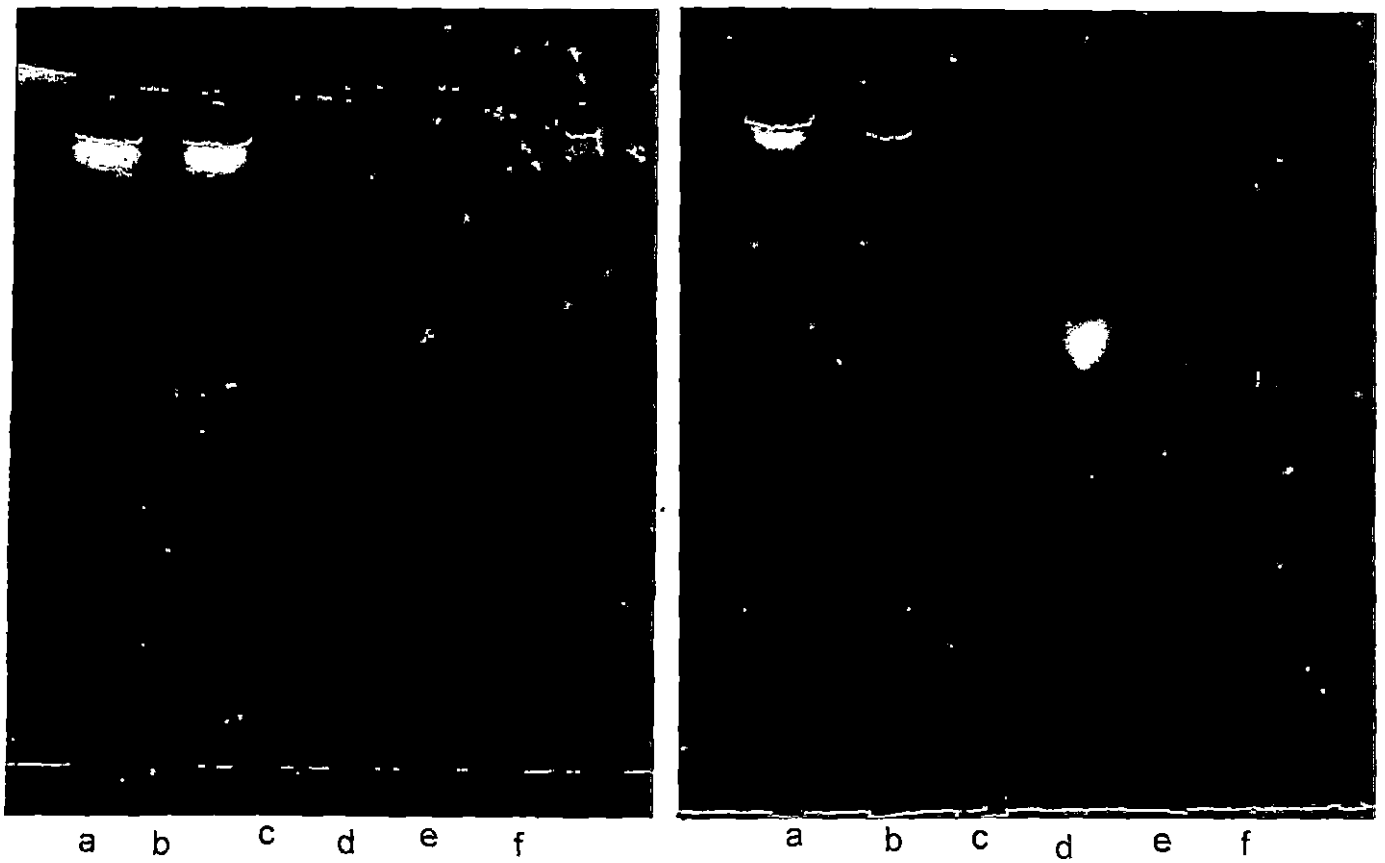


Plate 4 Thin layer chromatogram of roots of *Plumbago* spp. in different solvents

4.1 TLC of root extract of *P. rosea*
4.2 TLC of root extract of *P. zeylanica*

a. ethyl acetate extract
b. acetone extract
c. roots after washing in water
d. roots after washing in lime solution (0.5 % $Ca(OH)_2$)
e. water extract
f. lime (0.5 % $Ca(OH)_2$) extract

Table 13. Efficiency of different solvents on the extraction of plumbagin		
Solvents	Recovery of plumbagin (%)	
	P. rosea	*P. zeylanica*
Ethyl acetate	0.36	0.49
Acetone	0.49	0.25
Lime solution (5%)	0.05	0.03
Tap water	0.03	0.07

Infra Red Spectrum

In the infrared absorption spectrum of plumbagin (Fig. 17), presence of 1, 4, quinone group was supported by the strong band at 1663 cm^{-1} and a broad brand in the region 3300 to 3700 cm^{-1} indicated the presence of an intramolecular hydrogen bonded phenolic -OH group.

Thin layer chromatogram

Thin layer chromatogram of fresh, dry and cured root extracts in 3: 1 Hexane : Ethylacetate is given in Plate 5a and 5c. The Rf values are given in Table 14.

Table 14. Chromatogram of *P. rosea* and *P. zeylanica*							
	Colour of spot	*P. rosea*			*P. zeylanica*		
		Fresh	Dry	Cured	Fresh	Dry	Cured
Visible light	Yellow	0.89	0.89	0.89(faint)	0.89	0.89	0.89(faint)
	Light yellow	-	0.79	-	-	0.79	-
	Light yellow	-	-	0.64	-	-	-
	Pink	0.23	0.23	0.23	faint	0.2	0.2
	Yellow	-	-	0.04	-	-	0.04
Fluorescent light	366 nm	0.88	0.88	0.88	0.88	0.88	0.88
	366 nm	-	-	0.56	-	0.56	0.56
	254 nm	-	-	0.56	-	0.56	0.56

The fresh root extract of both the species expressed two spots at Rf values 0.89 and 0.23. The Rf 0.89 was identified as plumbagin by spotting purified plumbagin. The pink spot at Rf 0.23 was an unknown compound and this was recorded in both the species. The dry root sample gave an additional yellow spot at Rf 0.79 in both the species. When the plates were subjected to UV rays at 366 nm, fresh, dry and cured root samples of both the species gave a bright fluorescence at Rf 0.88 just below the plumbagin fraction. In addition to this there was a fraction of Rf 0.56 in cured samples of both the species and in dry

---------- PARAMETERS OF SPECTRUM -- 1997/10/29 14:34:00 ---
MEASURING MODE ; %T
RESOLUTION ; 4.0 cm-1
NO. OF SCAN ; 40
GAIN ; AUTO
DETECTOR ; DETECTOR 1 (2.8 mm/sec)
APODIZATION ; HAPP-GENZEL
REMARKS ; B
ANALYST ; SHAFI

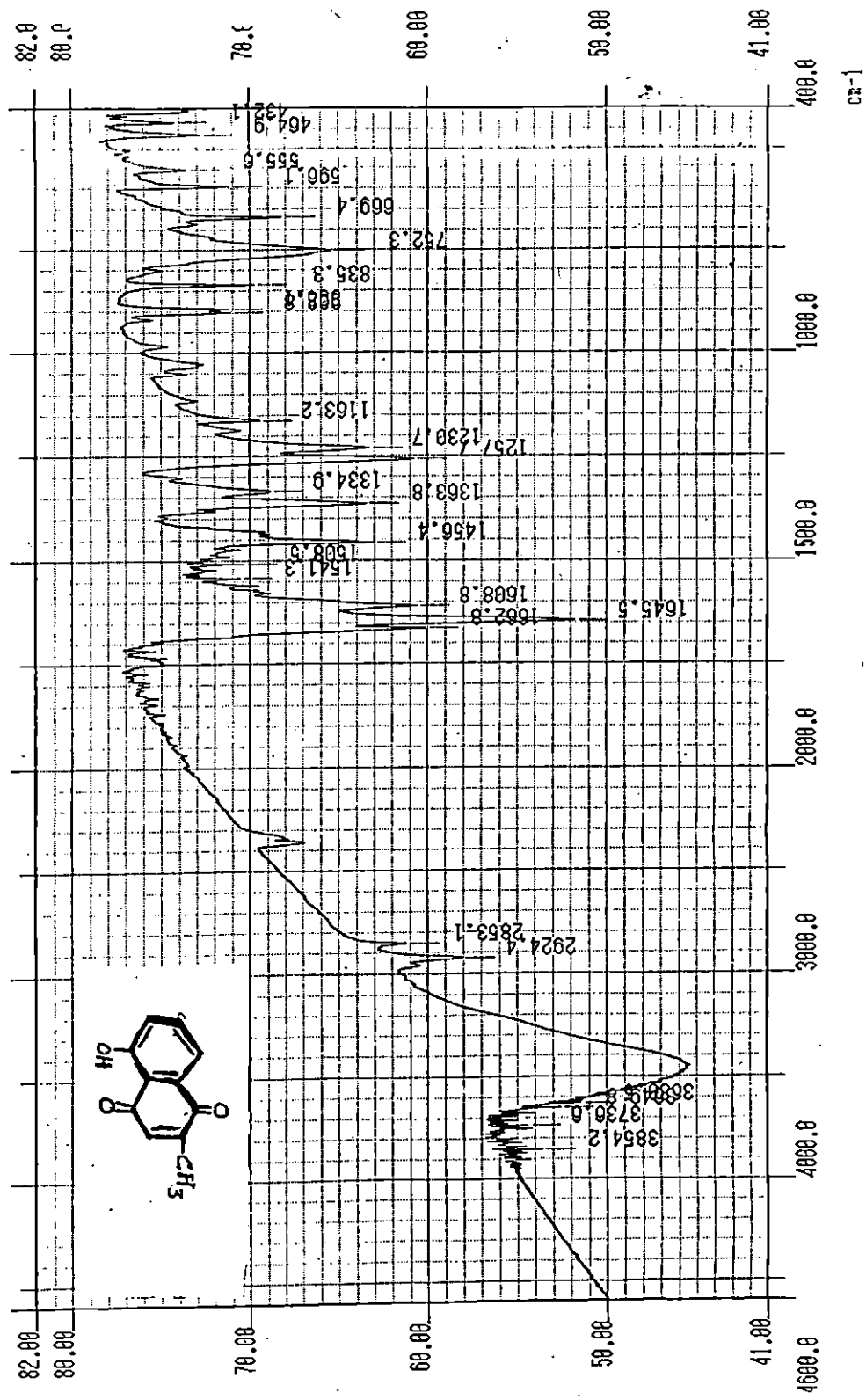


Fig. 17 IR spectrum of plumbagin

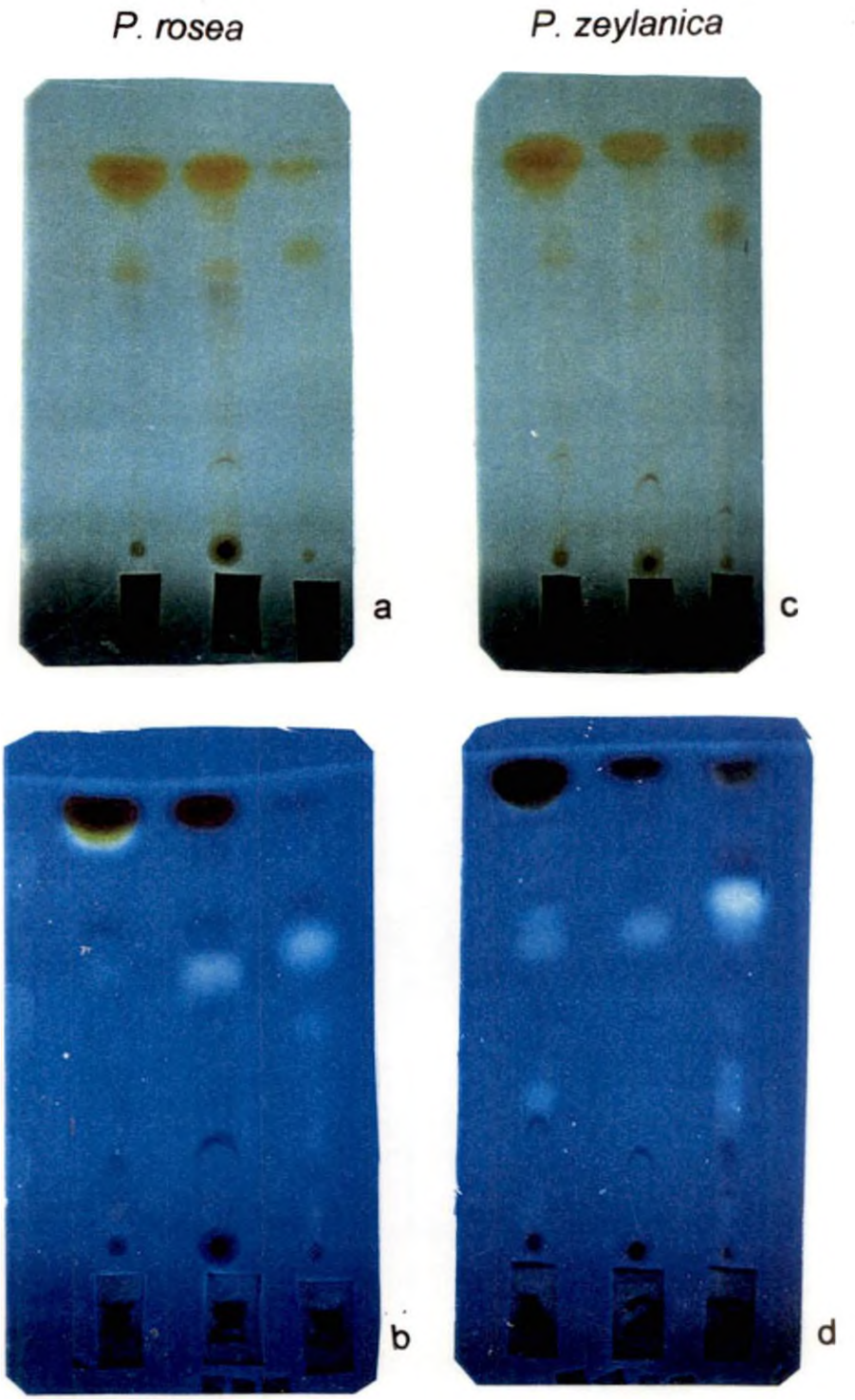


Plate 5 Thin layer chromatogram of fresh, dry and cured samples of *P. rosea* and *p. zeylanica* under visible and UV light

5a *P. rosea* under visible light
5b *P. rosea* under UV light
5c *P. zeylanica* under visible light
5d *P. zeylanica* under UV light

1. fresh sample
2. dry sample
3. cured sample

samples of *P. zeylanica* (Plate 5b and 5d). The dry samples of *P. rosea* did not exhibit the fraction at Rf 0.56.

Qualitative tests of other secondary metabolites were also carried out. The details are given in Table 15.

Table 15. Qualitative tests in fresh, dry and cured roots of *Plumbago* spp.

Group of compound	Spray reagent	Colour of spot	*P. rosea*			*P. zeylanica*		
			Fresh	Dry	Cured	Fresh	Dry	Cured
I. Alkaloids	Drangendroff	-	*	*	*	*	*	*
II. Amino acids	Nin hydrin	Pink	0.89	0.89	0.89	0.89	0.89	0.89
		Pink		0.79			0.79	
III. Carbohydrates	Phenol – Sulphuric acid	Brown	#	#	#	#	#	#
		Greenish black	0.84	0.84	0.84	0.84	0.84	0.84
IV. Phenol	Folin ciocalteau	Blue	#	#	#	#	#	#
V. Steroids	Antimony trichloride	Yellow	0.89	0.89	0.89	0.89	0.89	0.89
		Green	-	-	0.22	-	-	0.22
		Purple	0.62	-	-	-	-	-
		Purple	-	0.10	0.10	-	0.10	0.10
		Green fluorescence	-	-	0.62	-	-	0.62
	Vanillin Sulphuric acid	Pink	-	-	0.32	-	-	0.32
		Green	-	-	0.25	-		0.25
		Purple	-	0.77	-	-	0.77	-
VI. Flavonoids	Lead acetate	Pink	-	0.34	-	-	-	-
		Fluorescence	-		0.49	-	-	0.49

* no response
\# spot at origin

Drangendroff's test for alkaloid was negative for all samples. Ninhydrin reaction was observed in all samples and the Rf value was 0.89, which corresponds to plumbagin. There was an additional amino group in dry samples of both the species at Rf 0.79. Phenol Sulphuric acid test for carbohydrate was positive and showed brown spot at the origin and greenish black fraction at Rf 0.84 in all the samples. Phenol test (Folin ciocalteau) expressed a blue spot at the origin. Steroid test showed a variety of spots for both antimony trichloride and Vanillin Sulphuric acid tests. Yellow spot at Rf 0.89 was common for all samples, which indicated the presence of plumbagin and an amino group.

Chlorophyll content

The results on chlorophyll a, chlorophyll b and total chlorophyll at 10, 12, 16 and 18 MAP are given in Table 16.

Table 16. Chlorophyll content in the leaves of two species of *Plumbago* at varying stages of growth (fresh weight basis)

Plant growth stages (month)	Chlorophyll 'a' (mg g^{-1})		Chlorophyll 'b' (mg g^{-1})		Total chlorophyll (mg g^{-1})		Chlorophyll a: Chlorophyll b	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
June – July	1.80	2.03	0.90	0.78	2.69	2.81	2.02	2.61
August – September	1.85	2.19	0.85	0.98	2.62	3.18	2.18	2.23
December - January	2.59	2.34	1.20	0.97	3.79	3.32	2.15	2.41
February – March	1.92	1.63	0.86	0.99	2.78	2.62	2.19	1.66

The total chlorophyll was the highest at 16 MAP in both species. (3.79 mg g^{-1} and 3.32 mg g^{-1} respectively in *P. rosea* and *P. zeylanica*) (Fig.18). Chlorophyll a was also higher at 16 MAP in *P. rosea,* (2.59 mg g^{-1}). In *P.rosea* chlorophyl b also recorded highest value of 1.20 mg g^{-1} at 16 MAP. However, there was no such drastic difference in chlorophyll b content in leaves of *P. zeylanica.* When the chlorophyll a: chlorophyll b ratio was worked out, it was found that variation was negligible in *P. rosea* throughout the growth period. In *P. zeylanica* the highest ratio of 2.61 was noticed during June - July period (10 MAP) followed by 2.41 at 16 MAP.

Protein content

The protein contents in the leaves and roots of both the species at different growth stages are furnished in Table 17. In *P. rosea,* the root protein was 0.14 per cent on fresh weight basis at 10 MAP and it decreased to 0.01 per cent at 16 MAP, which coincided with flowering stage (Fig. 19). There after there was a slight increase in percentage (0.07 % at 18 MAP).

In the case of *P. zeylanica*, decrease in root protein occurred up to 12 MAP (0.10 %). Thereafter the protein content increased and reached the maximum of 0.18 per cent at 18 MAP.

The leaf protein in tender leaves of *P. rosea* was the lowest (0.05 %) at 16 MAP. The same trend was observed in *P. zeylanica* also, when it recorded the lowest protein content of 0.15 per cent. In respect of mature leaf, the same trend was observed in

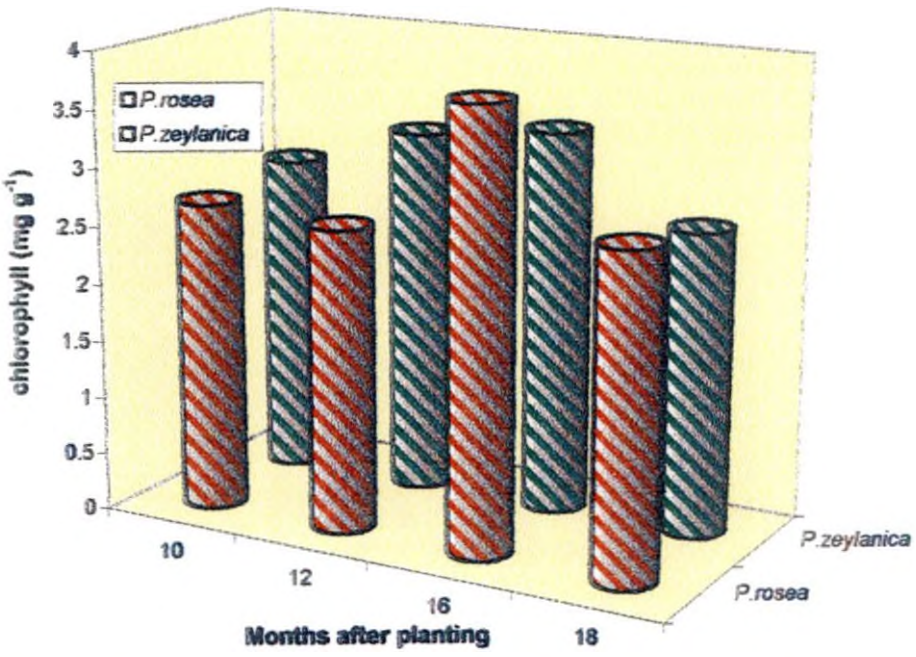


Fig.18. Total chlorophyll content in the leaves of *Plumbago* spp.

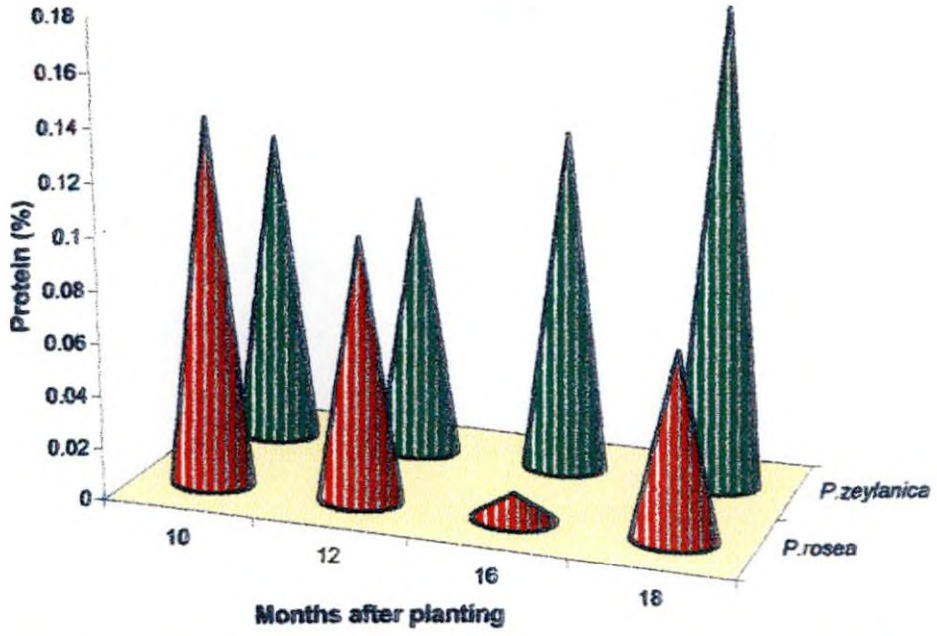


Fig.19. Protein content in roots of *Plumbago* spp. at different stages of growth.

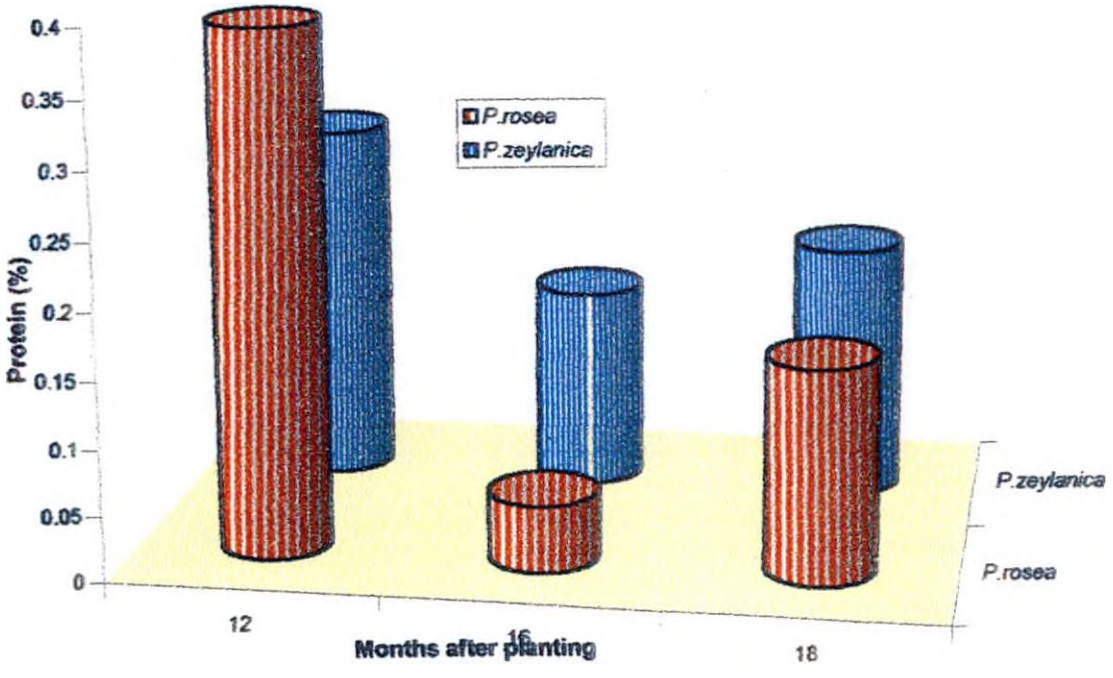


Fig.20. Leaf protein content of *Plumbago* spp. at different stages of growth.

P. zeylanica (0.129 %), whereas *P. rosea* showed a higher protein content at 16 MAP (0.225 %).

The average protein content of leaves in both the species, increased from 10 MAP to 12 MAP. At 16 MAP the leaf protein level declined and reached 0.138 per cent and thereafter the protein content increased to 0.169 per cent (Table 17 and Fig. 20).

Table 17. Protein in root and leaves of *Plumbago* spp. at different stages of growth (fresh weight basis)

Plant growth stage (month)	Root (%)		Tender leaf (%)		Mature leaf (%)		Leaf (on average)(%)	
	P. rosea	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*	*P. rosea*	*P. zeylanica*
June – July	0.14	0.12	Not estimated separately		not estimated separately		0.049	0.054
August – September	0.10	0.10	0.39	0.27	0.195	0.176	0.292	0.232
December – January	0.01	0.13	0.05	0.15	0.225	0.129	0.138	0.138
February - March	0.07	0.18	0.16	0.19	0.175	0.153	0.169	0.169

When a comparison was made on the root protein levels in the two species, it was noticed that the root protein content, in general, was higher in *P. zeylanica,* and leaf protein content was higher in *P. rosea.* Tender leaf recorded higher protein than mature leaves.

Malate dehydrogenase (MDH) activity

The tender leaves of *P. rosea* and *P. zeylanica* showed the presence of malate dehydrogenase enzymes. The decrease in absorbance readings at one minute interval at various concentrations of substrate, oxalo acetic acid in *P. rosea* and *P. zeylanica* are given in Table 18 and 19. The absorbance in *P. rosea* ranged from 0.001 to 0.029, while in *P. zeylanica* it ranged from 0.001 to 0.065.

Table 18. Effect of Oxalo acetic acid concentration on Malate de hydrogenase activity in *P. rosea*

Substrate concentration (%) (oxalo acetic acid)	Absorbance after				
	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
10	0.012	0.012	0.014	0.015	0.017
20	0.003	0.004	0.006	0.007	0.009
30	0.001	0.003	0.004	0.006	0.005
40	0.015	0.018	0.019	0.022	0.026
50	0.02	0.027	0.028	0.022	0.029

Table 19. Effect of Oxalo acetic acid concentration on Malate de hydrogenase activity in *P.zeylanica*

Substrate concentration (%) (oxalo acetic acid)	Absorbance after				
	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
10	0.007	0.012	0.019	0.027	0.034
20	0.007	0.016	0.024	0.029	0.037
30	0.005	0.011	0.016	0.023	0.030
40	0.001	0.005	0.011	0.012	0.021
50	0.021	0.026	0.042	0.054	0.065

It was also obvious that enzyme activity was independent of time at optimum substrate concentration. *In vitro* studies revealed that 30 per cent oxalo acetic acid was optimum for MDH activity in *P. rosea* (Fig. 21) and 40 per cent in *P. zeylanica* (Fig. 22). Thereafter, the enzyme activity was inhibited by substrate and thereby substrate inhibition was effected.

The specific activity of Malate dehydrogenase worked out for two minutes in the two species are furnished in Table 20. The data revealed that *P. zeylanica,* recorded higher activity. In *P. rosea* the activity was 1.370 and in *P. zeylanica* it was 1.410 (Fig. 23).

Table 20. Enzyme activity, protein content and specific activity of MDH and PEP carboxylase in *P.rosea* and *P.zeylanica*

Enyme	Species	Enzyme activity in 0.4 ml extract	Protein content (%)	Specific activity at 2 minutes
Malate dehydrogenase	*P. rosea*	0.004	0.530	1.370
	P. zeylanica	0.016	2.060	1.410
Phospho enol pyruvate carboxylase	*P. rosea*	0.003	0.987	0.550
	P. zeylanica	0.006	1.302	0.840

Phospho enol pyruvate (PEP) carboxylase activity

The tender leaves of *P. rosea* and *P. zeylanica* showed the presence of the enzyme phospho enol pyruvate carboxylase. The observations on absorbance at one minute interval at various concentrations of the substrate (6.67 to 33.33 %) in *P. rosea* and *P. zeylanica* are given in Table 21 and 22 respectively.

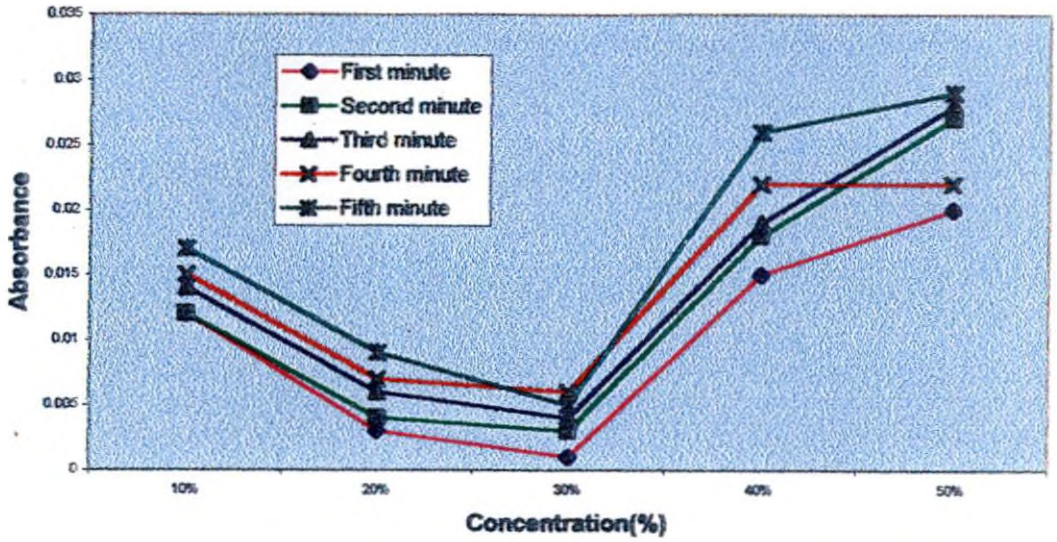


Fig.21. Effect of concentration of Oxalo Acetic Acid on MDH activity in P.rosea

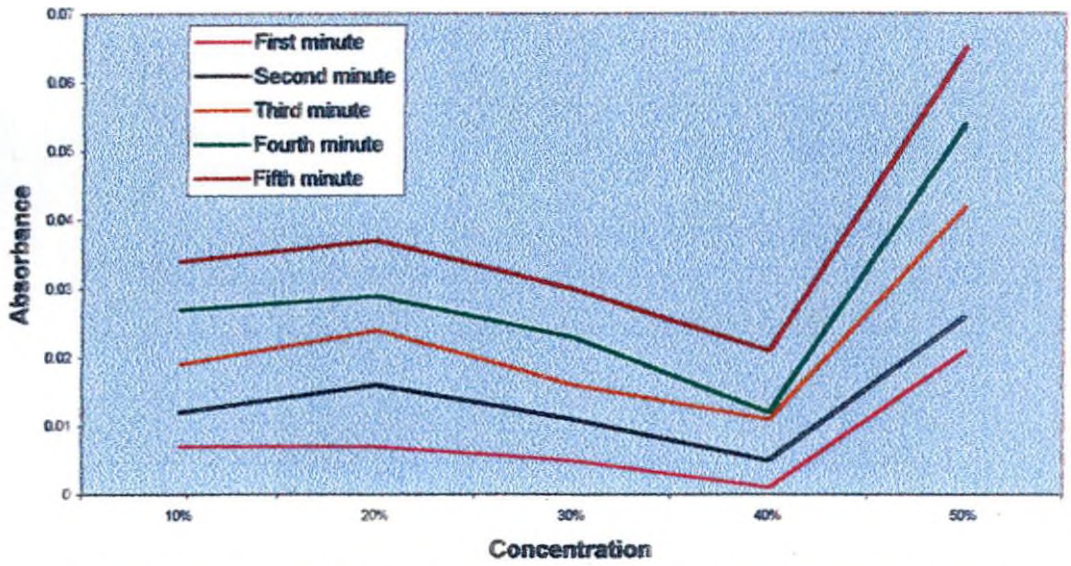


Fig.22. Effect of concentration of Oxalo Acetic Acid on MDH activity in P.zeylanica

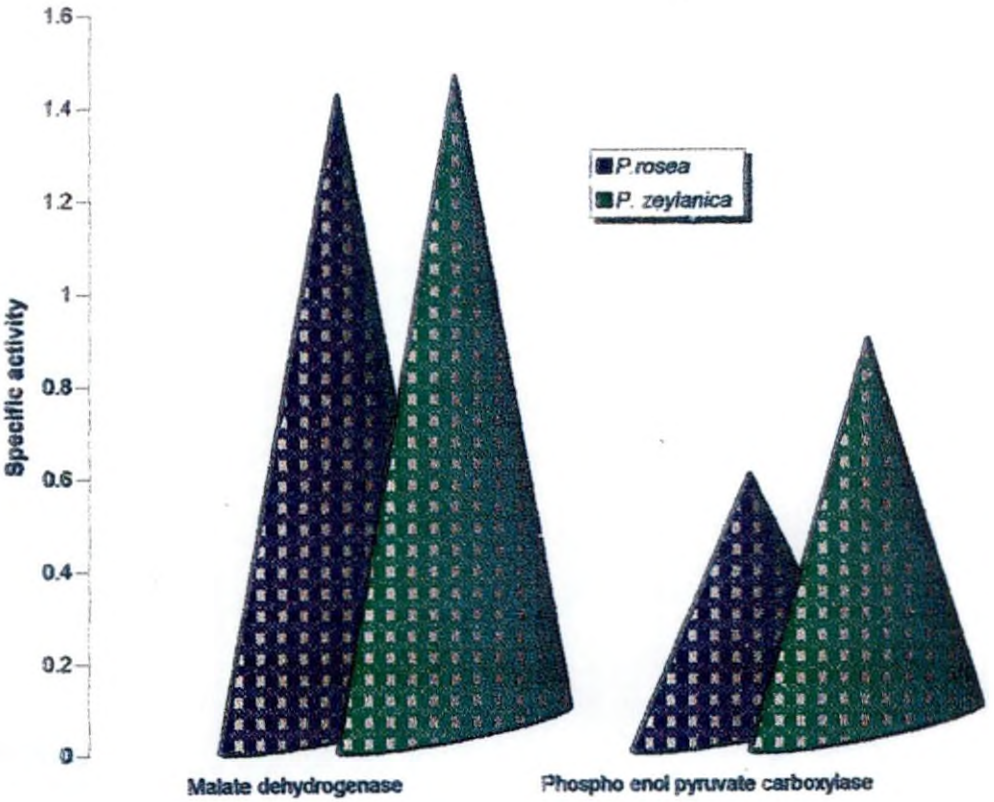


Fig.23. Specific activity of enzymes in Plumbago spp.

Table 21. Effect of concentration of Phospho enol pyruvate on Phospho enol pyruvate carboxylase enzyme activity in *P. rosea*

Substrate concentration (%) (phospho enol pyruvate)	Absorbance after				
	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
6.67	0.001	0.002	0.000	0.002	0.001
13.33	0.001	0.004	0.003	0.000	0.000
20.00	-0.005	0.003	0.002	0.002	0.004
26.67	-0.009	-0.005	-0.005	-0.005	-0.005
33.33	0.005	0.008	0.008	0.005	0.009

Table 22. Effect of phospho enol pyruvate concentration on phospho enol pyruvate carboxylase enzyme activity in *P.zeylanica*

Substrate concentration (%) (phospho enol pyruvate)	Absorbance after				
	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
6.67	-0.002	0.000	0.001	0.003	0.003
13.33	0.009	0.006	0.011	0.007	0.009
20	0.002	0.006	0.007	0.010	0.012
26.67	-0.001	0.002	0.004	0.004	0.004
33.33	0.009	0.008	0.008	0.007	0.009

It was observed that in both the species optimum concentration of PEP carboxylase was constant at 26.67 per cent (Fig. 24 and 25). The studies revealed that the PEP carboxylase activity was more in the first minute and that thereafter the rate of reaction was independent of time. The data showed that the activity was higher in *P. zeylanica*. The specific activity of Phospho enol pyruvate carboxylase in *P. rosea* was 0.55 and in *P. zeylanica* it was 0.84 (Table 20).

4.1.6 Number of stomates and diffusive resistance

The results furnished in Table 23 indicated that *P. zeylanica* had lower number of stomates per microscopic field (29.6). Diffusive resistance was higher in *P. zeylanica* (0.18 cm per second) as compared to *P. rosea,* which recorded a negative value (-0.42 cm per second). The rate of transpiration was also higher in *P. rosea* (10.99 µg water per cm per second) as against 9.15 µg water per cm per second in *P. zeylanica*. The leaf temperature was slightly lower in *P. rosea* (30.55^{0}C) when compared to *P. zeylanica* (30.59^{0}C).

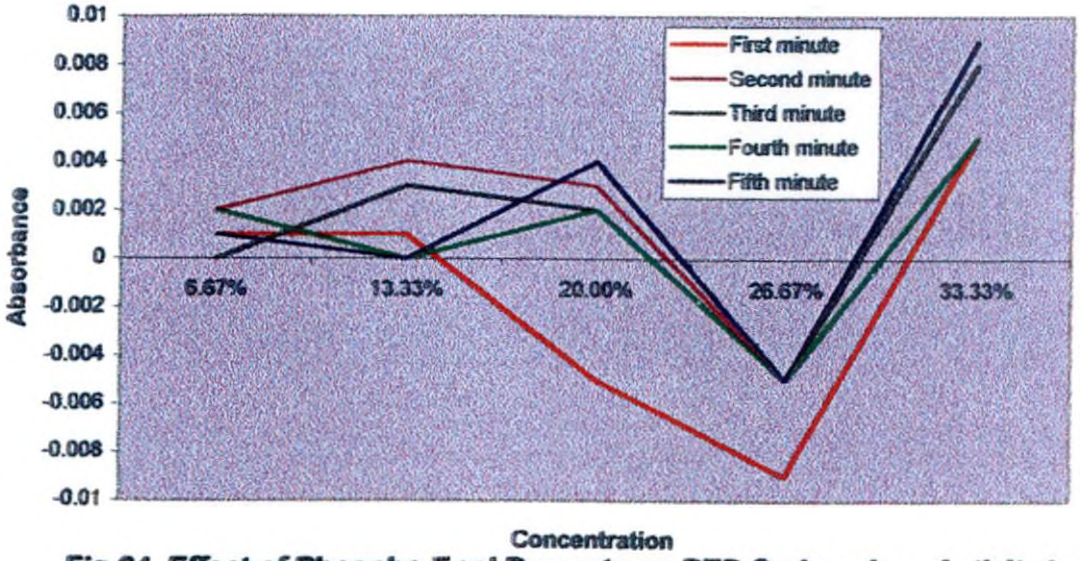


Fig.24. Effect of Phospho Enol Pyruvate on PEP Carboxylase Activity in *P. rosea*

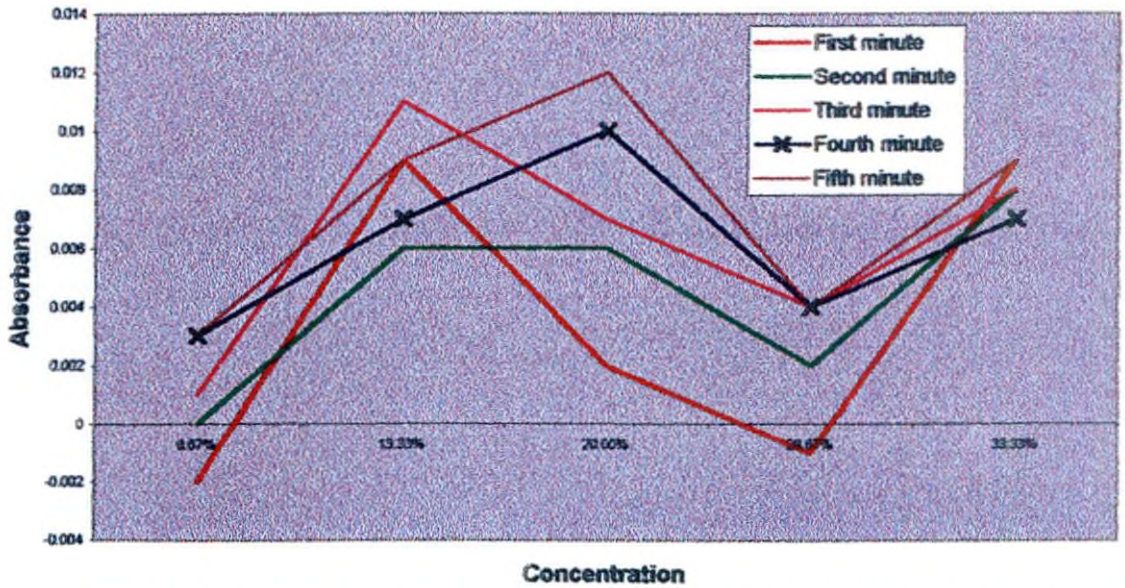


Fig.25. Effect of Phospho Enol Pyruvate on PEP Carboxylase Activity in *P. zeylanica*

Table 23. Stomatal number, diffusive resistance, transpiration and leaf temperature in two species of *Plumbago*

Species	Stomatal number per microscopic field (40 x)				Diffusive resistance ($cm\ s^{-1}$)	Transpiration (mg water $cm^{-1}g^{-1}$)	Leaf temperature (^0C)
	Tip	Middle	Base	Mean			
P. rosea	39.5	28.2	37.5	35.1	-0.42	10.99	30.55
P. zeylanica	31	27.3	30.5	29.6	0.18	9.15	30.59

4.1.7 Anatomy

The anatomy of *P. rosea* and *P. zeylanica* shows clear cut differences with respect to the arrangement of the different tissues.

Stem

The anatomy of *P. zeylanica* stem showed the following features. Transverse section of the stem of this species showed a corrugated or wavy outline while that of *P. rosea* appeared to be circular (Plate 6a and 6c).

In *P. zeylanica* the epidermal cells are very small and are covered with a thick layer of cuticle. The cortex is divisible into three clear-cut zones (Plate 6b). Just below the epidermis three to four layers of collenchymatous hypodermis are present. The number of collenchymatous layers in the ridged region of the stem is about 15 to 20. Just below the epidermis, in the regions of the furrows, about eight to ten layers of chlorenchymatous cortical zones are observed. In *P. zeylanica,* the pericycle is a continuous ring of sclerenchymatous tissue of about three to four rows of cells. The number of rows of cells in the region of the ridges is about five to seven. Vascular bundles are arranged in the form of a discontinuous ring and they are open and collateral (Plate 6b). There is a broad zone of pith that occupies about six-tenth of the area and the cells are parenchymatous.

In *P. rosea* the epidermis consists of cells that are comparatively larger in size as compared to the epidermal cells of *P. zeylanica.* The hypodermis is seen as a wavy layer (Plate 6d). The cortex consists of chlorenchyma and parenchyma and is traversed by groups of resin canals. The amount of chlorenchyma in the cortex is very less as compared to that of *P. zeylanica.* Resin canals are absent in *P. zeylanica.* The pericycle is seen as patches of sclerenchyma, above the vascular bundles and not continuous as in *P. zeylanica.*

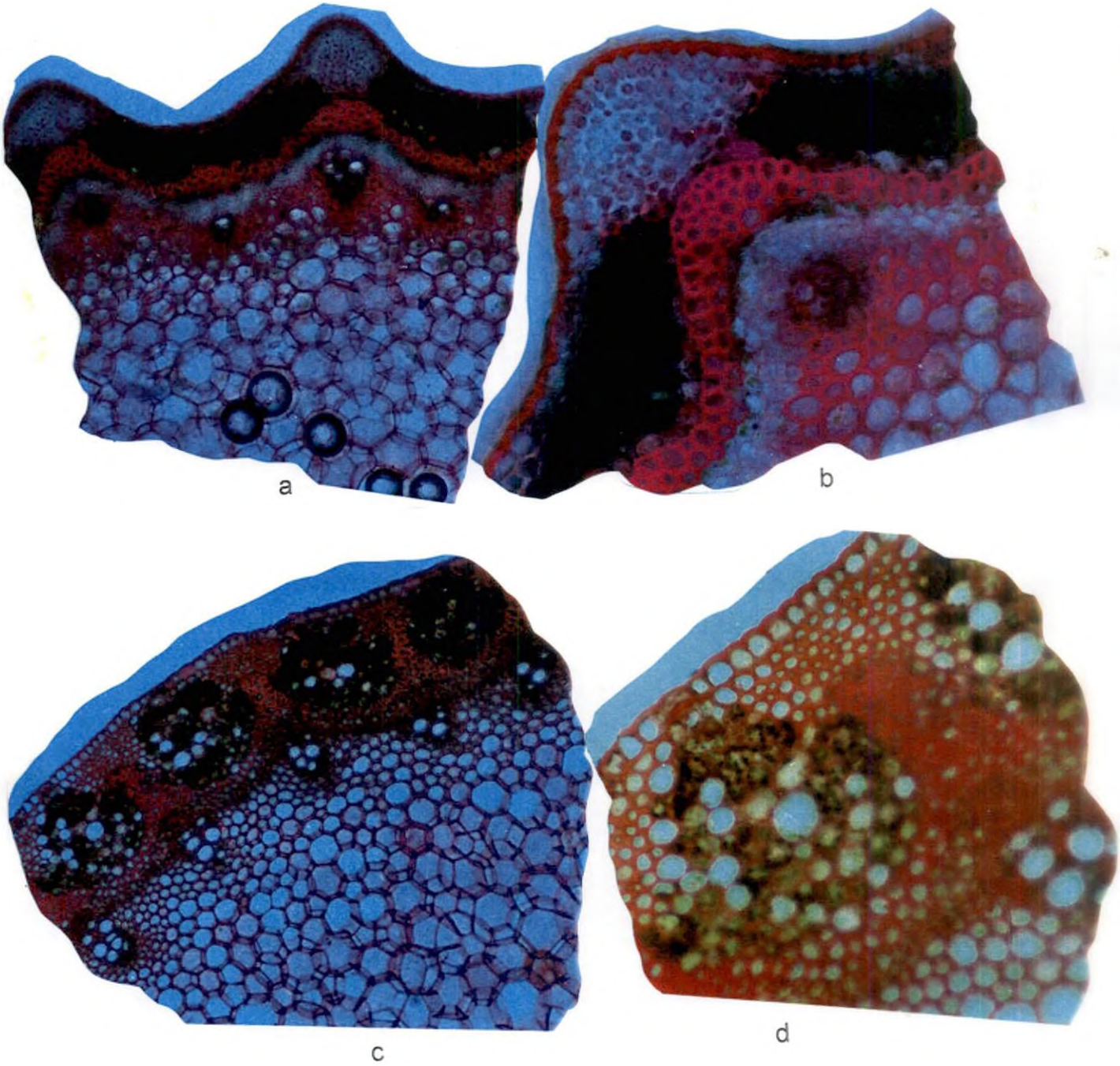


Plate 6 Anatomy of stem of *Plumbago* spp.

6a Cross section of *P. zeylanica* (20x)
6b Cross section of *P. zeylanica* (50x)
6c Cross section of *P. rosea*(20x)
6d Cross section of *P. rosea*(50x)

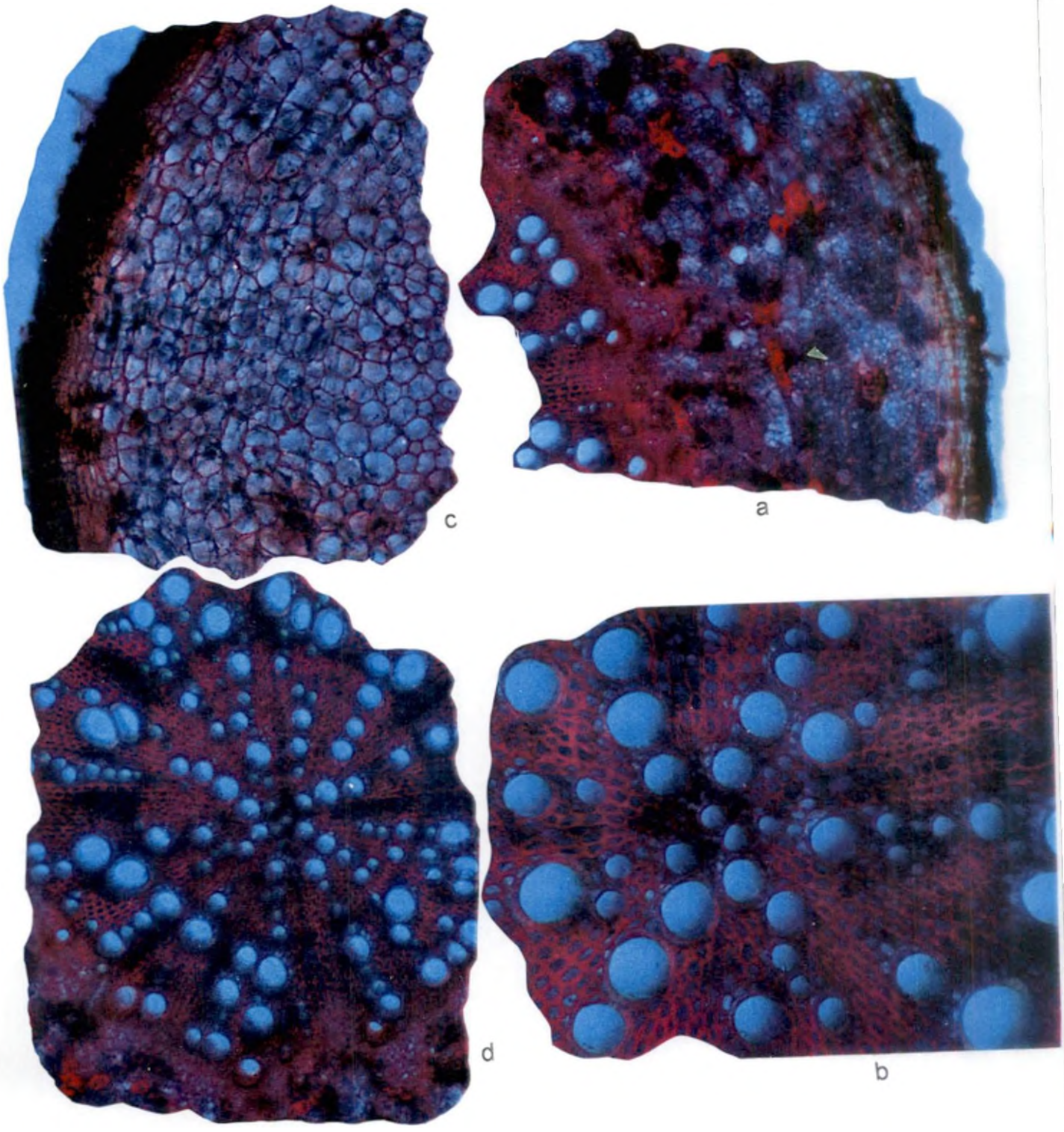


Plate 7 Anatomy of root of *Plumbago* spp.

7a Cross section of *P. zeylanica* (20x)
7b Cross section showing steel of *P. zeylanica* (50x)
7c Cross section of *P. rosea*(20x)
7d Cross section showing steel of *P. rosea*(50x)

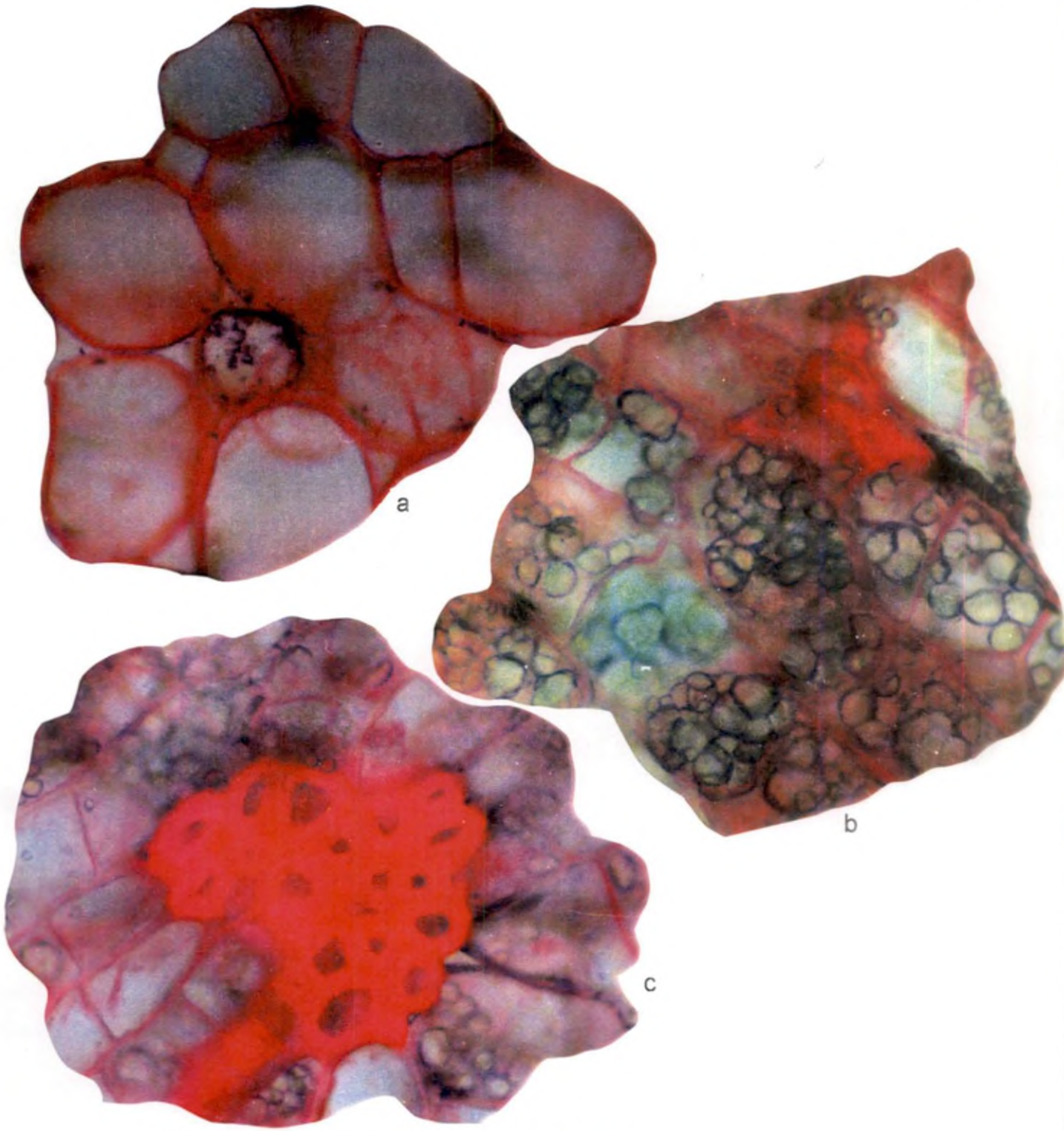


Plate 8 Anatomy of root of *Plumbago* spp.

8a Root cortex of *P. rosea*

8b Root cortex of *P. zeylanica* showing starch grains

8c Root cortex of *P. zeylanica* showing sclerenchyma patches

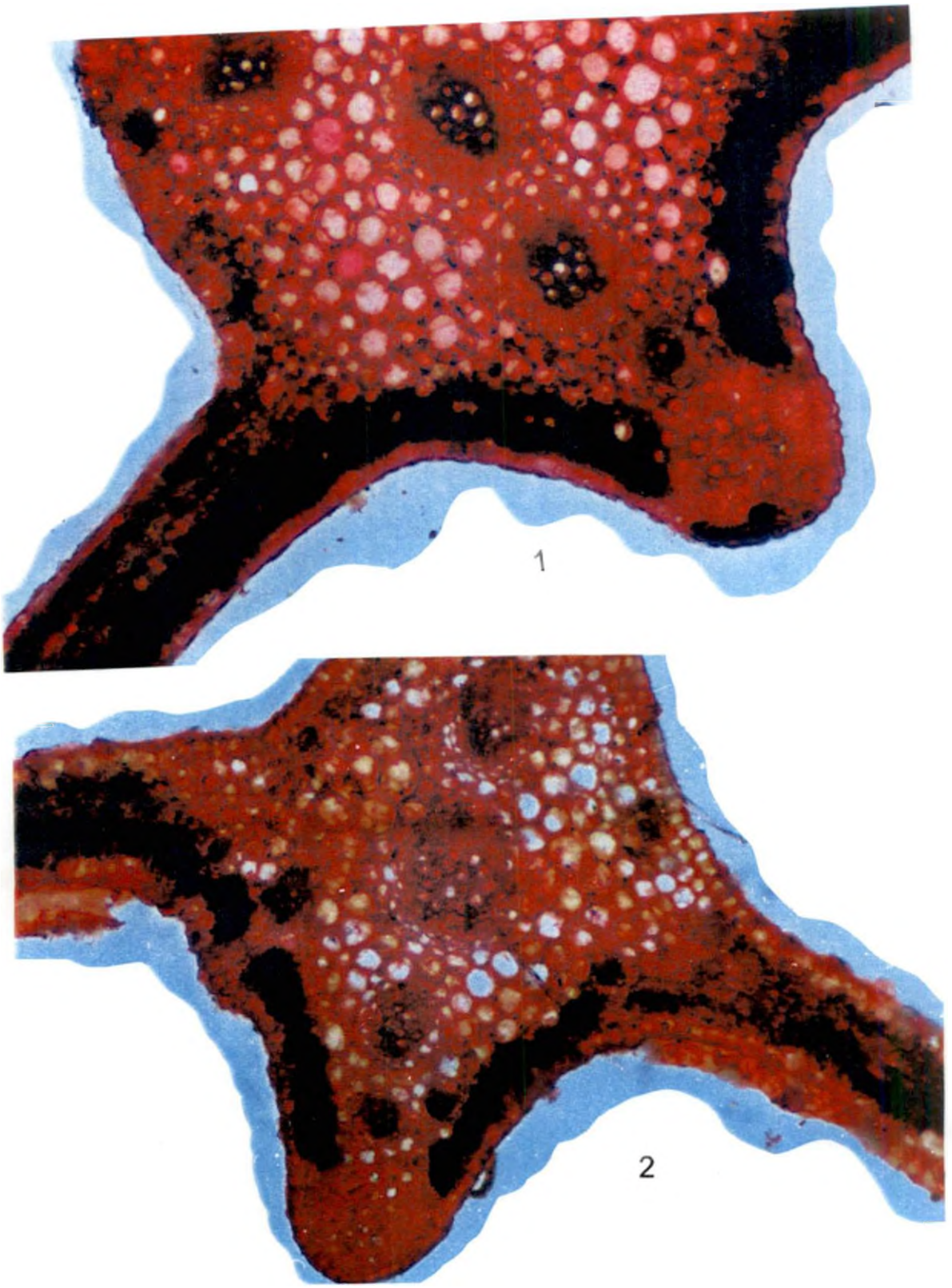


Plate 9 Leaf anatomy of *Plumbago* spp.

9.1 Cross section of leaf of *P. rosea*

9.2 Cross section of leaf of *P. zeylanica*

Vascular bundles are enlarged, collateral, open and are arranged in the form of a ring in *P. rosea*. Pith is very large, made of parenchyma with intercellular spaces and occupies about six-tenth of the area.

Root

The anatomy of the root of *P. zeylanica* revealed the presence of a well-defined cortex (Plate 7a). Most of the cortical cells are filled with large sized starch grains (Plate 8b). In the cortex of *P. zeylanica,* there are patches of sclerenchyma consisting of 20 to 30 cells distributed in a scattered manner (Plate 8c). The central region is completely occupied by secondary xylem consisting of very large vessels and trachieds (Plate 7b).

P. rosea has well-defined cortex consisting of parenchymatous cell with intercellular spaces (Plate 7c). The absence of patches of sclerenchyma and starch grains makes it quite different from *P. zeylanica* (Plate 8a). Development of secondary xylem is also less as compared to the other species (Plate 7d).

Leaf

Leaf anatomy of *P. zeylanica* and *P. rosea* reveal a typical dicot leaf structure (Plate 9). The palisade tissue consists of three to four layers of chlorenchyma. Intercellular spaces are absent.

4.2 Analysis of reproductive behaviour in *Koduveli* (*Plumbago* spp.)

The results obtained on flowering pattern, inflorescence and flower characters are furnished in Table 24. The results showed that the rooted cuttings of both the species transplanted in July 1996 started flowering by October 1996. In the case of *P. rosea,* flower production was continued and attained the peak during December – January and thereafter there was a decline in flowering. Flowers were not produced from third week of February. Thus, the study clearly indicated that *P. rosea* was strictly seasonal in respect of flowering. In the case of *P. zeylanica,* the plants started flowering by October 1996 and flowering was continued thereafter without any clear-cut peak.

Table 24. Variation in flowering pattern and floral characters in two species of *Plumbago*

Sl. No.	Characters	*P. rosea*	*P. zeylanica*
1	Flowering time	Seasonal October – February	Ever flowering / Continuous flowering Throughout the year
2	Month in which flowering commenced	October	October
3	Month of peak flowering	December – January	No clear cut peak
4	Month of completion of flowering	February	Continuous flowering
5	Type of inflorescence	mostly single spike	Single or branched spike
6	Site of production of inflorescence	terminal and rarely axillary	terminal and axillary
7	Length of inflorescence (cm)	15 – 65	12 - 27
8	Days for opening of first flower after visual emergence of inflorescence bud	8 - 13	8 - 14
9	Days for completion of flowering in an inflorescence	15 – 45	14 - 30
10	Number of flowers per inflorescence	15 – 77	19 - 60
11	Number of flowers opened per day	1 – 6	1 - 5
12	Colour of flower	Bright red	White
13	Length of flowers (cm)		
	Mean	3.2	2.4
	Range	2.8 - 3.7	2.2 - 2.6
14	Length of calyx (cm)		
	Mean	0.9	1.0
	Range	0.8 - 1.0	0.8 - 1.1
15	Colour of calyx	Greenish with red tinge	Green
16	Length of corolla tube (cm)		
	Mean	1.4	0.7
	Range	1.2 – 1.7	0.7 – 0.8
18	Width of corolla lobe (cm)		
	Mean	0.9	0.3
	Range	0.8 - 1.0	0.3 - 0.4
19	Colour of corolla	Bright red	White
20	Length of style (cm)		
	Mean	2.0	2.2
	Range	1.7 - 2.3	1.9 - 2.2

4.2.1 Inflorescence characters

The inflorescences of both the species are spikes, which may be single or branched. In *P. rosea*, the inflorescence is mostly terminal and rarely axillary. In *P. zeylanica* the inflorescence was either terminal or axillary. The spikes continue to elongate even after, reaching a length of 15 to 65 cm in *P. rosea* and 12 – 27 cm in *P. zeylanica.*

The mean number of days for opening of first flower after visual emergence of the inflorescence bud in the two species were 8 to 13 and 8 to 14 respectively. The mean number of days for completion of flowering per inflorescence was 15 to 45 in *P. rosea* and 14 to 30 in *P. zeylanica*. Similarly, the mean number of flowers per inflorescence was also higher (15-77) in *P. rosea* as against 19 to 60 in *P. zeylanica*. The number of flowers opened per day ranged from one to six in *P. rosea* and one to five in *P. zeylanica*.

4.2.2 Flower characters

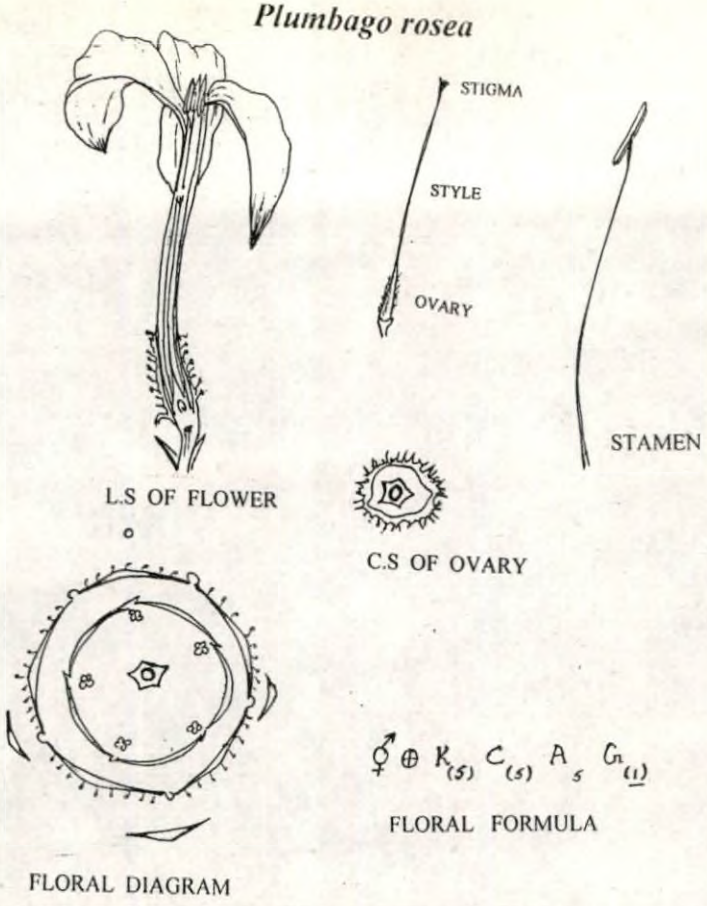
The floral biology of the two species of the *Plumbago* is furnished in Fig. 26. Flowers of both the species are produced on small pedicels on the inflorescence in a spiral manner. The colour of the flower is bright red in *P. rosea* and white in *P. zeylanica* (Plate 10). The individual flowers are complete, bracteate, bisexual and actinomorphic. The rachis of the inflorescence is covered with very short glutinous hairs or glands. The bracts are larger than bracteoles in *P. zeylanica* and equal in *P. rosea*. The bracts and bracteoles are also covered with sticky glandular hairs.

The calyx consist of five sepals which are valvate, united, tubular, five toothed and subsessile in both species. The calyx of *P. rosea* is 0.9 cm long whereas that of *P. zeylanica* 1.0 cm. They are greenish with reddish tinge in *P. rosea* and green in *P. zeylanica*. The calyx is persistent and covered with glutinous stalked glands in both species.

Corolla of both the species consists of five petals rarely six or seven and gamopetalous. The petals are twisted in *P. rosea* while they are valvate in *P. zeylanica*. It forms a slender long tube, which is larger than calyx (3.0 cm in *P. rosea* and 2.2 cm in *P. zeylanica*). At the end of corolla tube the corolla lobes are free, rotate, roundish and toothed. The lobes are 1.4 cm long and 0.9 cm wide in *P. rosea* and 0.7 cm long and 0.3 cm wide in *P. zeylanica*. Petals are bright red in *P. rosea* and white in *P. zeylanica*. The androeciums of both the species consist of five stamens that are hypogynous and free. The filaments are as long as corolla tube and connate at base into a lobed nectar- secreting disc.

Anthers are exerted beyond the throat of the corolla tube and dorsifixed in both species. Anthers are bluish and linear with two clefts, which dehisce longitudinally in both species.

Plumbago rosea



Plumbago zeylanica

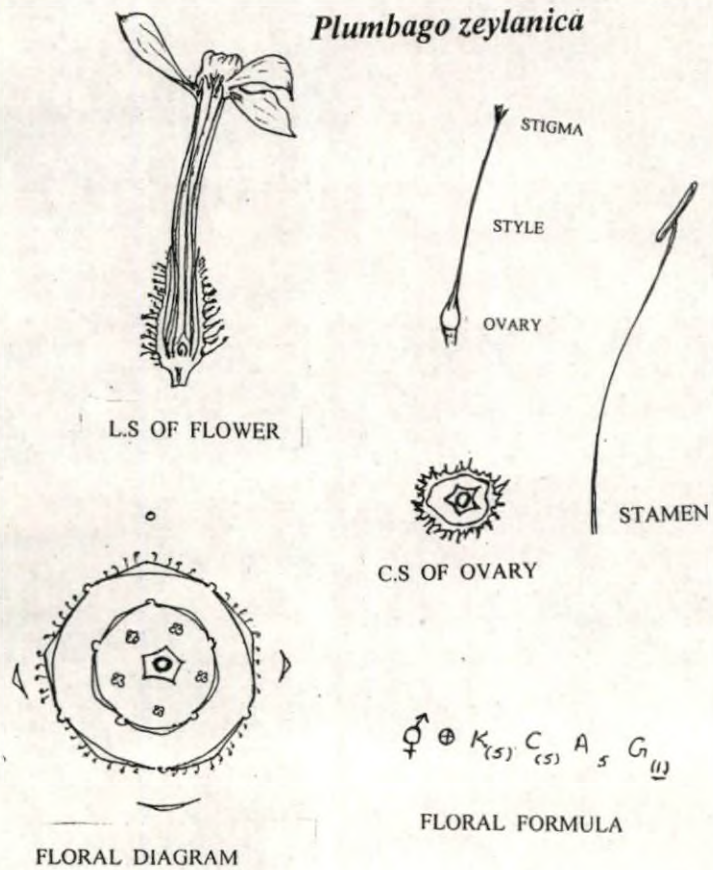


Fig. 26 Flower structure of *Plumbago* spp.

The gynoeciums of both species are monocarpellary, syncarpous and superior. Ovary is ovate or oblong, narrowed at apex and placentation is basal. In *P. rosea* style in shorter (2.0 cm) as compared to *P. zeylanica* (2.2 cm)(Plate 11). The stigma is five lobed and filiform. Stigma of *P. rosea* is deeply seated inside the corolla tube and thus the anthers and stigma are held at two levels hindering self-pollination. However, in the case of *P. zeylanica*, the styles (five in number) are held at the same level, at a slightly higher or lower level as that of the anthers. Thus the results indicated that the floral morphology favours self-pollination in *P. zeylanica*, in plants with stigma and anthers at the same level. In the case of flowers where stigma is at a higher level, cross-pollination can also occur. The study revealed that there is no fruit set in *P. rosea*. The fruits of *P. zeylanica* are enclosed within the persistent calyx. It is one seeded capsule and seed is solitary and ovate.

4.2.3 Anthesis

The data on time of anthesis of two the species of *Plumbago* are furnished in Table 25. The data showed that in *P. rosea*, anthesis started at 8.00 a.m. and reached the peak between 8.30 a.m. and 9.00 a.m. and declined after 9.30 a.m. In the case of *P. zeylanica*, anthesis started before 6.30 a.m. and attained the peak at 7.30 a.m. and 8.00 a.m.. The anthesis was found to decline after 9.00 a.m.

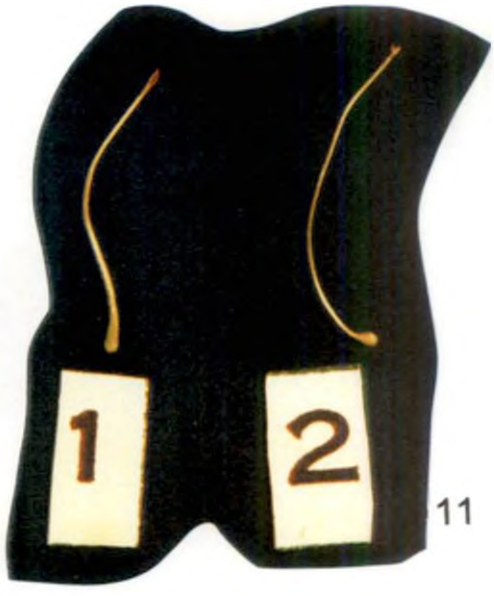
Table 25. Time of anthesis in two species of *Plumbago*

Time (a.m.)	Anthesis (%)	
	P.rosea	*P.zeylanica*
6.30	0.00	8.89
7.00	0.00	11.11
7.30	0.00	29.99
8.00	3.33	37.78
8.30	20.00	7.78
9.00	61.11	4.45
9.30	12.22	0.00

4.2.4 Pollen studies

The examination of pollen grains under microscope, revealed that the pollen grains of *P. rosea* are yellowish white, while those of *P.zeylanica* are bluish white. The average pollen size of *P. zeylanica* was 5.05 µm and 3.69 µm respectively in *P. zeylanica* and *P. rosea* (Plate 12). The data furnished in Table 26 showed that number of pollen grains

10



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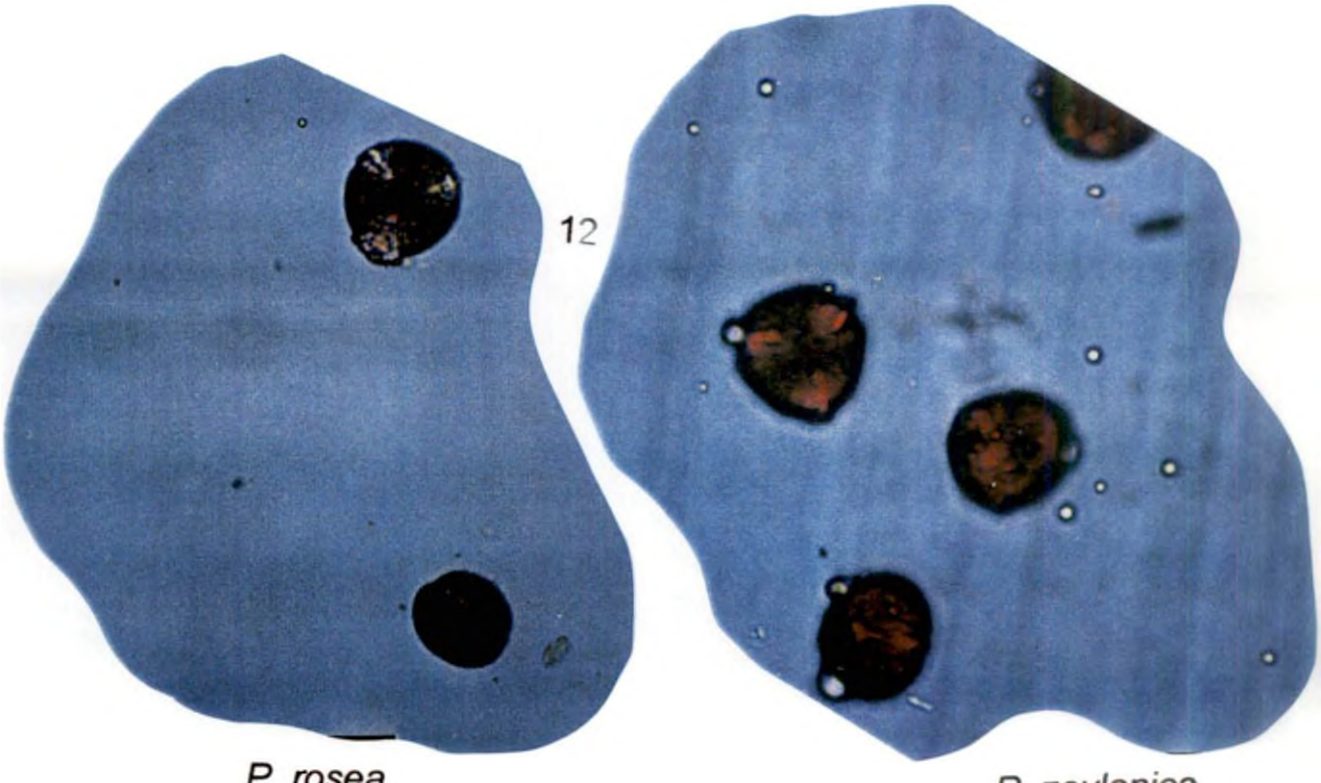

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P. rosea

P. zeylanica

Plate 10 **Single flower of *P. rosea* and *P. zeylanica***

Plate 11 **Gynoecium of 1. *P. rosea***
2. *P. zeylanica*

Plate 12 **Pollen grains of *Plumbago* spp.**

12.1 *P. rosea*
12.2 *P. zeylanica*

per anther was higher in *P. zeylanica* (750) as compared to that in *P.rosea* (463). The pollen fertility in *P. rosea* was only 28.13 per cent as against 89.98 per cent recorded by *P. zeylanica.* Though *in vitro* pollen germination was tried using 19 media as listed earlier none of them was found to be effective in inducing germination of pollen grains under *in vitro* conditions.

Table 26. Variation in pollen morphology and pollination habit in *Plumbago* spp.

Characters	*P. rosea*	*P. zeylanica*
Colour of pollen grain	Yellowish white	Bluish white
Pollen shape	Spherical	Spherical
Pollen size (μ M)	3.69	5.05
Number of pollen grain per anther	463.00	750.00
Pollen fertility (%)	28.13	89.98
In vitro pollen germination in 19 media tried	0.00	0.00
Fruit set after self pollination (%)	0.00	70-80
Fruit set after open pollination (%)	0.00	85-90
Fruit set after natural cross pollination (%)	0.00	30-40

In vivo pollen germination

Artificial pollination was attempted between the two species. Examination of the excised pistils after 24 hours under fluorescent microscope suggested that the pollen grains of *P. zeylanica* germinated on the stigmatic surfaces of both *P. rosea* and *P. zeylanica* (Plate 13). However, the germination percentage was low and even after 24 hours of pollination, the pollen tube failed to reach the ovule. It was also found that pollen grains of *P. rosea* failed to germinate on stigmatic surfaces of both the species.


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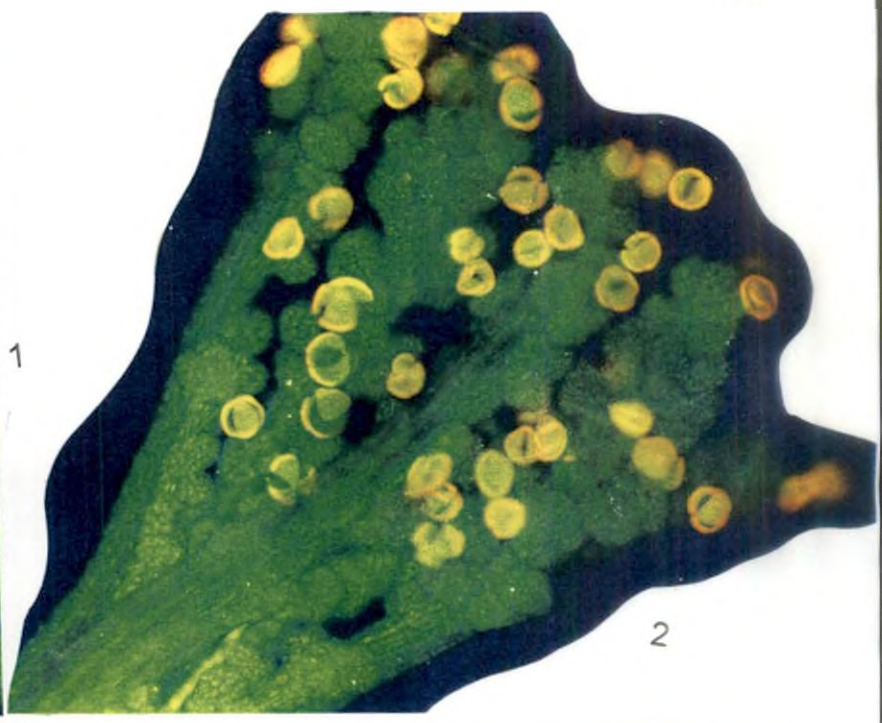
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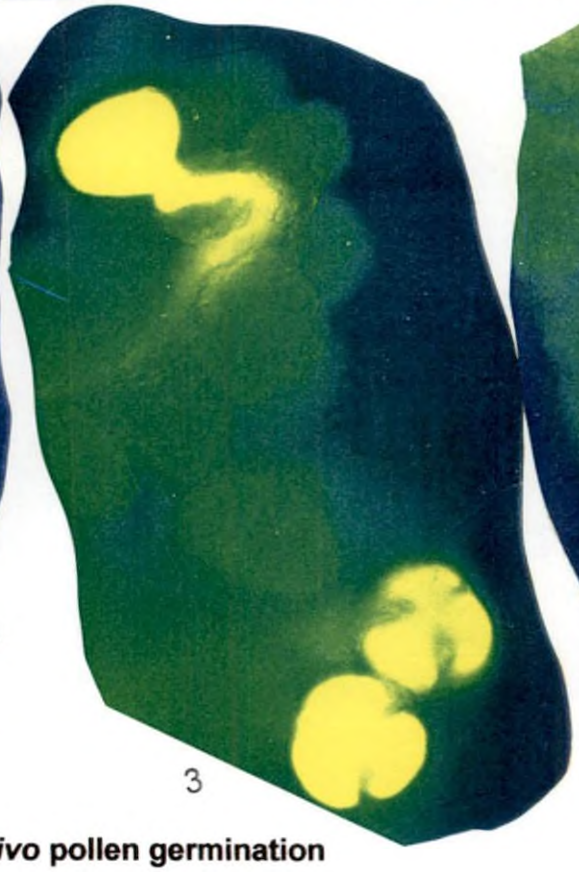
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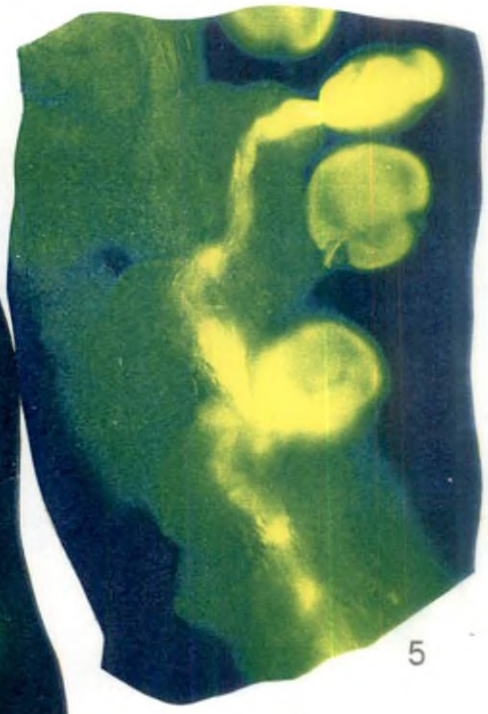
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Plate 13 *In vivo* pollen germination

13.1 Stigmatic surface under epifluorescence microscope

13.2 Stigmatic surface with sterile pollen grains

13.3 Stigmatic surface of *P. zeylanica* after self pollination

13.4 Stigmatic surface of *P. rosea* after cross pollination using pollen grains of *P. zeylanica* (20×)

13.5 Stigmatic surface of *P. rosea* after cross pollination using pollen grains of *P. zeylanica* (50×)

4.2.5 Pollination studies

Effect of different methods of pollination on fruit set

The results furnished in Table 26 showed that *P. zeylanica* set fruit under open pollination, self- pollination and natural cross pollination (Plate 14). But *P. rosea* failed to set fruit under all conditions tried.

Under open pollination 85 to 90 per cent of tagged flowers set fruit in *P. zeylanica*. Self-pollination observed in the tagged inflorescences ranged from 70 - 80 per cent and natural cross pollination reduced fruit set to 30 - 40 per cent.

The data on fruit set in artificially cross- pollinated flowers after 24, 48, 72 and 96 hours of cross pollination are furnished in Table 27. The results showed that in crosses among *P. rosea* the sepals were retained up to 72 hours. In a cross of *P. rosea* with pollen grains of *P. zeylanica,* only 3.4 per cent of flowers persisted after 96 hours, which rose to 23.8 per cent after 72 hours. In *P. zeylanica* x *P. rosea* none of the flowers was retained up to 72 hours.

Table 27. Fruit set in artificially cross pollinated flowers at different intervals after pollination

Crosses	Number of flowers crossed	Percent flowers retained after different intervals (hours)			
		24	48	72	96
P. rosea x *P. rosea*	25	78.90	52.63	21.00	0.00
P. rosea x *P. zeylanica*	25	89.60	27.50	23.80	3.44
P. zeylanica x *P. rosea*	25	58.80	5.80	0.00	0.00

4.2.6 Germination of seeds of *P. zeylanica*

The seeds of *P. zeylanica* failed to germinate when they were sown fresh. Therefore, different treatments were imposed. The data (Table 28) indicated that when the seeds were given cuts at the micropylar or chalazal ends, cent percent germination was recorded.

Table 28. Seed germination of *P. zeylanica* under different treatments

Seed treatment	Medium	Seed	Germination (%)	Number of days to initiate germination
Control	Moist filter paper	Fresh seeds	0	0
24 hr soaking	Moist filter paper	Fresh seeds	0	0
Control	Moist filter paper	Stored seeds	40	10
Cut at micropylar end	Moist filter paper	Fresh or stored seeds	100	3
Cut at chalazal end	Moist filter paper	Fresh seeds	100	4.5
Scarification using sand	Moist filter paper	Fresh seeds	60	9
Cut at the micropylar end	Soil: Sand mixture	Fresh or stored seeds	100	3

However, the data indicated that the pattern of germination was different. When the micropylar end of seeds were cut and kept in petri -dishes with moist filter paper or in soil: sand mixture, germination started by the third day. In the case of seeds, which were cut at the chalazal portion, it took 4 to 5 days for starting germination (Plate 15). In respect of scarification using sand and keeping the seeds in a glass petri-dish over moistened filter paper, 60 per cent of the seeds germinated and it took almost nine days for initiating germination. More over, the incidence of fungal growth was observed to be severe in this treatment. When the seeds were stored for three months and kept on petri-dishes over moist filter paper, 40 per cent of the seeds germinated and it took ten days for starting germination. The control seeds (fresh) did not germinate. The results revealed that seeds of *P.zeylanica* are characterised by hard seed coats, which are impermeable to water.

The rate of growth in terms of number of leaves produced and size of leaves was lower in seedlings produced from seeds cut at chalazal end (Plate 16). The seedlings produced only three to four leaves in a month while seedlings produced from seeds cut at micropylar end produced four to five leaves.

4.2.7 Ancillary observations at flowering

Two pests attacking the flower bud and flowers of *P. zeylanica* were identified. The larvae of *Anisephyra ocularia,* belonging to family Geometridae (Plate 17a) were found to feed on petals of flowers. This pest was rarely observed in *P. rosea.* They were found to feed on petals only and not on the other plant / flower parts.

14

15

16

Plate 14 **Fruit development in *P. zeylanica***

Plate 15 **Germination of seeds of *P. zeylanica* after cutting chalazal end**

Plate 16 **Seedlings of *P. zeylanica***
1. after cutting at chalazal end
2. after cutting at micropylar end

17a

17b

Plate 17 Pests of plumbago

17a *Anisephyra ocularia* F. Geometridae

17b *Syntarcus* sp. F. Lycinidae

Another pest *Syntarcus* sp. of Lycinidae (Plate 17b) was found to attack the floral buds and calyx of opened flowers of *P. zeylanica.* But they were not found on *P. rosea.*

4.3 Post-harvest storage of roots of *Plumbago rosea*

The efficiency of different packaging systems for storage of dried roots of *P. rosea* was studied. Moisture absorption as measured by the change in weight, fungal contamination and loss of plumbagin were recorded.

The moisture content of fresh roots was 69.00 per cent. The air-dried roots recorded 18 per cent moisture, which was again reduced to 8 per cent by oven drying. The initial plumbagin content was 0.65 per cent on fresh weight basis.

4.3.1 Moisture absorption

The change in weight of samples observed at bimonthly interval is given in Table 29 (Fig. 27). The dried roots kept under ambient conditions as control absorbed moisture very quickly and after four months of storage it recorded a weight of 31.60 g. From 6 MAS onwards the samples showed progressive decrease in weight. At the end of the experiment (18 MAS) the weight of the sample was reduced by 46.60 per cent.

Table 29. Changes in weight of root samples after different periods of storage in *P.rosea*

Storage period	Storage treatments				
	Control	Plastic bottles	Poly ethylene	Polyporpylene	Polythene- lined gunny
0 MAS	30.00	30.00	30.00	30.00	30.00
2 MAS	31.50	30.50	30.50	30.80	31.30
4 MAS	31.60	30.50	30.80	300.80	32.10
6 MAS	25.23	30.20	31.70	31.50	36.70
8 MAS	23.30	31.60	32.00	32.00	31.40
10 MAS	22.70	30.80	32.10	32.50	29.10
12 MAS	18.00	31.10	31.60	31.90	23.50
14 MAS	18.00	31.90	31.20	32.00	18.90
16 MAS	17.00	31.80	32.00	32.50	16.90
18 MAS	14.00	31.90	33.50	29.50	16.70

* MAS – Months After Storage

The roots stored in polyethylene lined gunny bags, showed the same trend as that of control except that the decrease in weight began at 10 MAS (29.10 g) and recorded 16.70 g at 18 MAS.

Samples stored in polypropylene containers also showed the same trend and maximum increase in weight was observed at 10 MAS (32.00 g).

The roots stored in polyethylene indicated increase in weight from 30.50 g at 2 MAS to 32.10 g at 10 MAS. Thereafter, there was a gradual decline in weight and at 18 MAS an increase in weight (11.70 %) was observed.

However, the samples stored in plastic containers showed only a slight increase in weight. The maximum increase was noted at 14 MAS (31.90 g). At 18 MAS the increase was 6.30 per cent only.

4.3.2 Fungal contamination

In order to study the influence of season on microbial contamination of roots stored under ambient conditions, fresh roots were collected during October 1996 and February 1997. The roots were oven-dried and stored for varying periods. The results indicated that the samples stored during October 1996 were affected by fungi during July 1997 (10 MAS) after the commencement of south west monsoon in June. However, samples collected and stored during February showed symptoms of fungal contamination during July 1997 (6 MAS).

The weight of sample increased steadily up to 4 MAS in control and subsequently the weight decreased after development of fungal mycelia in July at 6 MAS. Of the other four containers, polythene lined gunny bags showed the presence of mycelia at 8 MAS. The fungal growth and spread was comparatively faster in the control and entire material was covered with the fungal mycelia there after (Plate 18).

All the four containers were effective in protecting samples from fungal infestation upto 8 MAS. At 10 MAS only a few mycelia could be noticed in polythene-lined gunny bags. In polypropylene containers fungus infestation was noticed up to 16 MAS and no fungal growth could be noticed in samples stored in polyethylene bags and plastic bottles.

The spores were of four different colours (Plate 19) viz., yellow, chocolate, light green and black. *Pencillium* sp. and *Aspergillus* spp. were observed. Bacterial and actinomycete colonies were also observed.

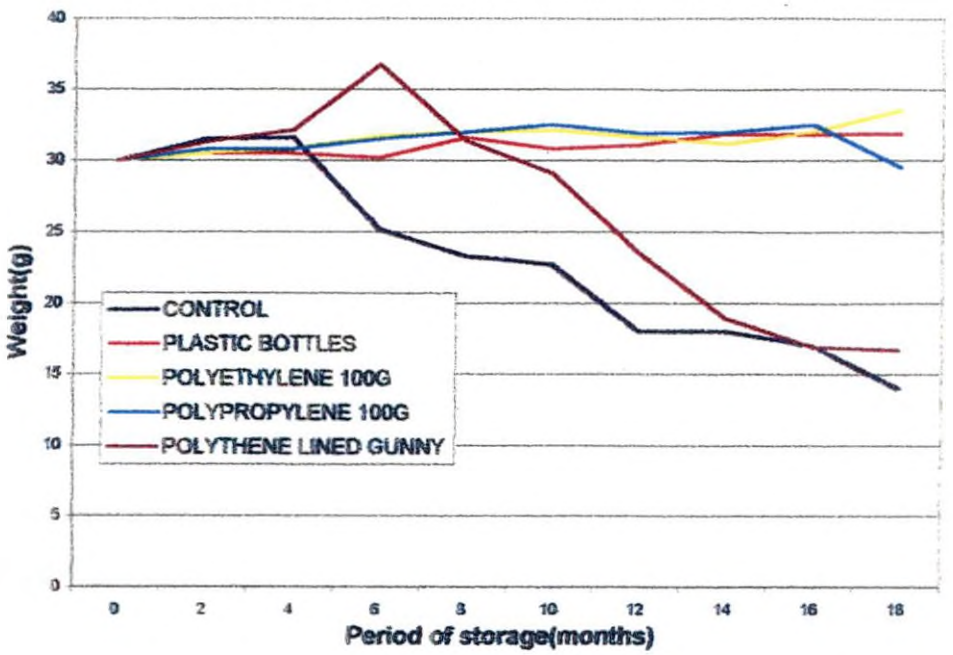


Fig.27. Change in weight of root samples during storage

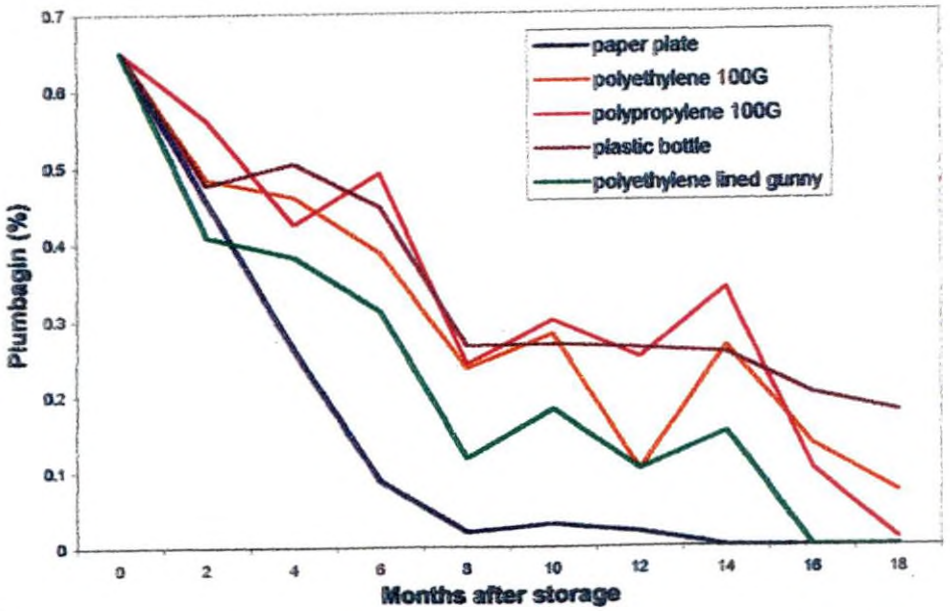
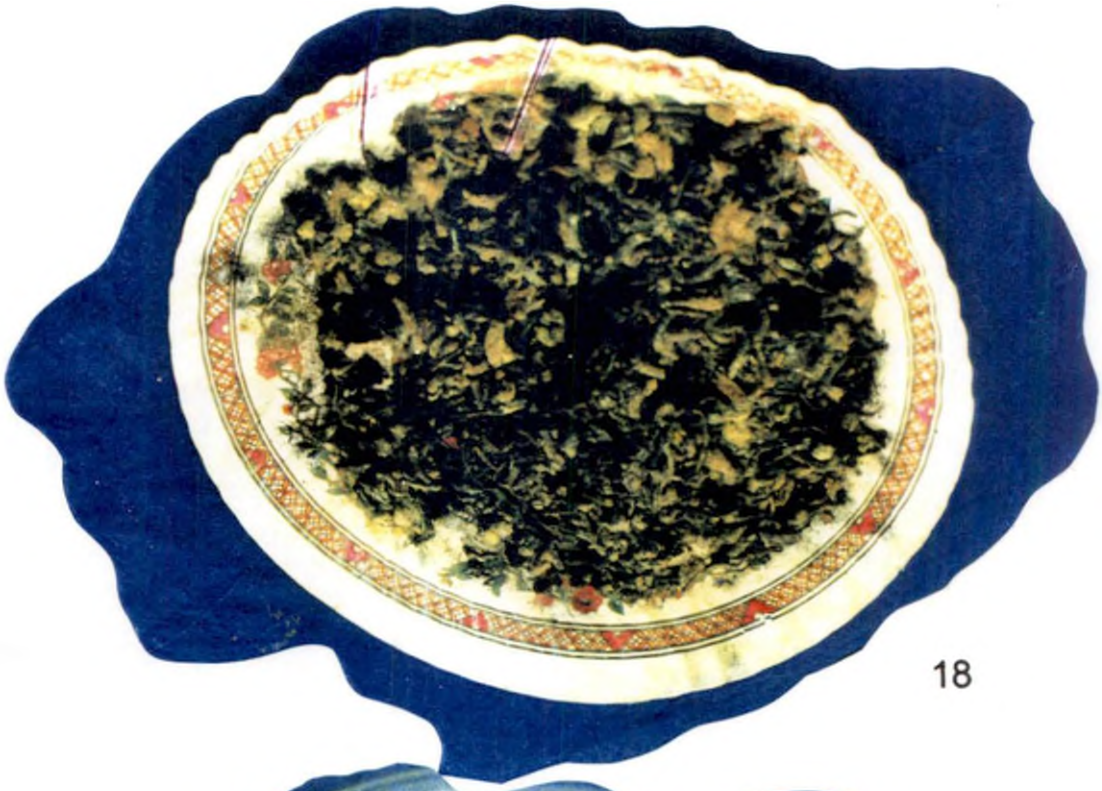


Fig.28. Variation in levels of plumbagin under different storage conditions



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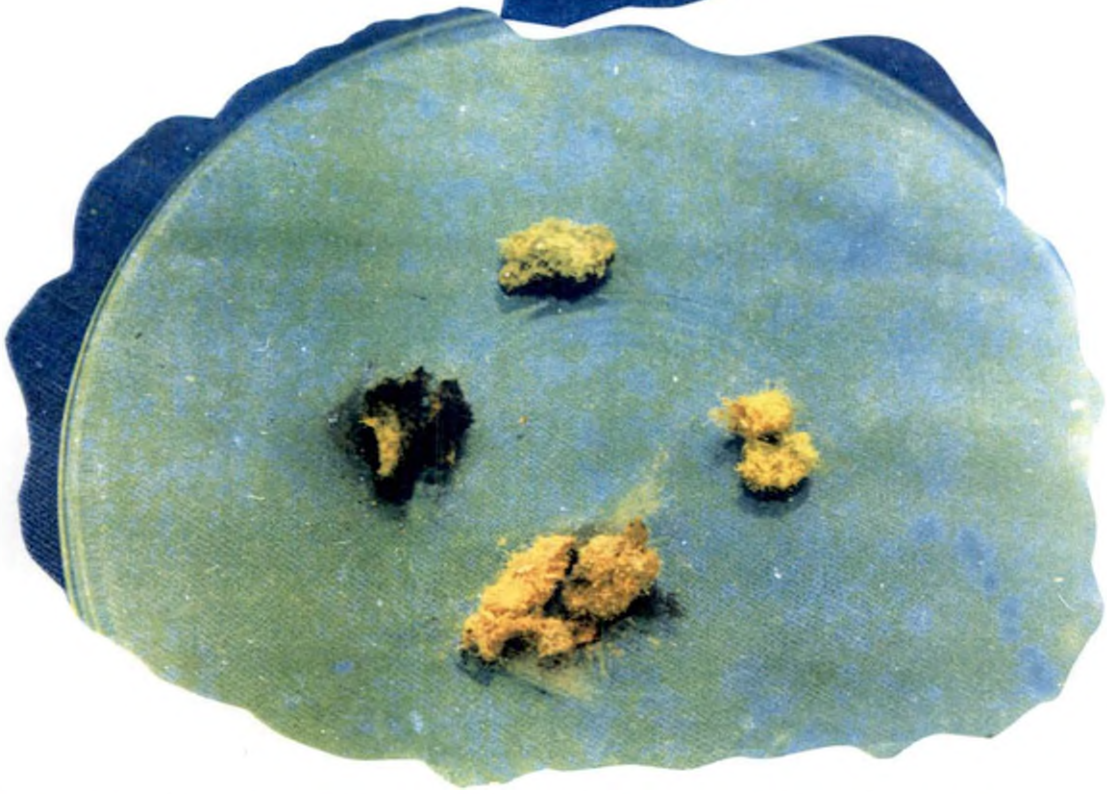

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Plate 18 **Samples stored under ambient condition after one year of storing**

Plate 19 **Root samples infected with different species of *Aspergillus***

4.3.3 Plumbagin content

Plumbagin content of dry sample was estimated to be 0.65 per cent before storage. The variation in plumbagin content of samples stored in different containers from 0 MAS to 18 MAS are furnished in Table 30 and Fig. 28.

Table 30. Variation in Plumbagin content of *P. rosea* roots during storage

Storage period	Plumbagin content (%)				
	Paper plate (control)	Polyethylene 100 G	Poly propylene 100 G	Plastic bottle	Polythene-lined gunny bag
0 MAS	0.65	0.65	0.65	0.65	0.65
2 MAS (March April)	0.456	0.484	0.562	0.476	0.408
4 MAS (May June)	0.26	0.46	0.424	0.504	0.381
6 MAS (July Aug)	0.087	0.387	0.491	0.447	0.31
8 MAS (September October)	0.02	0.234	0.24	0.264	0.116
10 MAS (November December)	0.03	0.28	0.298	0.266	0.181
12 MAS (January February)	0.02	0.102	0.249	0.261	0.103
14 MAS (March April)	traces	0.263	0.34	0.255	0.15
16 MAS (May June)	traces	0.133	0.101	0.199	trace
18 MAS (July August)	traces	0.071	0.01	0.176	trace

MAS - Months after storage

The results revealed that roots stored in all containers showed a drastic reduction in plumbagin content during the course of storage. In the case of samples stored under ambient conditions, the reduction in plumbagin content was so sharp that after 14 months, the plumbagin content was only trace.

In the case of polythene-lined gunnies also, the same trend was observed. The plumbagin content progressively reduced. The results in respect of other containers also suggested that though the samples recorded lower weight difference and lesser microbial infestation, the plumbagin content reduced progressively. However the decrease was not so marked. At the end of the study (18 MAS) the samples recorded 0.010 – 0.176 per cent plumbagin.

Discussion

DISCUSSION

Plumbago spp. valued very much in different ayurvedic preparations, have been introduced for cultivation in recent times. Since it is a new crop, it is essential to have knowledge on growth pattern, yield, flowering and post harvest handling techniques. The present study was an attempt to gather the above information.

The results obtained are briefly discussed here under.

5.1 Analysis of growth and quality '*Plumbago* spp.'

5.1.1 Biometric characters

The major differences between two species at 16 MAP and 18 MAP are furnished in Appendix 11 and 12. *P. zeylanica* was taller than the other species. Both species recorded a steady increase in plant height up to 16 MAP. The maximum height was recorded at 16 MAP when the plant started flowering. In peppermint, Marotti *et al.* (1993) also reported maximum height at full flowering.

The pattern of growth was linear in both the species even after one year. The crop is recommended to be grown as an annual in Kerala. The present study indicated that harvesting the plant at 12 MAP was not worthwhile to realise maximum yield and returns. However, Subha (1990) recommended that plumbago is suitable for growing as an annual. The conclusion was based on cost benefit analysis after 12MAP.

P. rosea produced longer internodes. The length of internode was the highest at 8 MAP in both the species. It was also observed that *P. rosea* produced suckers at definite intervals and branching was rarely seen. At the end of the study (18 MAP), the plant produced about 6.3 suckers per plant. In contrast, *P. zeylanica* rarely produced suckers and it produced new branches at regular intervals.

P. zeylanica was more vigorous in growth and produced higher total leaf area per plant. However, as against the height, the results on leaf area per plant indicated that the rate of increase in leaf area was not proportional to the increase in plant height. The total leaf area per plant in *P. zeylanica* increased up to 12 MAP, and the highest value was noted at 12 MAP, i.e. after the commencement of south west monsoon. Then it

showed slight decline at 14 MAP and thereafter increased up to 16 MAP in December. By 18 MAP, as dry season commenced, leaf area declined. In the case of *P.rosea,* the total leaf area increased up to 8 MAP and with the commencement of summer, a marked decline in leaf area was noticed. Later, with the onset of south west monsoon, there was a progressive increase in leaf area up to 16 MAP and declined thereafter. Reduction in leaf area during dry season is a common observation in many crop plants. The present study also revealed that leaf area that contributes the major assimilatory surface of plant was influenced mainly by season. In *Rauvolfia tetraphylla,* it was found that the total foliage was markedly higher during rainy season and minimum during dry season (Granda, *et.al.*1986). In *Holostemma annulare,* Meera (1994) also reported a progressive increase in leaf area up to 12 MAP and decline in leaf area thereafter.

P. zeylanica produced comparatively longer and higher number of roots (Table 2). But the girth of roots was comparatively smaller than *P.rosea.* In both species, number of roots and girth of roots tended to increase with age up to 16 MAP and thereafter the values were on par. However, in respect of length of root, the observations showed that, the roots continue to grow up to 18 MAP. Meera (1994) reported similar results in *Holostemma annualare* where in all root characters increased progressively with age. Similar results were reported in *Costus speciosus* also (Joseph, 1983).

In respect of fresh weight of roots, the two species did not differ significantly. However, the dry root weight was significantly higher in *P zeylanica,* which is due to the higher dry recovery. Fresh and dry weights of root increased progressively with age of the crop and maximum values were observed at 18MAP. Meera (1994) observed progressive increase in fresh and dry weights of root in *Holostemma annulare.* But it could be noticed that in *P. rosea,* dry weights at 16 and 18 MAP were on par. This showed that *P. rosea* can be harvested at 16 and 18 MAP as the yields did not vary significantly. In *P. zeylanica,* further studies are required to arrive at the optimum stage of harvest, as the root yield continued to increase with age of plant up to 18 MAP. Most of the earlier studies in this crop suggested the suitability of growing this crop as annual (Subha, 1990). In the present study, it was found that the rate of growth in respect of economic characters showed a linear trend with age. The comparison of fresh and dry yields at 12 and 18 MAP stressed the usefulness of retaining the crop up to 18 months to

realise higher yield. Such a practice increased dry root yield by 2.27 times in *P. rosea* and 2.01 times in P.zeylanica.

P. zeylanica recorded significantly higher fresh and dry weight of stem. In both species the values showed a progressive increase with age. In respect of fresh weight of shoot, significant difference was noticed up to 14 MAP and after that the values were on par. The significant increase in fresh weight up to 14 MAP is attributed to favourable environmental conditions that prevailed during the period (Appendix 11). After 14 MAP, the values were not significantly different, which might be due to the high metabolic rate during dry season. However, the increase in dry weight was significant up to 18 MAP and this is due to the high dry recovery.

P. zeylanica showed vigorous growth as compared to *P. rosea*. The fresh weight of leaf was significantly higher in *P. zeylanica*. During different stages of harvest the values showed the similar trend as that of total leaf area per plant. The influence of wet and dry seasons on leaf area was reflected in fresh and dry weights of leaf also. Similar results were reported by Granda *et al.* (1986) in *Rauvolfia tetraphylla*.

5.1.2. Physiological characters

The total dry matter production was comparatively higher in *P. zeylanica* and showed a linear increase till the end of the study. In *P. zeylanica* the increase was due to the significant increase in root dry weight, stem dry weight and leaf dry weight. Path coefficient analysis revealed the direct influence of fresh weight of shoot on total dry matter. However, in *P. rosea* though the increase in root dry weight was not significant after 16 MAP, the total dry matter accumulation showed steady increase due to the drastic increase in dry weights of shoot and leaf. The observation on root: shoot ratio indicated the influence of shoot dry weight at 18 MAP on dry matter accumulation. The path coefficient analysis also suggested that fresh weight of shoot had direct effect on total dry matter production in *P. rosea*.

Root: shoot ratio was high during May. This might be due to high water stress experienced during summer. It is reported that the root: shoot ratio of plants increase with water stress (Turner,1979). This usually arises from a greater decrease in rate of growth of tops in relation to roots. Turner (1979) has provided the evidence for an increase in root growth in dry season. An increase in root weight may indicate greater

intensity of root or greater depth of root, which are important morphological adaptations to water deficit, in that they can extract soil water more efficiently and maintain high plant water potential.

Harvest index of *P. rosea* was comparatively higher. Both the species recorded the highest harvest index at 10 MAP (May) followed by a decline at 12 MAP and a marginal increase at subsequent intervals. The highest harvest index recorded in both the species during May might be due to rapid translocation of assimilates to the roots to tide over severe dry weather. In cereal crops it was found that when water stress occurs at seed filling stage, an increased proportion of assimilates is transferred to the seed (Turner,1979). With the onset of south west monsoon the assimilates might have been re-translocated to the vegetative parts resulting in a decline in harvest index at that period. In subsequent stages, the vegetative growth was continued due to favourable weather conditions and thereby translocation of assimilates to the roots was only marginal.

Leaf weight ratio of the two species did not differ significantly. In both the species this character did not show a definite pattern during the various stages of harvest. However, in *P. rosea* the leaf weight ratio was the lowest at 10 MAP, which coincided with the end of summer. In *P. zeylanica,* the lowest value was recorded at 18 MAP. The ratio was reduced after 12 MAP. In the second year, the plant could produce only less number of leaves and after 18 months senescence might have commenced.

Being more vigorous *P. zeylanica* recorded higher leaf area ratio. In this species the highest leaf area ratio was recorded at 12 MAP during which it recorded the highest leaf weight ratio also. In *P. rosea* the highest leaf area ratio was observed at 16 MAP when it recorded the higher leaf weight ratio also.

In terms of leaf area index also *P. zeylanica* was significantly better. Changes in leaf area index at different harvesting intervals followed the same trend as in the case of leaf area ratio.

Crop Growth Rate and Net Assimilation Rate were significantly higher in *P. zeylanica* as compared to *P.rosea*. This generally shows an increase in the efficiency of available leaf area and relatively high dry matter production. In *P. rosea* NAR and

CGR showed negative values at 10 MAP i.e. during May. This might be due to the fact that during dry season rate of growth was the least. In *P. zeylanica* negative values were not observed due to the adaptability of crop to stress conditions.

The response function fitted in both the species revealed that fresh weight of root per plant was directly influenced by fresh weight of leaf and root length in *P. rosea* and root girth in *P. zeylanica.* The total dry matter per plant of *P. rosea* was directly influenced by fresh weight of shoot, root length and plant height. In *P. zeylanica* fresh weight of shoot, root and number of roots directly influenced total dry matter production of plant.

Leaf Diffussive Resistance (LDR) is considered as a direct indicator of stomatal response of plants. The lower number of stomates of *P. zeylanica* was reflected in higher diffussive resistance of the plants and thereby the rate of transpiration was also low (Appendix 8 and 9). This directly indicated the drought tolerant nature of *P. zeylanica.* The anatomical investigations also confirmed these results.

5.1.3 Biochemical attributes

The major differences in biochemical attributes of the two species are furnished in Appendix 7 and 8. At 10 and 12 MAP total chlorophyll contents were higher in *P. zeylanica* which was also followed by higher total dry matter production. The higher total dry matter production in this species was also due to higher total leaf area recorded. However, root protein content was low during these stages. This may be due to the fact that the assimilates at these stages are utilised for vigorous growth of the species. Accumulation of protein in roots commenced after this stage.

The total chlorophyll was the highest at 16 MAP in both the species. The total chlorophyll, chlorophyll a and chlorophyll b contents were higher in *P.rosea.* The ratio of chlorophyll a: chlorophyll b was higher in *P. zeylanica* at 16 MAP. But the root protein and tender leaf protein contents were higher in *P. zeylanica,* which also recorded a higher MDH and PEP activity. The total dry matter content was also higher in *P. zeylanica* at 16 MAP. But the plumbagin content was low and a high plumbagin was noticed in *P. rosea* (Appendix 8). In *P. rosea* the chlorophyll content was higher but the protein content was low and plumbagin content was high. The reverse trend was noticed in *P. zeylanica.* This is contradictory to the results reported by Narayanan

(1993), who has established a negative relationship between leaf chlorophyll and root alkaloid content in different species and types of *Rawolfia sp.*

Though the chlorophyll content of *P. zeylanica* was lower, the effect was compensated by the production of larger leaf area, which had contributed to higher dry matter accumulation. The tender leaf protein content was also higher in this species. The higher dry matter accumulation in *P. zeylanica* might have increased the synthesis of protein. Dinda and Saha (1987) isolated nine amino acids from the aerial part of *P. zeylanica.* The low plumbagin content in this species indicated that the conversion of assimilates to plumbagin, the secondary product, was low in this species.

The alkaloids are frequently considered to be shaped through decomposition of protein (Winterstain and Trier, 1931) and experimental evidence suggests the existence of a relation between decrease of total and protein N and increase of total alkaloid and soluble nitrogen. In the present study, *P. rosea* recorded higher leaf protein (mature leaf) as compared to *P. zeylanica* during all stages of growth. This species recorded higher plumbagin content also. Though *P. rosea* recorded a lower value in respect of most of growth parameters, the higher plumbagin content recorded can be explained as due to the higher rate of conversion of assimilates to secondary metabolites.

At 18 MAP, the ratio of chlorophyll a: chlorophyll b was higher in *P.rosea.* The plumbagin content was also higher in *P.rosea.*

In *P. rosea*, low leaf protein was observed during flowering season, which was accompanied by an increase in plumbagin content in roots. It could be observed that there exists inverse relationship between leaf protein and plumbagin content in the roots of both the species. However, there was no relation between root protein and plumbagin content. This is due to the conversion and utilisation of amino acids as the precursor for plumbagin. As a result both the species expressed high plumbagin at flowering.

The low MDH activity observed in *P.rosea* may be due to the low concentration of substrate or excess accumulation of the substrate, oxalo acetic acid. Plumbagin, a secondary metabolite was higher in *P.rosea* and this is a character, which is governed by genetic or environmental factors. For increased production of plumbagin in plant system there must be higher precursors of plumbagin, mainly amino acids. Since the accumulation of organic acids such as oxalo acetic acid are instrumental for protein or

enzyme formation and these organic acids seem to be diverted for synthesis of plumbagin, the MDH activity was substantially reduced in this species. One of the main reasons for the reduced growth rate and less vigorous nature of this species can be low enzyme activity.

In the case of *P. zeylanica*, up to 40 per cent oxalo acetic acid could be utilised effectively for normal metabolism as against 30 per cent in *P. rosea*. The higher dry matter accumulation in *P. zeylanica* may be a reflection of this character. This is supported by the activity and specific activity differences in both the species.

In the case of phospho enol pyruvate carboxylase, at optimum substrate concentration (26.67 per cent) fast reaction was noticed in the first minute. The activity was independent of time thereafter. The PEP carboxylase activity and specific activity were higher in *P. zeylanica*. As a result it can be presumed that *P. zeylanica* having higher PEP accumulation has a more efficient normal metabolism.

Though the specific activity of PEP carboxylase was detected in both the species, the anatomical studies did not reveal Kranz anatomy. Therefore, the results are not confirmatory and more detailed investigation are required to arrive at a conclusion. In C_4 species, *Amaranthus retroflexus* (Usuda *et al.*, 1971) and *Portulaca oleracea* (Kennedy, 1976) C_4 acids are major products of CO_2 fixation though they lack Kranz anatomy. C_4 plants have higher stomatal resistance in comparison to C_3 species. There are also reports of occurrence of C_3, C_4 and C_3-C_4 intermediate species within a single geneus *Flavaria* (Mc Gowgh and Nelson, 1995) and *Alternanthera* (Rajagopal *et al.*, 1993).

The quantity of crude extract and purified plumbagin were found to be higher in *P. rosea*. In *P.rosea* the highest plumbagin recovery was obtained at 16 MAP which coincided with the flowering season. The plant was strictly seasonal in respect of flowering and flowers were produced only during the period from October to January in *P.rosea*. In *P.zeylanica* flowering was a continuous process (Table 26). Reproductive stage of development permits the biogenesis of active principles. Similar results have been reported in *Cinchona, Datura, Atropa, Hyosyamus, Catharanthus, Costus* and *Digitalis* (Chatterjee, 1992), in *Costus* (Sarin *et al.*, 1977) and *Adonis amurensis* (Skurzoa and Shnyakina, 1985). While analysing the habit and habitat of medicinal

plants of Peechi forest, Raj (1997) observed that the tribes collect different medicinal plants when they produce flowers.

In *P. zeylanica*, the quantity of crude extract and purified plumbagin content showed fluctuation during different intervals of harvest. The plant produce flowers at regular intervals. Thus the correlation between flowering and plumbagin content could not be established in this crop as in *P.rosea*.

5.1.4 Curing

The dry recovery of roots was higher in *P. zeylanica* when compared to *P. rosea* at 12 and 18 MAP. A reduction in plumbagin level up to 68.33 per cent by drying and 87.71 per cent by curing in lime water was observed in *P. rosea* roots at 18 MAP. Curing of *P. zeylanica* roots in lime water removed the plumbagin up to the extent of 97.22 per cent while drying removed only 60.97 per cent. In ayurvedic preparations, *Plumbago* spp. is mainly used against abdominal complaints. The roots are usually cured in lime water and dried (Pandey, 1960) or simply dried before being used. The main objective behind this practice might have been reduction of plumbagin content. Sasidharan (1996) suggested that curing in lime water reduced toxicity of *P. rosea* roots. Plumbagin in small doses has a stimulant action on central nervous system, on plain muscles and on the secretion of sweat, urine and bile. Blood pressure shows a slight fall and the peripheral vessels are found to dialate (CSIR, 1969). With large doses, plumbagin causes paralysis leading ultimately to death. An extract of sandal and vetiver in water was served as antidote to plumbago poisoning (Nesamani, 1985).

Chemical analysis also indicated the formation of organic compounds having steroidal properties, which in turn may contribute to medicinal properties. TLC and UV scanning studies throw light on these aspects. Steroids are derived triterpenoids which possess a number of methyl groups (Daniel, 1991). Steroids in general have anti-inflammatory activity. The effectiveness of plumbago against rheumatic complaints could thus be explained.

The pattern of spot development in fresh, dry and cured root samples showed variation. The cured root extract of both the species gave an additional yellow spot at Rf 0.04 and a light yellow spot at Rf 0.64. The cured roots showed the presence of additional compound with a very low content of plumbagin. When the same

chromatogram was viewed under UV at 254 and 366 nm, the cured root extracts showed a third fraction at Rf 0.56, which was very close to the light yellow spot at visible range.

Even though loss of plumbagin was high in the curing process, the leachate contained only traces of plumbagin . The additional spot in the chromatogram of cured samples at 0.04 and 0.64 in visible and 0.56 in UV suggested that there might be some degradation or interconversion of natural metabolites to a compound having medicinal properties. The loss in plumbagin content of cured sample was compensated by the formation of new components as it was evident from the above results.

The compound at Rf value 0.79 in dry sample and the compounds at Rf 0.56 of cured sample clearly indicated the formation of new components in the root samples while drying and curing.

The chromatograms were analysed for the presence of different groups of compounds (Table 25) as suggested by Krichner (1978). All the three root extracts showed the presence of amino acids, carbohydrates, phenols and one steroid group (yellow spot) at Rf. 0.89 with antimony trichloride.

The dry root extract gave an additional pink spot at Rf. 0.79 using ninhydrin spray. The purple spot at Rf 0.1 with antimony trichloride spray and with Vanillin sulphuric acid spray (Rf 0.77) indicated the presence of steroid group. The spot at Rf 0.1 was observed in cured sample also. A pink spot at Rf 0.34 was expressed when lead acetate was sprayed in dry sample. The above results revealed the presence of steroid groups having medicinal properties.

The presence of steroid group after curing was noticed as it was indicated by green spot developed at Rf 0.22 and a purple spot at 0.1 using antimony chloride spray and a purple spot of Rf 0.32, green spot of Rf 0.25 developed with vanillin sulphuric acid spray. The yellow spot of plumbagin was also very faint, which indicated the presence of low quantity of plumbagin. Flavonoid test was carried out by spraying with lead acetate and when viewed under UV (366 nm), a bright fluorescent spot at Rf 0.49 was observed.

Even though dry and cured samples expressed a spot at Rf 0.1 in antimony trichloride, the spot at 0.2 and 0.25 in both antimony trichloride and vanillin sulphuric

acid test showed the presence of steroid groups which might have an important role in therapeutics. One of the flavonoid tests showed it to be positive in both dry and cured samples where Rf values observed were different (0.34 in dry and 0.49 in cured).

Quinones, lignans, coumarins, flavonoids and tannines are the major groups of phenolics exhibiting marked pharmacological activities (Daniel, 1991). Bioflavonoids increase the capillary resistance and thus prevent subcutaneous capillary bleeding. Flavonoids with multiple methoxy groups play an important role in circulatory system by reducing the aggregation (Daniel, 1991).

The *Plumbago* spp. exhibit digestive and stomachic properties. In ayurvedic preparations it is used only after curing. The cured samples showed low plumbagin, some steroid and flavonoid groups of compounds. However, the dry and cured samples did not give same results.

5.1.5 Anatomical characters

Anatomical characterisation is recognised as an effective way of studying adaptation of plant to different stress conditions. The stem sections of *P.zeylanica* and *P. rosea* showed distinct variations. The T.S. of the stem of *P.zeylanica* had a corrugated or wavy outline but that of *P.rosea* was circular. The epidermal cells of *P. zeylanica* were small and covered with thick cuticle. In *P.rosea* the epidermal cells were comparatively larger. The cortex of *P.rosea* was distinct in that it was traversed by resin canals. Resin canals were absent in *P. zeylanica.* The quantity of collenchyma cells was very less as compared to that of *P. zeylanica.* The pericycle was seen in patches of chlorenchyma in *P. rosea* whereas it was continuous in *P.zeylanica.* Vascular bundles were arranged in discontinuous rings in *P.zeylanica* whereas it was arranged in the form of a ring in *P.rosea.*

The roots of both species showed the presence of well-defined cortex. In *P. zeylanica* cortical cells were filled with starch grains consisting of 20 to 30 cells distributed in scattered manner. However, in *P. rosea* the starch grains and patches of sclerenchyma were absent. The presence of sclerenchyma in the cortex of root is an indication of drought tolerant nature of *P. zeylanica* (Plate 8). Jaffe and Biro (1979) reviewed the adaptation of drought tolerant plant to withstand stress. Among the

different factors, they stressed the role of number and thickness of sclerenchyma rows as one of the important factors for imparting drought tolerance.

Iyer and Kolammal (1960) described the histology of roots of two the species of *Plumbago* and they also recorded the presence of starch grains and sclerenchyma patches on the roots of *P. zeylanica.*

The anatomy of leaves of both the species reveal leaves showed typical dicot anatomy and that there was no difference in structure of leaf of both the species.

5.2. Analysis of flowering and breeding behaviour

P. rosea was strictly seasonal and *P. zeylanica* did not show seasonal behaviour and flowering continued through out the year. The inflorescences were often terminal and rarely axillary in *P. rosea.* But, in *P. zeylanica* they were either terminal or axillary. The spikes of *P. rosea* were indeterminate and continued to elongate even after completion of flowering. In *P. zeylanica* the inflorescence was determinate. The duration of flowering per inflorescence was longer in *P.rosea.* The mean number of flowers per inflorescence was higher in *P. rosea.* The colour of flowers are bright red in *P. rosea* and white in *P. zeylanica.* In contrast to the vegetative characters, the flower was comparatively smaller in *P. zeylanica.* The bracts were larger than bracteoles in *P. zeylanica* and equal in *P. rosea.* Aestivation of petals was twisted in *P. rosea* whereas it was valvate in *P. zeylanica.* The stamens of *P. zeylanica* were as long as corolla tube and the anthers were exerted beyond the throat of corolla tube in both the species. The styles of *P. rosea* were very short when compared to *P. zeylanica.* The stigmas of *P. rosea* were deeply seated inside the corolla tube and thus anthers and stigma were held at two levels preventing self- pollination. In the case of *P. zeylanica,* three types of styles were observed. In first type, styles were produced at the same level as that of anthers favouring self pollination. In the other two types, the styles were held at a higher or lower level. As such, the chances for cross- pollination are high. So *P. zeylanica* could be considered as an often cross- pollinated crop. This type of heteromorphism has been reported in angiosperm families namely Oxalidaceae, Lythraceae and Pontederiaceae. In *P. zeylanica,* incompatitility is not observed. However, heteromorphic self incompatibility was reported in the family Plumbaginaceae

(Frankel and Galun, 1977). Detailed investigations have to be undertaken to confirm whether incompatibility mechanism exists in *P. zeylanica.*

The time of peak anthesis of *P. rosea* was between 8.30 to 9.00 hours and in *P. zeylanica* the peak time was between 7.30 to 8.00 hours. The pollen grains of *P. rosea* were smaller than *P. zeylanica* and number of pollen grains per anther was less in *P. rosea.* The pollen grains of both species were trizonocolpate. Moore and Webb (1978) reported the presence of trizonocolpate pollen in Plumbaginaceae. Pollen fertility of *P. rosea* was only 28.10 per cent as against 90 per cent in *P. zeylanica.* Usha (1983) identified lack of fertile pollen grain as one of the reasons for lack of fruit set in ginger. On *in vivo* pollination the pollen grains of *P. zeylanica* could germinate on stigmatic surfaces of both the species. However, there was poor pollen germination. The pollen tube growth was so slow that even after 24 hours of pollination none of the pollen tubes could reach the ovule. It was also found that the pollen grains of *P. rosea* did not germinate on stigmatic surfaces of both the species. This might be due to the low pollen fertility and poor pollen production in *P. rosea.* Difficulties in adherence of pollen grains on stigmatic surface was observed in *P. rosea.* It is to be noted that pollen grains have to lodge and adhere to the stigma papillae before they can germinate. When adhesion is a chemical reaction, the initial lodgement is a mechanical function. The difficulty in adherence of pollen grains on stigmatic surface have been reported in the family Plumbaginaceae (Dulberger, 1975, Maltson, 1983). According to Sathyabhama (1988) the reason for lack of seed set in ginger, was spiny nature of stigma. She found that the pollen grain applied on the stigma were either sticking to spine tips or were damaged by spines. However, in the present study such a situation was not noticed. It may be worth while to note that the flowers also contain plumbagin, which is caustic in nature. Kitanov and Pashankov (1994) reported the presence of plumbagin in the flower (3.06 %) of *P. europea.* In inter specific crosses involving *P. rosea* and *P. zeylanica* the reason for lack of fruit set may also be due the difference in chromosome number of the two species(CSIR, 1969).

In *P. zeylanica,* seed set was observed under natural conditions and also under all pollination methods tried. Under open pollination 85 to 90 per cent fruit set was observed. Self pollination reduced the fruit set to 70 to 80 per cent and there was a further reduction to 30 to 40 per cent on natural cross pollination. The heterostyly that is

observed in the population of *P. zeylanica* and the damages caused on emasculation might have contributed to the low fruit set under self and natural cross pollinations respectively. Heterostyly was reported to be the factor for low fruit set in the family Plumbaginaceace (Frankel and Galun, 1977). The fruit set occurred under different pollination systems with in the species may be due to the favourable floral morphology, higher pollen fertility and pollen production. It is also to be noted that the flowers of *P. zeylanica* contain only low plumbagin as compared to *P. rosea.*

5.2.1 Germination of seeds of *P. zeylanica*

In the present study, it was observed that when the seeds were given cuts at micropyle or chalazal ends, there was cent per cent germination. The fresh seeds without any treatments failed to germinate. However, seeds stored for three months started to germinate in ten days and recorded 40 per cent germination. The seeds which were cut at micropyle end took three days. In the case of those seeds, which were cut at chalazal end, took 4½ days to initiate germination. Scarification using sand also recorded cent percent germination but with a delay in initiation of germination. The results of the present study indicated that seeds of *P. zeylanica* are characterized by the presence of hard seed coat which are impermeable to water. Scarification of seeds helped to rupture the seed coat and seeds could imbibe water more efficiently. When the seeds were cut at micropyle end imbibition took place at a faster rate and there by germination was also faster. In this treatment the seedling produced radicle first and the plants produced were normal, healthy and vigorous. In the case of seeds which were cut at chalazal end, the imbibition was not fast and germination was delayed (Plate 15). Also it was observed that the seedlings produced plumules first and plants produced were less vigorous and unhealthy (Plate 15).

The lack of germination of *P. zeylanica* seed can also be due to the presence of germination inhibitors (Evenari, 1949). When the seeds were ruptured at either ends germination occurred due to the leaching of inhibitors from the seeds. The seeds of *P. zeylanica* are characterised by the presence of plumbagin, which is reported to be germination inhibitor. The plumbagin reduces the germination per cent and seedling length of small seeded plant species (Dornbos and Spencer, 1990). Soaking in low concentration of 200 μM of 5 – hydroxy naphtho quinone have been reported to delay

germination of seeds of Norway spruce (Segura *et al.*, 1992). In Iris, seed germination could be improved when the germination inhibitor was leached from seed with water.

The seeds on storing for three months recorded 40 per cent germination. Studies on the effect of storage on plumbagin content indicated that there was progressive reduction in the level of plumbagin with advance in period of storage in *P. rosea* (Table 30 and Fig. 28). So the storage of seeds for three months might have helped to reduce the plumbagin content (inhibitor level) inside the seed and helped in germination of stored seeds.

5.3 Effect of different containers on prolonging the storage life

Among the five different containers tried, the control samples kept under ambient conditions continued to show increase in weight up to 4 MAS. From 6 MAS the samples show progressive decrease in weight. At the end of the experiment the weight of the sample was reduced by 46.6 per cent. In contrast to the common observation that hygroscopic dry samples absorb moisture and gain weight under high RH, the present observations showed progressive decrease in weight from 6 MAS onwards. This was due to the severe fungal infestation. This period (6 MAS) actually coincided with the SW monsoon showers, thereby an increased relative humidity was built up which indirectly invited pathogens.

The moisture content is an important factor, which influences the growth of storage microorganisms in agricultural commodities. Considerable reduction in dry weight, starch content and reducing sugars were noticed in paddy seeds infested with fungi. (Vidyasekharan and Govindaswamy, 1966). The reduction in dry weight was due to reduction of starch. A progressive weight loss was also noticed in stored methi seeds (*Trigonella foenum-graecum*) and this weight loss increased with increase in relative humidity (Komaraiah and Reddy, 1986).

The fungi identified in the stored roots were *Aspergillus* spp. and *Penicillium* sp.. Occurrence of these two fungi in stored products has been reported in black pepper (Martinez and Christensen, 1973, Estellita, 1982) dried copra (Fishlock, 1929) and vegetable seeds (Ambika, 1991).

In the case of samples kept in polythene-lined gunny bags, the same trend of moisture absorption was noticed except that the decrease in weight was noticed at 10 MAS and samples registered a loss of 44.3 per cent after 18 MAS. Here also fungus infestation has contributed for the weight loss.

In the case of samples kept in polypropylene containers, the weight reduction was not very conspicuous. This may be due to the delayed fungal infestation.

The roots stored in polyethylene bags and plastic bottles did not show any reduction in weight. Till the end of the study it recorded a slow increase in weight. In these two treatments fungal contamination was not recorded. The samples kept in plastic bottles could retain the freshness even after 18 MAS.

The results on the effect of containers on plumbagin content indicated that the roots stored in all containers showed a drastic reduction in active principle with period of storage. In the case of samples stored under ambient conditions the reduction was so fast that after 14 MAS plumbagin content was negligible. This can be due to the fungal infestation from 6 MAS onwards and also due to conversion of plumbagin to newer compounds. In polythene lined gunny bag also the plumbagin got reduced to a negligible quantities at 16 MAS.

The qualitative deterioration of stored agriculture products by infestation with storage microflora is a common phonomenon. The microflora which occur on copra were found to be capable of utilizing coconut oil as a source for their growth, leading to reduction in oil. (Eyre, 1932., Sreemulanathan and Nair, 1971). The oleoresin content of stored black pepper sample was found to be decreasing due to the microbial infestation (Estellitta, 1982).

In respect of other containers also in spite of lower weight difference and lesser microbial contamination, the plumbagin content reduced progressively. However, decrease was not so marked. The results thus suggested that there was drastic reduction in plumbagin content with period of storage and this can be due to chemical changes taking place in the active principle on storage.

Summary

SUMMARY

The present study entitled "Analysis of growth, flowering and quality in *Plumbago* spp." was carried out in the Department of Plantation Crops and Spices, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur during 1995-1998.

The programme envisaged analysis of growth, physiological growth attributes, biochemical changes during different growth phases and post harvest handling of *Plumbago rosea* and *Plumbago zeylanica* up to 18 months after planting. The other aspects were studies on reproductive behaviour of the two species and standardization of post harvest storage practices in *P. rosea*. The salient findings are furnished below:

1. Analysis of growth and quality in *P. rosea* and *P. zeylanica*

1) *Plumbago zeylanica* recorded significantly higher plant height and total leaf area. However, *P. rosea* recorded higher internodal length. Plant height at 16 and 18 months after planting (MAP) were on par in both the species. In *P. rosea* total leaf area progressively increased with age and the highest value was observed at 16 MAP ($0.17 m^2$ pl^{-1}). In *P. zeylanica* the total leaf area increased up to 12 MAP and there after it showed declining trend.

2) The root length and root girth increased with growth stages and maximum value was observed at 18 MAP. The number of roots also increased steadily up to 16 MAP and thereafter no significant change. *P. zeylanica* recorded higher values in respect of length of roots and number of roots during all stages of growth when compared to *P. rosea*. However, *P. rosea* recorded the highest girth of roots.

3) Fresh and dry weights of leaves were significantly higher in *P. zeylanica* and the maximum weight was noticed at 12 MAP. In *P. rosea* leaf weight steadily increased up to 14 MAP and thereafter there was no significant difference.

4) Fresh and dry weights of stem were significantly higher in *P. zeylanica*. Maximum dry weight of shoot was obtained at 18 MAP in both the species. However, fresh weights at 16 and 18 MAP were on par.

5) There was no significant difference in fresh root yield between the two species. However, this was slightly higher in *P. rosea*. With regard to dry root yield, *P. zeylanica* recorded higher value due to the higher recovery of dry roots.

6) Growth was found to be linear up to 18 MAP in both species. Retention of the crop in the field up to 18 months increased dry root yield up to 2.27 times in *P. rosea* and 2.01 times in *P. zeylanica* as compared to harvesting at 12 months after planting.

7) *P. zeylanica* recorded significantly higher total dry matter production. The highest weights were noticed at 18 MAP in both the species.

8) In contrast to all the above characters, root: shoot ratio and harvest index were higher in *P. rosea*. Maximum values were noticed at 10 MAP.

9) Leaf growth parameters such as leaf area index and leaf area ratio were higher for *P. zeylanica* and higher values were observed at 16 MAP.

10) *P. zeylanica* recorded higher crop growth rate and net assimilation rate. These parameters did not show significant difference at different stages of harvest.

11) A linear model of growth was observed up to 18 MAP in both species. The rate of growth was higher in *P. zeylanica* except for fresh weight of root, fresh weight of leaf and girth of root.

12) Stepwise regression analysis revealed that the fresh weight of roots per plant was directly influenced by fresh weight of leaf and root length in *P. rosea* and root girth in *P. zeylanica*. The prediction equation for fresh weight of root (g pl^{-1}) in *P. rosea* was worked out to be [1.994 x fresh weight of leaf (g pl^{-1})] + [1.472 x length of root (cm)] – 21.13. In *P. zeylanica*, the prediction equation for fresh root yield (g pl^{-1}) was [51.522 x girth of root (cm)] – 28.43.

13) The stepwise regression analysis in *P. rosea* suggested that total dry matter per plant was directly influenced by fresh weight of shoot, length of root and plant height. The results in respect of *P. zeylanica* showed the influence of fresh weight of shoot, fresh weight of root and number of roots on the above attribute. The prediction equation for total dry matter per plant (g pl^{-1}) in *P. rosea* was worked out as [0.465 x fresh weight of shoot (g pl^{-1})] + [0.375 x length of root (cm)] + [0.181 x Plant height (cm)]

– 11.04. The prediction equation in respect of *P. zeylanica* was found to be [0.373 x fresh weight of shoot (g pl^{-1})] + [0.595 x fresh weight of root] + [0.964 x number of roots] – 9.02.

14) Path coefficient analysis in *P. rosea* suggested that in both the species fresh weight of shoot had a direct effect on total dry matter production.

15) *P. zeylanica* showed higher diffusive resistance, lower number of stomates and lower rate of transpiration.

16) The active principle, plumbagin was higher in *P. rosea* and it ranged from 0.69 to 1.4 per cent (on dry weight basis). In *P. zeylanica* the range was from 0.19 to 0.33 per cent (on dry weight basis). In *P. rosea* plumbagin level varied significantly at different stages of growth and the maximum value of 1.4 per cent was noticed at 16 MAP. However, in *P. zeylanica* the plumbagin level did not exhibit conspicuous variation during different stages of growth.

17) *P. rosea* recorded lower total chlorophyll content during early periods of growth and *P. zeylanica* recorded higher values during early stages. Maximum total chlorophyll was recorded at 16 MAP in both species.

18) The root protein content increased steadily with age in *P. zeylanica*. But in *P. rosea* protein content decreased up to 16 MAP. Tender leaf protein was also the lowest at 16 MAP in both the species.

19) The specific enzyme activity of malate dehydrogenase (MDH) followed similar trend in both the species. However, specific activity of phospho enol pyruvate carboxylase was higher in *P. zeylanica*.

20) Curing the roots in lime water significantly, reduced the plumbagin levels by 87 to 93 percent in *P. rosea*. In *P. zeylanica* a reduction of 93 to 97 percent was noticed. The plumbagin content of cured samples varied between 0.01 to 0.07 per cent on fresh weight basis. Drying the roots also reduced the plumbagin to the tune of 60 to 80 per cent.

21) Thin layer chromatogram of cured roots showed the presence of an additional compounds as indicated by the fluorescence spot at Rf 0.56, steroid groups (Rf 0.22)

and flavonoid group (Rf 0.49). Drying the roots gave one more amino group (Rf 0.79) and a steroid group (Rf 0.77).

22) The anatomical studies revealed variation in respect of stem and root of the two species. Cuticle of *P. zeylalnica* was thicker compared to *P. rosea.* Vascular bundles of *P. zeylanica* were in the form of discontinuous ring while in *P. rosea* they were arranged in continuous ring. In the pericycle, there was a continuous ring of sclerenchyma in *P. zeylanica* where as in *P. rosea* patches of sclerenchyma were present.

23) The roots of *P. zeylanica* were characterized by the presence of large sized starch grains and patches of sclerenchyma The stele was comparatively larger in *P. zeylanica* as compared to that of *P. rosea.*

24) The leaf structure of both the species did not show any significant difference.

2. Analysis of flowering and breeding behaviour

1. Flowering in *p. rosea* was observed to be seasonal (October – February) while it was continuous in *P. zeylanica.*

2. In *P. rosea* inflorescence was shown to be single spike which were terminal and rarely axillary. In *P. zeylanica* inflorescences were single or branched spikes, either terminal or axillary.

3. Inflorescences were longer in *P. rosea* and took longer periods for completion of flowering in a spike. It produced more number of flowers per inflorescence.

4. Flowers were found to be bright red and bigger in size in *P. rosea* while flowers were white and smaller in *P. zeylanica.*

5. Calyx was greenish in *P. zeylanica* and reddish in *P. rosea.*

6. The stigma was deeply seated inside the corolla tube in *P. rosea.* However, the stigma and anthers were seen at same level, slightly higher level or at slightly lower level in *P. zeylanica.*

7. The Peak period of anthesis was found to be between 7.30 and 8.00 a.m. in *P. zeylanica* and 8.30 and 9.00 a.m. in *P. rosea.*

8. Pollen grains were yellowish-white in *P. rosea* and bluish-white and larger in *P. zeylanica.*

9. *P. zeylanica* produced more pollen grains per anther and recorded a fertility of 89.98 per cent. *P. rosea* recorded a low pollen fertility of 28.13 %. Pollen grains of both the species failed to germinate in 19 media tried. Pollen grains of *P. zeylanica* recorded germination under *in vivo* conditions on the stigma of both the species.

10. In *P. zeylanica* fruit set after self-pollination was 70 to 80 per cent. It recorded 85 to 90 per cent fruit set on open pollination and 30 to 40 per cent on natural cross pollination.

11. Seeds of *P. zeylanica* failed to germinate when sown fresh. Stored seeds could germinate within 10 days and germination was only 40 per cent. However, when micropylar tip of seed (fresh or stored) were cut and sown, they started to germinate within 3 days and 100 per cent germination was attained. Normal seedlings could also be obtained by this treatment. Cutting the seeds at chalazal end also produced 100 per cent germination. However, the plumules emerged first and the seedlings were not healthy.

3. Post harvest storage studies

1) The moisture absorption by the root samples stored in paper plates and polythene lined gunny bags were so fast that fungal infestation was noticed by 6 months after storage and by 10 months after storage (MAS) respectively. The weight of root samples stored in the above containers reduced significantly with storage. The samples stored in plastic bottles and polyethylene bags showed comparatively lesser change in weight during storage.

2) The fungi associated with roots were identified as *Aspergillus* spp. Four types of spores, yellow, chocolate, light green and black were noticed. Apart from this *Pencillium* sp., Actinomycetes and bacterial colonies were also observed.

3) The plumbagin content of fresh roots was 0.65 per cent (on fresh weight basis). During storage, irrespective of containers, the plumbagin content was significantly reduced. The reduction in plumbagin level was most rapid in the case of samples stored under ambient conditions and polythene lined gunny bags.

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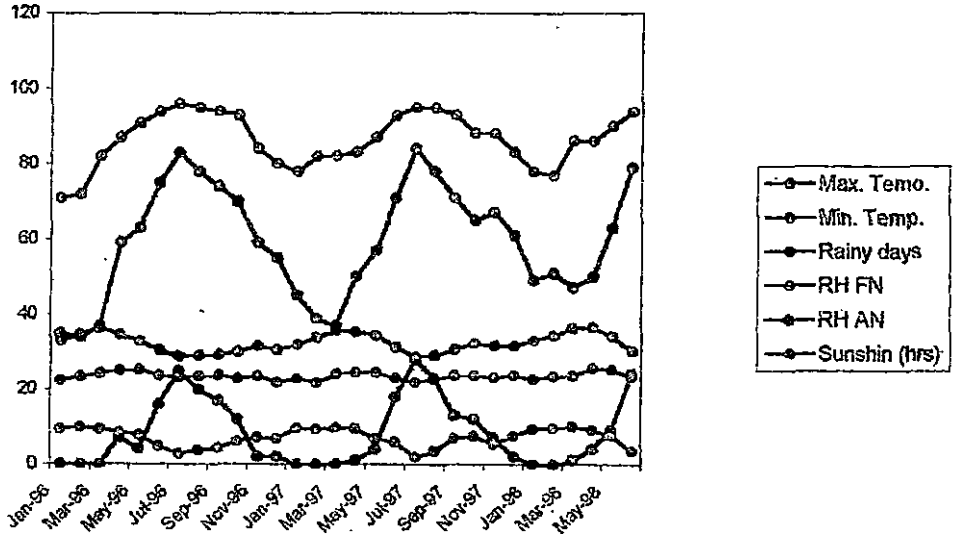
* Originals not seen

♣ Appendices

Apendix 1.



Weather data at Vellanikkara during the period of study

Appendix 2. Mean sum of square (M.S.S.) and F values of plant height, internodal length and total leaf area

Source	Degrees of freedom	Plant height		Internodal length		Total leaf area	
		M.S.S	F	M.S.S.	F	M.S.S	F
Stages	6	2737.753	15.1401**	4.609	5.5639**	0.087	921.6585**
Error	14	180.828		0.828		0.000	
Species	1	46078.532	260.3380**	8.595	22.1337**	0.421	7179.6639**
Species Vs Stages	6	553.417	3.1269*	1.166	3.0035*	0.069	1181.4503**
Error	14	176.995		0.388		0.000	

Appendix 3. Mean sum of square (M.S.S.) and F values of root girth, root number and root length

Source	Degrees of freedom	Root girth		Root number		Root length	
		M.S.S	F	M.S.S.	F	M.S.S	F
Stages	6	1.073	36.9399**	74.653	18.4123**	543.514	15.0162**
Error	14	0.029	224.4500**	4.055		36.195	
Species	1	9.619	0.5667	380.404	40.3001**	825.487	27.1495**
Species Vs Stages	6	0.024		13.709	1.4524	12.363	0.4066
Error	14	0.043		9.439		30.405	

** significant at 1% level
* significant at 5% level

Appendix 4. Mean sum of squares (M.S.S.) and F value of fresh weight of root and dry weight of root

Source	Degrees of freedom	Fresh weight of root (g plant^{-1})		Dry weight of root (g plant^{-1})	
		M.S.S	F	M.S.S.	F
Stages	6	3182.88	156.4091**	423.199	78.2769**
Error	14	20.35		5.406	
Species	1	166.802	4.3852*	1715.204	171.0683**
Species Vs Stages	6	63.117	1.6593	35.653	3.5565*
Error	14	38.038		10.026	

Appendix 5. Mean sum of square (M.S.S.) and F value of fresh and dry weights of shoot and leaf

Source	Degrees of freedom	Fresh weight of shoot		Fresh weight of leaf		Dry weight of shoot		Dry weight of leaf	
		M.S.S	F	M.S.S.	F	M.S.S	F	M.S.S	F
Stages	6	3348.438	27.2253**	705.01	21.8202**	673.415	28.0501**	33.519	15.6142**
Error	14	122.99		32.31		24.008		2.147	
Species	1	27142.46	168.2818**	2837.215	117.6942**	6756.411	259.3585**	268.03	112.7299**
Species Vs Stages	6	1111.325	6.8902**	548.282	22.7440**	303.514	11.6510**	26.472	11.1337**
Error	14	161.292		24.107		26.05		2.378	

** significant at 1% level
* significant at 5% level

Appendix 6. Mean sum of square (M.S.S.) and F value of total dry matter, root:shoot ratio and harvest index

Source	Degrees of freedom	Total dry matter (g plant^{-1})		Root:shoot ratio		Harvest index	
		M.S.S	F	M.S.S.	F	M.S.S	F
Stages	6	2444.132	111.1616**	0.219	4.3849*	0.011	2.9574
Error	14	21.987		0.050		0.004	
Species	1	19106.134	411.8905**	0.639	227.3320**	0.427	69.8618
Species Vs Stages	6	539.836	11.6378**	0.108	2.5475	0.001	0.217
Error	14	46.386		0.042		0.006	

Appendix 7. Mean sum of square (M.S.S.) and F values of leaf weight ratio (LWR), leaf area index (LAI) and leaf area ratio (LAR)

Source	Degrees of freedom	Leaf weight ratio		Leaf area index		Leaf area ratio	
		M.S.S	F	M.S.S.	F	M.S.S	F
Stages	6	0.006	4.1510*	7.927	953.0569**	0.000	46.0604**
Error	14	0.001		0.008		0.000	
Species	1	0.000	0.2006	37.873	6818.2632**	0.000	13.2613**
Species Vs Stages	6	0.007	4.4281*	6.314		0.000	31.0432**
Error	14	0.002		0.006	1136.6497**	0.000	

Appendix 8. Linear model fitted for two species of *Plumbago*

Characters	*P.rosea*		*P. zeylanica*	
	R^2	Y = a + bx	R^2	Y = a + bx
Fresh weight of root	0.97	8.56 + 11.74 sh	0.97	14.06 + 9.373 sh
Fresh weight of shoot	0.92	-4.89 +4.97 sh	0.92	0.27 + 16.392 sh
Dry weight of root	0.93	1.31 + 2.746 sh	0.95	5.70 + 4.844 sh
Dry weight of shoot	0.85	-1.12 + 1.574 sh	0.97	-1.67 + 8.051 sh
Dry weight of leaf	0.80	0.17 + 0.675 sh	0.13	5.10 + 0.706 sh
Total dry matter	0.94	0.90 + 4.995 sh	0.99	9.13 + 13.601 sh
Girth of root	0.97	1.64 + 0.218 sh	0.97	0.88 + 0.169 sh
Number of root	0.82	6.01 + 1.492 sh	0.82	11.21 + 1.696 sh
Length of root	0.95	22.68 4.414 sh	0.94	32.21 + 4.246 sh

sh - stages of harvest

Appendix 9. Multiple regression analysis of fresh weight of root on different biometric characters

Character	*P.rosea*			*P. zeylanica*		
	Regression coefficient	Standard error	T value	Regression coefficient	Standard error	T value
Fresh weight of leaf (X_1)	1.994	0.362	5.51**	-	-	-
Root length (X_2)	1.472	0.203	7.25**	-	-	-
Root girth (X_3)	-	-	-	51.522	3.669	14.042**
Intercept constant :	-21.13			-28.43		
Multiple R.Sq :	0.937			0.9121		
F value for R :	133.81			197.18		
Adjusted R.Sq :	0.93			0.9075		
Prediction equation Fresh weight of root (g/plant	1.994 X_1 + 1.472 X_2 - 21.13			51.522 X_3 - 28.43		

Appendix 10. Multiple regression analysis of total dry matter production

Character	*P.rosea*			*P. zeylanica*		
	Regression coefficient	Standard error	T value	Regression coefficient	Standard error	T value
Fresh weight of shoot (X_1)	0.465	0.132	3.510**	0.373	0.063	5.918**
Fresh weight of root (X_2)	-	-	-	0.595	0.126	4.723**
Root length (X_3)	0.375	0.134	2.804**	-	-	-
Root number (X_4)	-	-	-	0.964	0.300	3.207**
Plant height (X_5)	0.181	0.069	2.617**	-	-	-
Intercept constant :	-11.04			-9.02		
Multiple R.Sq :	0.9231			0.9754		
F value for R :	68.02			224.97		
Adjusted R.Sq :	0.9095			0.9711		
Total drymatter (g/plant)	-11.04 + 0.465 X_1 + 0.375X_3 + 0.181 X_5			-9.02 + 0.378 X_1 + 0.595 X_2 + 0.964 X_4		

Appendix 11. Comparison of various parameters of growth in two species of Plumbago at 16 MAP

Growth parameters	*P.rosea*	*P.zeylanica*
Plant height (cm)	69.87	157.20
Total leaf area (m^2 $plant^{-1}$)	0.17	0.51
Girth of root (cm)	3.03	1.90
Number of roots per plant	17.53	20.50
Length of roots (cm)	47.70	54.17
Fresh weight of root (g $plant^{-1}$)	79.43	70.83
Fresh weight of shoot (g $plant^{-1}$)	24.37	98.50
Dry weight of root (g $plant^{-1}$)	17.70	33.67
Dry weight of shoot (g $plant^{-1}$)	7.63	45.40
Total dry matter (g $plant^{-1}$)	30.23	90.20
Diffusive resistance (cm s^{-1})	-0.42	0.18
Stomatal number	35.10	29.60
Total chlorophyll (mg g^{-1})	3.79	3.32
Chlorophyll a (mg g^{-1})	2.59	2.34
Chlorophyll b (mg g^{-1})	1.20	0.97
Chlorophyll a : Chlorophyll b	2.15	2.41
Root protein (%) (fresh weight basis)	0.01	0.13
Tender leaf protein (%) (fresh weight basis)	0.05	0.15
Plumbagin (%) (dry weight basis)	1.40	0.27
Dry recovery (%)	low	high
Enzyme activity MDH	low	high
Enzyme activity PEP carboxylase	low	high
Specific activity MDH	low	high
Specific activity PEP carboxylase	low	high

Appendix 12. Comparison of various parameters of growth in two species of Plumbago at 18 MAP

Growth parameters	*P.rosea*	*P.zeylanica*
Plant height (cm)	68.07	145.30
Total leaf area (m^2 $plant^{-1}$)	0.14	0.14
Girth of root (cm)	3.13	2.00
Number of roots per plant	14.47	25.60
Length of roots (cm)	57.07	64.37
Fresh weight of root (g $plant^{-1}$)	88.63	77.26
Fresh weight of shoot (g $plant^{-1}$)	34.20	108.17
Dry weight of root (g $plant^{-1}$)	22.73	41.73
Dry weight of shoot (g $plant^{-1}$)	12.13	36.26
Total dry matter (g $plant^{-1}$)	39.90	101.70
Diffusive resistance (cm s^{-1})	-0.42	0.18
Stomatal number	35.10	29.60
Total chlorophyll (mg g^{-1})	2.78	2.62
Chlorophyll a (mg g^{-1})	1.92	1.63
Chlorophyll b (mg g^{-1})	0.83	0.99
Chlorophyll a : Chlorophyll b	2.19	1.66
Root protein (%) (fresh weight basis)	0.07	0.18
Tender leaf protein (%) (fresh weight basis)	0.16	0.19
Plumbagin (%) (dry weight basis)	0.72	0.26
Dry recovery (%)	25.30	54.10
Enzyme activity MDH	low	high
Enzyme activity PEP carboxylase	low	high
Specific activity MDH	low	high
Specific activity PEP carboxylase	low	high

Analysis of growth, flowering and quality in 'Koduveli', *Plumbago* spp.

By

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ABSTRACT OF A THESIS

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ABSTRACT

Analysis of growth, flowering and quality in *Plumbago rosea* and *Plumbago zeylanica* were undertaken in the College of Horticulture, Kerala Agricultural University, Thrissur during 1995 to 1998. The study analysed the variation in vegetative, physiological, biochemical, anatomical and reproductive characters of the two medicinally important species of *Plumbago*. The post harvest storage techniques of *P. rosea* were also studied.

Studies on growth up to 18 months after planting revealed that the two species exhibited a linear pattern of growth. There was no significant difference in fresh root yield of the two species. However, *P. rosea* recorded 7.78 per cent higher yield than *P. zeylanica* at 18 months after planting. The dry root yield was found to be significantly higher in *P. zeylanica* and the percentage of increase in yield in *P. rosea* was 83.5. It was observed that retention of crop in the field up to 18 months increased dry root yield by 2.27 times in *P. rosea* and 2.01 times in *P. zeylanica* as compared to harvesting at 12 moths after planting.

Plumbago zeylanica was found to be more vigorous and robust with respect to most of the vegetative characters. Total dry matter production, LAR, LAI, CGR and NAR were higher in *P. zeylanica.* However, *P. rosea* was shown to be more efficient in partitioning photosynthetic to the economic part as indicated by higher fresh root yield, harvest index and root: shoot ratio.

The root plumbagin level was higher in *P. rosea* (0.69-1.40 %) as compared to *P. zeylanica*(0.19-0.33 %). The roots of *P. rosea* showed significant variation during different phases of growth with the maximum accumulation at flowering. However, *P. zeylanica* did not exhibit conspicuous variation root plumbagin levels during different stages of growth and it was more or less uniform through out the period of investigation. Total chlorophyll, chlorophyll a and chlorophyll a: chlorophyll b ratios were higher in *P. rosea.* The root and leaf protein levels were higher in *P. zeylanica.* This species also recorded higher enzyme activity (MDH, PEPC and specific activity) also.

The post harvest practices such as drying and curing could significantly bring down the root plumbagin levels. Drying alone could reduce the plumbagin content to the extent of 61.0 per cent in *P. zeylanica* and 68.3 per cent in *P. rosea.* Curing the roots in lime solution recorded a further reduction of plumbagin level by 97.87 per cent in *P. zeylanica* and 87.7 per cent in *P. rosea.* The thin layer chromatograms of cured

samples showed the presence of some additional groups of compounds like steroids, flavonoids and amino groups.

Plumbago zeylanica was found to be more drought tolerant as evidenced by thicker cuticle, lower number stomates, higher diffusive resistance, lower rate of transpiration and presence of sclerenchymatous patches in the root cortex. *P. zeylanica* showed the presence of starch grains also in the root.

The two species differed markedly in most of the inflorescence and flower characters studied. The causes for the lack of seed set in *P. rosea* were identified as (1) spatial separation of stigma and anther in the flower, (2) lack of adherence of pollen grains on the stigmatic surfaces, (3) low pollen fertility and lower number of pollen grains per anther and (4) incompatibility. *P. zeylanica* produced viable seeds on open pollination, cross pollination and self pollination. Seed dormancy in *P. zeylanica* was identified to be due the presence of hard impermeable seed coat and inhibitor, plumbagin. Studies on breaking seed dormancy revealed the effectiveness of cutting the micropylar end of seeds (fresh or stored) before sowing. This treatment resulted in 100 per cent germination in three days. The seedlings recovered were also healthy and quick growing.

Different storage treatments differed markedly with respect to their effectiveness in maintaining quality. The roots stored under ambient conditions started to absorb moisture at a rapid rate and fungal contamination set in after 6 months. The samples showed 46.6 per cent reduction in weight after 18 months. The roots stored in polythene line gunny bags started to deteriorate in 10 months. The samples stored in other containers did not show drastic reduction in weight up to 18 months. The fungi associated with the roots of *P. rosea* were identified as *Penicillium* sp. and *Aspergillus* spp. The root plumbagin levels in the stored samples reduced progressively with period of storage. However the rate of reduction varied with the container. Plastic bottles were most effective in maintaining the quality and freshness of roots.