MINISTRY OF HEALTH & FAMILY WELFARE, GOVT. OF INDIA

CENTRAL SECTOR SCHEME FOR

DEVELOPMENT OF AGROTECHNIQUES AND CULTIVATION OF MEDICINAL PLANTS USED IN AYURVEDA, SIDDHA, UNANI AND HOMOEOPATHY (1998-2002)

Final Report

KERALA AGRICULTURAL UNIVERSITY

AROMATIC AND MEDICINAL PLANTS RESEARCH STATION

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CENTRAL SCHEME FOR DEVELOPMENT OF AGROTECHNIQUES AND CULTIVATION OF MEDICINAL PLANTS USED IN AYURVEDA, SIDDHA, UNANI AND HOMOEOPATHY 1998-2002

(FINAL REPORT)

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2	Name of allocated Medicinal Plants	**1. *Curculigo orchioides*** **2. *Holostemma ada-kodien*** **3. *Saraca asoca*** **4. *Strychnos nux - vomica***

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Executive Summary

The Central Sector Scheme for Development of Agrotechniques and Cultivation of Medicinal Plants used in Ayurveda, Siddha, Unani and Homeopathy was sanctioned to the Kerala Agricultural University initially for a period of three years (1998-2001) and later extended till December 2002. The project was located at the Aromatic and Medicinal Plants Research Station (Kerala Agricultural University), Asamanoor Post, Ernakulam District, Kerala.

The objective of the scheme was to develop suitable agrotechniques for the commercial cultivation of selected medicinal plants - *Curculigo orchioides, Holostemma ada-kodien, Saraca asoka* and *Strychnos nux*-vomica. A total of 33 experiments were conducted to cover various aspects of agronomic requirements for the economic cultivation and quality considerations of the candidate plants in a phased manner. Thin Layer Chromatographic fingerprints were developed to characterise the crude drugs, chemically. Studies were also undertaken to elucidate the differences in the quality of the raw drugs obtained from wild and that produced by cultivation. Quality variation among the market samples of raw drugs was also estimated.

1. ***Curculigo orchioides.*** The growing season for *Curculigo orchiodes* is June to February. Suitable spacing is 10x10 cm and the seed requirement is about 600-750 kg/ha. The planting is to be done on flat beds. The crop comes up well under 25% shade. The optimum stage of harvest of the roots is 7 months after planting. Application of farmyard to the level of 30 tons/ha increases the yield of root stock. Substitution of one-fourths of the organic manures with inorganic fertilisers can be done. A crude drug yield of 400- 500 kg/acre can be expected. Since the root tubers are highly relished by rodents, adoption of measures to control them is necessary

2. .to protect the crop.

From local collections, two species of *Curculigo* were identified. The commonly used species *Curculigo orchioides* is seen in the midlands of the state where the soil is generally lateritic whereas the other species, *Curculigo trichocarpa* was found exclusively in forests. The morphological differences between two species were studied in detail and described.

2. ***Holostemma ada-kodien*** The plant can be propagated through seeds or through cuttings. The seedlings can be planted in June. The plant produces thin hairy roots and

root thickening is induced when it strikes a hard pan/mechanical obstruction. When raised in polybags, excessive root elongation is curtailed and roots grow thicker. Farm Yard Manure at 30 tons/ha promotes growth. The roots can be harvested after one year and a crude drug yield of about 192 kg/acre can be realised.

3. *Saraca asoca*. Asoka is a slow growing tree. The plant responded well to manuring. About 2.5 kg of dried crude drug can be collected from a fully grown tree. A large variation was observed among crude drug in the market. Anatomical and chemical distinctions between the crude drug (bark) of Asoka and that of the common adulterant, *Polyalthia longifolia* were elucidated. The pattern of distribution of chemical components in various parts of the tree and the possible quality changes as a result of ageing of the plant were studied.

4. *Strychnos nux-vomica* also is a slow growing tree. This plant responded positively to organic manures. About 50-75 kg dry seed can be collected from a well-grown tree per year. A method was standardised for the assay of the major alkaloids in various plant parts by HPLC. Variation in the content and distribution of the major alkaloids in various parts of the tree was also estimated.

TLC fingerprinting. The TLC fingerprints of the crude drugs developed in this study is a scientific method for their identification and documentation which will be of great help to the crude drugs trader and drug manufacturer. It further aids in the identification of adulterants and substitutes.

Quality studies in medicinal trees. Certain classical observations were made in this project on the quality of stem bark of *Saraca asoca* and *Strychnos nux-vomica* which may apply to medicinal trees in general. As a general practice, the stem bark of such trees is collected from the main trunk of more than 25-year old trees, which often leads to the sacrifice of the tree itself. The results of this study showed that there is no relationship between the age of the tree or the part of the tree trunk on the quality of stem bark and that bark of good quality is available from branches and twigs as well. This calls for a change in the method of collection of bark of trees, which will be sustainable.

Dr. Samuel Mathew,
Principal Investigator

PROFORMA FOR FINALISED AGRO-TECHNIQUES

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2	**Name of allocated Medicinal Plants**	1. *Curculigo orchioides* 2. *Holostemma ada-kodien* 3. *Saraca asoca* 4. *Strychnos nux-vomica*

I. BLACK MUSALE

Curculigo orchioides Gaertn. Family : Amarillidaceae

a. Botanical Name *Curculigo orchioides* Gaertn.

b. Vernacular names

Sanskrit: Musali	Hindi: Kalimusali, Musali
Ben: Talamuli	Guj: Musalikand
Tel: Nelatadi Kelangu	Kan: Nladali
Tam: Nilappanai	Mal: Nilappana

c. Parts used for medicinal purpose: The rootstock of curculigo is used as a rejuvenating and aphrodisiac drug. It cures morbid vata and pita and improves complexion and is useful in general debility, deafness, cough, asthma, piles, skin diseases impotence, jaundice, urinary disorders, leucorrhoea and menorrhagia

3. Morphological characteristics including diagnostic characters for identification:

a. Habit: Musale is a small herbaceous geophilous plant with cylindrical rootstock

b. Root stock: Root stock is straight, cylindrical, tuberous, 5-22 cm long, 0.5 to 0.8 cm thick, brownish surfaces marked with closely spaced prominent transverse wrinkles in the upper or basal half. It bears a few stout lateral roots of 5 or more cm long. Lateral roots are dull white in colour and spongy externally. The fresh cut surfaces of the root stock has a starch white colour and

mucilaginous. A few fibrous roots also occur. Root stock elongate up to 1 foot along.

c. Leaves: Leaves simple, sessile, crowded on the short stem with sheathing leaf bases, tapering in to a short or petioled with sheathing bases, 15-45x1.2-2.5cm size, linear or linear lanceolate, pilose, membranous, glaberous or sparsely softy hairy and plicate in bud. The leaf tips when contacts the soil, develops roots and produce adventitious buds.

d. Flowers: Epigynous bright yellow, bisexual or unisexual with lanceolate, membranous bract. Perianth gamophyllous, rotate and six lobed, locate at the top of a slender sterile long extension of the ovary by means of which the perianth is exposed above the ground. Perianth lobes similar, elliptic, oblong 1.2-1.6 cm long, 0.2-0.3 cm broad, outer lobes hairy on the back, inner ones sparsely hairy along nerves. Stamens 6 in number, filamentous filiform, short 2 mm long, agnate to the base of the perianth lobes, anthers linear or linear lanceolate, basifixed and sagitate, ovary inferior, hidden among the leaves usually below the ground, tricarpellary syncarpous, lanceolate and trilocular with a fairly long slender beak or extension the stipe. Ovules many in each cell attached by a distinct long funicle. Style short columnar, 2 mm with a three lobed stigma. Lobes elongate, erect and appressed.

e. Fruits: Fruit is a capsule about 1.5-2cm long, 8mm broad, oblong, glabrescent with a slender beak and spongy septa. It is subulate to ovate, indehiscent, sacculent, few to many seeds, oblong, subglobose, black, striate, shining with crustaceous testa grooved deeply in wavy lines.

4. a. Habitat : It grows in shady forest areas and rubber plantations. The plant is considered as an endangered species.

b. Geo-climatic conditions The plant is found in all districts of India from near sea level to 230 m altitudes, especially in rock crevices and laterite soil.

c) Best soil and land preparation It grows well moist and humus rich soils. The land is to be ploughed well with onset of monsoon and organic manure at the rate of 10 t ha^{-1} is added and planting done on raised beds.

	d) Recomm-ended varieties, if any:	Nil
5.	**Best planting material(s) recommended for cultivation :**	Fresh tuber bits of 1.5-2 cm are used for cultivation.
6.	**Best method & time of raising the plants:**	*a. Best treatment recommended* : Planting of tubers have to be done with the onset of south west monsoon. Raised be ds of 15 cm height, 1 m width and convenient length are to be taken. FYM @ 20 t ha^{-1} is incorporated in to the soil. 25% shade is required for proper growth. *b. Optimum spacing recommended* **:** The tubers are to be planted at a spacing of 10x10 cm. The seed rate required per hectare is 600-750 kg.
7.	**Best period for sowing/planting seed/planting material:**	May-June
8.	**Viability of seed/planting material:**	Fresh tubers gave the best germination percentage.
9.	**Recommended seasonal practices and interculture operations**	
10.	**Maximum number of plants appropriate for plantation per acre through cropping/intercropping system**	Pure plantation : 2,50,000 Intercropping : 80,000

Practice	***Time***
Land preparation and planting	May- June
Weeding and fertilization	July- August
Rodent control measures	August to February
Providing shade	August to February
Weeding and fertilization	October- November
Irrigation	December to January
Harvesting	February

11. Manure & Nutrient requirements including time & method of application	FYM 20 t ha^{-1} is to applied at the time of land preparation. Nutrient source have no significant influence on the growth of the crop. Among the sources, poultry manure had distinct superiority over other sources in terms of rhizome yield. Substitution of 25% dose of FYM with chemical fertilizers is found advantageous.
12. Recommended irrigation practices including quantity and frequency of application:	The crop is grown as a rainfed crop during the monsoon period. It is to be irrigated with 5 cm water on alternate days after the cessation of monsoon for better yield.
13. Weed control method including chemical control:	Hand weeding is usually adopted. Weeding twice at 2 and 4 months after planting is necessary to keep the crop weed free
14. Diseases, insect, pests, nematodes, physiological disorders, if any and their control measures:	Seedling rot is found during rainy season and spraying 1% Bordeaux mixture can control it. Black rot disease can be controlled by spraying 0.05% calyxin. Rhizomes are found eaten by rodents and hence proper control measures are to be taken for their control.
15. Time of flowering & fruiting:	The plant starts flowering one month after planting and maximum numbers of flowers are noted during second and third month of planting. Flowers are produced throughout the year.
16. Period required for maturity:	Production of primary tubers starts from fourth month of planting and they get ready for harvest after six months. During summer months the above ground portion becomes dried up. If it is not harvested during the current year, it will put forth new shoots with the onset of next monsoon. The plant can thus remain as a perennial.
17. Best time for harvesting medicinally useful part (s) keeping in view the active chemical ingredients:	During January – February the rhizomes will be well formed and the above ground portion gets dried up and the crop is ready for harvest

18.	**Best harvesting, storage/packing techniques for useful part(s):**	The plant is perennial. It can be harvested as annual after 10 months or as biennial after 2 years. Harvesting is done by digging the soil and the rhizomes are collected. The shoot portion and the roots are removed and the separated rhizome are cleaned of the soil particles, dried in sun and stored in gunny bags.
19.	**Concentration of active constituents in different parts of the plant:**	The crude drug of curculigo contains: a) Acetone extractives : 1.5- 1.8% b) ash : 3.3-3.9 % c) curculigoside : 0.2%
20.	**Per acre yield of useful part(s) :**	The dried tuber yield is 400-500 kg per acre if harvested during first year. Double the yield is obtained if harvested during second year.
21.	**Per acre cost of cultivation :**	Rs. 8000/-
22.	**Whether the germplasm will be available for supply, if yes, name & address of the place where it will be available:**	Yes. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala – 683 549
23.	**Name & address of the gene bank where the germplasm is deposited and their accession number :**	The material is being deposited at the gene bank of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi

24. Photographs (plantation as a whole, single plant as a whole, only flowering & fruitinig parts, part used for medicinal value e.g. roots, stem, leaves, etc.) :

Kindly see Page 12

25 Any other relevant information

a. TLC fingerprint of Curculigo crude drug

A thin layer chromatographic fingerprint was developed to characterise the crude drug of Curculigo. An acetone extract of the drug was subjected to alkali hydrolysis and the hydrolysate was partitioned into chloroform. TLC was developed on silica plate with a mixture of hexane, chloroform, acetone and diethylamine in the ratio 2.5 : 6 : 0.5 : 1. The spots were visualized by dipping in 1% alcoholic sulfuric acid followed by heating at 100^{o}C for 5 minutes.

b. Variation in the quality of Curculigo available in different markets in Kerala

A large variation was observed in the quality of Curculigo crude drug collected from four important markets in Kerala.

Fig. 1. *Curculigo orchioides*

Field view

Habit

Plant at bloom

Whole plant

Root stock

Crude drug

I. *Curculigo orchioides*

Expt. 1. Phenotypical and development physiological studies on *Curculigo orchioides* grown in hot humid tropics.

Expt 1.1 Morphology of *Curculigo orchioides* types collected from different regions of Kerala

a. Vellanikkara Collection (Thrissur District): Leaf size 18.3x2.9 cm. Overall plant size is higher in Vellanikkara collection than the collections from the local area. The rhizome size varies from 5-9 x 0.6-0.7cm.

b. Panamkuzhy Collection (Ernakulam District): Leaf size 25x 1.9cm. Overall plant size is smaller when comparing with Vellanikkara collection and almost similar with the local collection. Rhizome size varies from 7-9x0.6-0.7cm.

c. Mannarkad Collection (Palaghat District): Leaf size 28x1.6cm. Overall plant size is almost similar with Vellanikkara collection. Rhinome size varies from 4-10x 0.7-0.8cm.

d. Anamooli Collection (Wyanad District): Leaf size 28x 1.5 cm. The overall morphology of both the collections Mannarkadu and Anamooli are almost similar. The rhizome size varies from 5-7x 0.8x 0.9cm.

e. Mukkali (Palaghat district): A sample of *Curculigo trichocarpa* collected from Mukkali (Palaghat district) shows slight variation in its morphology with the collections from the local area. The leaf size 28x 6cm and the petiole length 6cm and rhizome size is 13x 1.3cm.

f. Market sample from Preumbavoor: A study of the market sample of *Curculigo orchioides* has undertaken. The study showed that the rhizome is elongate, black rhizome with slightly ridged and furrowed outer margin. The thickness of rhizome varies from 0.8 to 2cm. In a cross section, there is well marked two regions. A dark brown central region and a light brown outer region below the black outer skin. The outer skin is slightly covered with hairs.

Expt. 1.2 Morphological description of *Curculigo* species

During collection of *C. orchiodes* from wild, another type could be identified as one belonging to the species *tricocarpa*. Detailed morpholociacal studies were undertaken and the comparative description is detailed below.

Curculigo orchioides

Rhizome elongate, 5-25 cm, vertical. Leaves lanceolate, 5-20x 0.8-1.5 cm plicate or flat, prominently nerved below, densely or sparsely villous sometimes glabrescent, narrow at the base, margin entire, apex acuminate. Petiole usually short to 3cm, often absent. Leaf sheath persistent, fibrous, imbricating. Scape 3-5 or more, slender to 6cm, hidden by leaf sheaths. Flowers light yellow, bisexual, sessile, regular, 1.2 cm across. Perianth 6 lobed to the base, lobes yellow, oblong –elliptic, 0.6-1x0.2-0.3 cm; outer ones pilose, inner ones sparsely pilose along nerves. Stamen 6; filaments filiform,to 2mm; anthers to 2mm. Ovary 3 celled, oblong, to4mm, pilose, apex rostrate, 3.5-6 cm;

ovules numerous per cell. Style to 2mm, stigmas-3, lobes elongate. Fruit oblong,1.5-2cm long 8mm broad, glabrescent, pericarp membranous. Seeds 8 globose, to 2mm, glossy, beaked. Fruit through the year.

Plains to 1600 M. Common on the floor of scarb jungles(Plains) and on slops(hills). Abundant in disturbed ground.

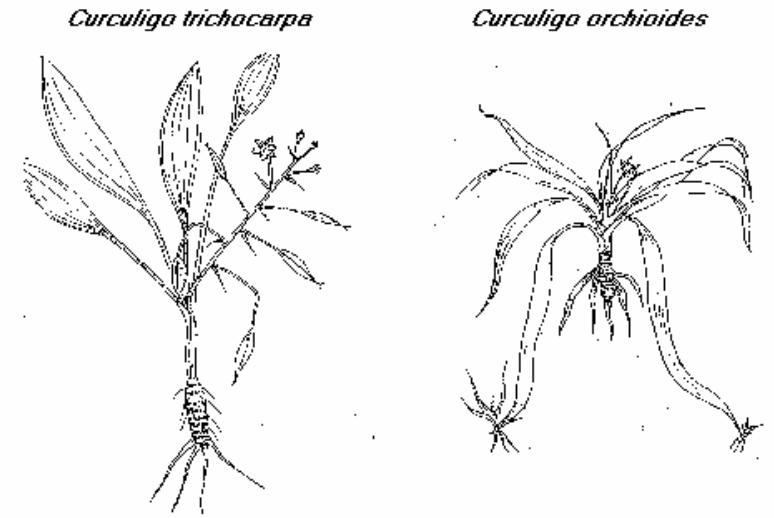


Fig.2. Line drawing showing morphological differences between two species of Curculigo

Curculigo trichocarpa

Rhizome erect, elongate. Leaves lanceolate, 9-25x 1.5-3.5 cm, flat-plicate, thin-coriaceous, prominently 7 nerved below, bordered in between by several thin nerves, usually glabrous, rarely pilose, base narrow, apex gradually acuminate. Petiole usually very elongate to 25 cm rarely reduced to 3cm. Leaf sheath membraneous. Scape 3-7cm, hidden among leaf base. Raceme 5-8 flowered, bracts linear, elongate,1.5-2cm, densely pilose; pedicel to1.5cm, filiform, curved. Flowers to 1cm across, regular, usually bisexual sometimes male ones at the top. Perianth persistent; lobes six yellow, oblong- elliptic, 3.8x2mm, 3-5 nerved apex obtuse; outer ones pilose without inner ones glabrous. Stamens 6; filaments erect, to1.5mm, anthers2.5mm. Ovary 3-celled, to 2mm; ovules numerous per cell. Style columnar to 1.5 mm. Stigma capitate, obscurely 3-gonous. Fruit oblong- ellipsoid, subterete, to 1.8x0.4cm, glabrous, striate, seeds globose,2mm, striate, not beaked.

The plant is common in hills above 1000 m, in shade. It flowers and produces fruit throughout the year.

Expt. 2. Standardization of propagation techniques in *Curculigo orchioides*

Objective : To standardise propagation technique.

This was an attempt to stimulate germination and to accelerate seedling growth by seed treatment with plant hormones.

Design : CRD

Replication : 3

Treatments : 6

Hormone treatment: IAA 1000ppm & 2000ppm

IBA 1000ppm & 2000ppm

NAA 1000ppm & 2000ppm

Ten-tubers per treatment were soaked in 1000 ppm and 2000 ppm for half an hour and dibbled on raised beds taken in the field. The beds are mulched with coconut fronts and were regularly watered. Control plots were also dibbled without any treatment.

Table 1. Effect of hormone treatments on the germination of *Curculigo orchioides*

Treatment	Number of plants germinated		
	After 30 days	After 37 days	After 44 days
I. Hormone			
1. IAA	3.83	5.50	7.17
2. IBA	1.50	4.17	6.50
3. NAA	0.50	1.67	2.50
$CD_{0.05}$	1.63	2.419	2.441
II. Dose (ppm)			
1. 1000	2.56	4.89	6.56
2. 2000	1.33	2.67	4.22
$CD_{0.05}$	NS	1.975	1.993
III. Controls			
1. Control	4.67	5.67	6.00
2. Vernallization	4.00	6.67	8.00
$CD_{0.05}$	NS	NS	NS
Control mean	4.33	6.17	7.00
Rest mean	1.94	3.78	5.39
Control vs Rest	Sig.	Sig.	NS

Results obtained: Sprouting and seedling establishment are highly protracted in this crop. Fresh tubers of *Curculigo orchioides* planted in the field took about one month for sprouting. In the first 30

days, germination was around 40%. This increased to around 60% by 37th day and about 70% at the 44th day after planting. In about two months after planting, 70-80% germination was obtained.

Fresh tubers gave the best germination percentage and vernalisation had no effect. Treating the tubers with growth hormones had significant inhibition effect on the germination of the tubers. Suppression of germination is more marked with NAA followed by IBA. IAA had the least adverse effect on germinability. It is worth noting that with time the germination percentage increased and by 44th day after planting, the inhibitory effects of the hormones were overcome.

Conclusion: About 70-80% of germination of fresh tubers of *Curculigo orchioides* could be obtained after two months after planting in humid tropical areas like Kerala. Growth hormone treatment or vernalisation did not increase germination of tubers.

Exp 3. Optimum spacing and planting density in *Curculigo orchioides*

Objective : To find out the optimum spacing for maximum yield in *Curculigo orchioides*.

Design :RBD

Replications : 3

Treatments : 4 spacings - 10 x 10cm, 20 x 10cm, 20 x 20cm, 30 x20cm

Results : Data on the effect of different plant spacings on the growth and root yield of *Curculigo orchioides* is presented in table 2 and represented graphically in Fig. 3.

Table 2 Effect of spacing on the growth and yield of *Curculigo orchioides*

Spacing (cm)	Plant height (cm)	Conopy spread (cm)	No. of Suckers/ plant	No. of leaves/ plant	Fresh rhizome yield (kg/ha)	Dry Rhizome yield (kg/ha)
10 x 10	18.57	20.30	2.45	5.48	1896.32	796.60
20 x 10	19.91	19.82	2.20	5.50	1137.65	499.53
20 x 20	20.11	20.53	2.15	5.85	648.62	259.88
30 x 20	18.49	19.82	2.08	5.65	353.03	146.36
Sem	0.679	0.709	0.106	0.199	37.759	9.770
CD(0.05)	NS	NS	NS	NS	110.211	28.517

Fig.3. Effect of spacing on the yield of Curculigo orchioides

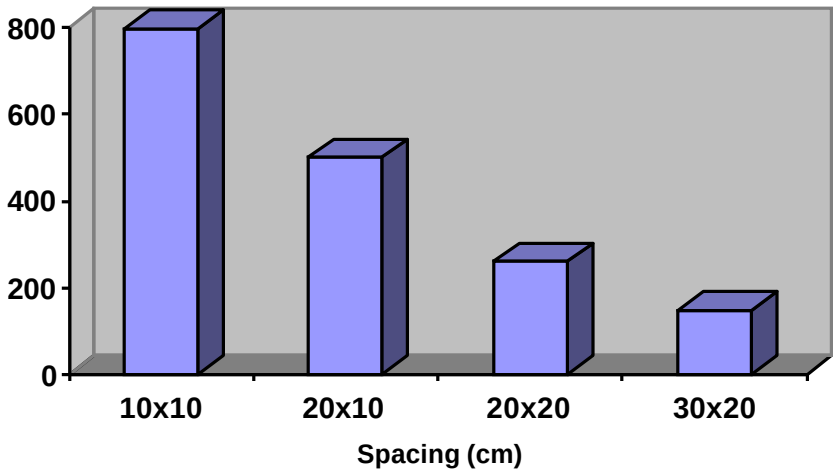


Table 3 and 4 presents the data on the quality of the crude drug at harvest as influenced by spacing treatments.

Table 3. Effect of spacing on the proximate composition of *Curculigo orchioides* rhizome

Spacing (cm)	Proximate composition of dry rhizome at harvest (%)				
	Glucose	Sucrose	Starch	Crude Fibre	Ash
10 x10	1.241	0.994	59.409	2.746	4.015
20 x 10	1.289	1.036	53.304	2.806	3.838
20 x 20	0.994	0.721	55.238	2.395	3.644
30 x 20	0.937	0.771	54.730	2.387	3.795
Sem	0.008	0.008	0.235	0.024	0.022
$CD_{(0.05)}$	0.0023	0.025	0.725	0.073	0.067

Table 4. Effect of spacing on the mineral content of *Curculigo orchioides* rhizome

Spacing (cm)	P (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
10 x10	0.192	0.569	0.238	0.138	1034.7	79.12	79.52	48.15
20 x 10	0.206	0.689	0.254	0.139	1120.7	85.47	68.00	42.15
20 x 20	0.192	0.546	0.281	0.124	1055.7	84.35	109.50	30.47
30 x 20	0.186	0.652	0.236	0.112	1158.3	77.30	93.32	34.37
SEm	0.003	0.009	0.003	0.002	147.32	2.625	1.958	2.784
$CD_{(0.05)}$	0.008	0.028	0.009	0.006	NS	NS	NS	8.578

Conclusion: Spacing had no significant effect on the growth of *Curculigo orchioides.* The yield was maximum of 1896.32 kg/ha (796.60 kg/ha dry) at the narrowest spacing of 10 x 10 cm due to higher

number of plants per unit area. Ash and starch contents were also higher at this spacing. P and Ca contents were highest at 20 x 10 cm spacing whereas, Cu and S contents were higher at 10 x10 cm spacing. Other micronutrients were not affected significantly.

Exp. 4 Optimum time of harvest of *Curculigo orchioides*

Objective: To find out the best time of harvest for maximum yield in *Curculigo orchioides.*

Design : RBD

Replication:3

Treatments: Harvesting at monthly interval from 1 to 12 months after planting.

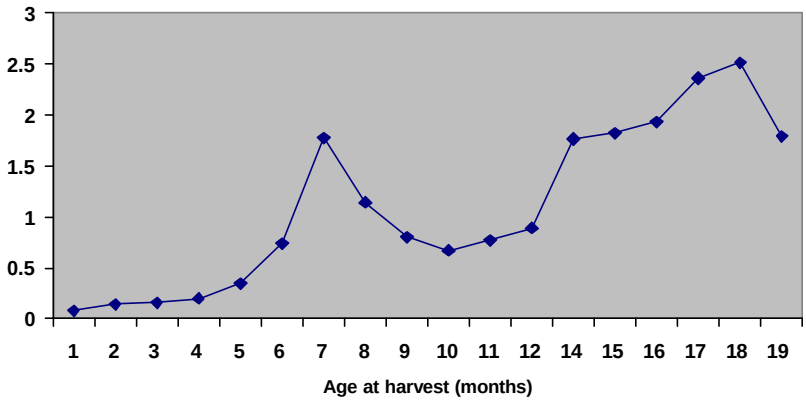
Results : Separate plots of the crop were harvested every month and the crude drug yield was recorded. The data are presented in table 5 and shown graphically in Fig.4.

The yield of roots increased slowly following planting in the field to reach a peak of 1.77 g/plant on the 7th month. Thereafter, it decreased sharply. . If the crop is left as such in the field, the yield tended to decrease up to the 10th month. Thereafter, it showed a steady increase to reach an all time high of 2.5 g/plant at the 18th month.

Table 5. Effect of age at harvest on the yield of *Curculigo orchioides*

Age at harvest (months)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Dry rhizome yield (g plant^{-1})	0.08	0.14	0.16	0.20	0.35	0.74	1.77	1.14	0.80
Age at harvest (months)	**10**	**11**	**12**	**14**	**15**	**16**	**17**	**18**	**19**
Dry rhizome yield (g plant^{-1})	0.67	0.77	0.89	1.76	1.82	1.93	2.36	2.51	1.79

Fig.4.Root yield of Curculigo orchioides when harvested after different periods of time



Conclusion: If the crop is to be grown on the annual mode, it is to be harvested seven months after planting to yield about 1.77 g/plant (1.77 t/ha). If the crop is grown perennially, about double the yield (2.5 t/ha) is obtained on the 18th month.

Exp. 5 Effect of shade on the yield and quality of *Curculigo orchioides*

Objective: To study the effect of shade on the yield and quality of *Curculigo orchioides*

Design : RBD

Replications : 3

Treatments : 4 shades (0, 25, 50 and 75 %) provided artificially by keeping under shading nets Results obtained: The data on the growth and yield of the plant as influenced by shade are presented in table 6.

Shading enhanced the growth and yield of *Curculigo orchioides.* Plant height and canopy spread were maximum at 75% shading. The number of leaves and suckers per plant were not significantly affected by shade. However, the rhizome yield was highest (1482.81 kg/ha fresh and 643.33 kg/ha dry) at 25% shading.

Table 6. Effect of shade on the growth and yield of *Curculigo orchioides*

Shade (%)	Plant height (cm)	Canopy spread (cm)	No. of suckers /plant	No. of leaves /plant	Fresh Rhizome yield (kg ha^{-1})	Dry Rhizome yield (kg ha^{-1})
0	15.67	16.30	1.92	5.13	420.33	129.19
25	18.20	19.77	1.92	5.95	1482.81	643.33
50	20.14	19.82	2.58	5.75	965.85	419.93
75	23.08	24.57	2.47	5.65	1166.63	509.93
Sem	1.134	0.784	0.208	0.177	39.005	9.874
$CD_{(0.05)}$	3.924	2.712	NS	NS	134.973	34.169

Data on the effect of the treatments on quality is given in table 7 and 8. The results of proximate analysis also demonstrated the superiority of 25% shade in respect of ash, fibre, starch and sucrose in the rhizome at harvest. However the nutrient contents were higher under open conditions exc ept in case of S and Cu.

Table 7. Effect of shade on the proximate composition of *Curculigo orchioides* rhizome

Shade (%)	Proximate analysis of dry rhizome at harvest (%)				
	Glucose	Sucrose	Starch	Fibre	Ash
0	0.592	0.549	53.838	2.908	4.424
25	1.275	1.061	59.880	2.615	3.782
50	1.353	0.908	53.385	2.350	3.418
75	1.243	1.003	55.578	2.462	3.668
SEm	0.006	0.005	0.437	0.010	0.019
$CD_{(0.05)}$	0.025	0.021	1.969	0.046	0.085

Table 8. Effect of shade on nutrient content of *Curculigo orchioides* rhizome at harvest

Shade (%)	N (%)	P (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
0	0.250	0.666	0.314	0.128	1824.0	94.07	157.37	34.32
25	0.211	0.621	0.238	0.106	802.8	65.80	71.60	42.30
50	0.172	0.587	0.225	0.138	825.0	76.72	61.10	45.05
75	0.144	0.582	0.233	0.141	917.7	89.65	60.27	33.47
Sem	0.001	0.012	0.003	0.001	71.62	2.508	1.891	2.789
$CD_{(0.05)}$	0.003	0.053	0.013	0.005	322.36	11.285	NS	NS

Conclusion: Highest crude drug yield and optimun quality of *Curculigo orchioides* is obtained when the plant is grown under 25% shade.

Exp. 6 Manurial requirements of *Curculigo orchioides*

Objective : To find out the manurial requirements of *Curculigo orchioides*

Design : RBD

Replications : 3

Treatments : 16

Three levels of FYM : 10, 20 and 30 t/ha

Five levels of substitution of FYM with fertiliser : FYM : Fert proportion 100:0, 75:25, 50:50, 25:75 and 0:100

Results: the experiment is an effort to standardise the optimum level of farm yard manure for the crop. It is also intended to see whether a portion of the organic manure can be substituted with NPK fertilisers.

Data on the effect of the treatments on growth parameters is presented in table 9 and that on yield and quality are given in table 10 and 11.

Conclusion: The growth parameters were not significantly affected by FYM, except plant height, which was maximum with the application of 30t/ha. Rhizome yield increased up to 20 t/ha of FYM and decreased thereafter indicating that 20 t/ha is optimum for the crop. Sucrose and fibre contents were also higher at this rate of application. N content increased with increase in FYM application whereas P and Mn contents decreased.

With regard to the proportion of FYM and fertilizer, 75:25 proportion was significantly superior to all other treatments in terms of rhizome yield indicating that both FYM and fertilizers are required and the optimum level of substitution is 25% of the nutrients with fertilizer for realizing maximum yield in *Curculigo orchioides*. This also ensured maximum protein content in the rhizome with a slight reduction in the contents of ash, fibre and starch. However, N, P and Ca contents in the rhizome were higher at this level of substitution.

Table 9. Effect of levels of FYM and proportions of FYM and fertiliser on the growth of *Curculigo orchioides*

Treatment	Plant height (cm)	Canopy spread (cm)	No. of suckers /plant	No. of leaves /plant
FYM (t ha $^{-1}$)				
10	15.15	18.99	2.000	5.24
20	15.69	19.29	1.853	5.35
30	17.30	21.10	1.933	5.48
SEm	0.454	0.740	0.109	0.200
$CD_{(0.05)}$	1.311	NS	NS	NS
FYM:Fert ratio				
100:0	17.61	20.06	1.911	5.78
75:25	16.40	19.48	2.111	5.44
50:50	14.40	20.11	1.867	5.02
25:75	15.24	18.82	1.867	5.16
0:100	16.57	20.51	1.889	5.38
SEm	0.586	0.956	0.140	0.258
$CD_{(0.05)}$	1.693	NS	NS	NS
Interaction	NS	NS	NS	NS
Control	15.85	19.66	2.000	4.67
Rest	16.05	19.80	1.929	5.36
Control x Rest	NS	NS	NS	NS
G. Mean	16.04	19.79	1.933	5.31

Table 10. Effect of levels of FYM and proportions of FYM and fertiliser on the rhizome yield of *Curculigo orchioides* and its proximate composition

Treatment	Rhizome yield (kg ha^{-1})		Proximate analysis of dry rhizome at harvest (%)					
	Fresh	Dry	Glucose	Sucrose	Starch	Fibre	Protein	Ash
FYM (t ha^{-1})								
10	405.27	151.34	1.268	0.821	50.636	2.655	11.286	3.979
20	518.95	201.62	1.253	0.892	50.336	2.656	11.578	3.875
30	490.22	191.95	1.387	0.770	51.830	2.530	11.973	3.819
Sem	7.324	2.500	0.007	0.013	0.281	0.026	0.118	0.045
$CD_{(0.05)}$	21.152	7.221	0.020	0.039	0.847	0.079	0.357	0.136
FYM:Fert								
100:0	448.39	167.29	1.348	0.835	48.835	2.860	11.533	4.132
75:25	736.45	293.37	1.247	0.764	51.423	2.695	11.609	3.836
50:50	567.99	222.93	1.307	0.915	52.647	2.585	11.494	4.013
25:75	340.75	130.47	1.283	0.810	51.408	2.232	11.197	3.529
0:100	363.82	135.80	1.328	0.813	50.357	2.698	12.227	3.946
Sem	9.455	3.228	0.008	0.017	0.363	0.034	0.153	0.058
$CD_{(0.05)}$ Interaction	27.308 **	9.322 **	0.026 **	0.050 **	1.094 **	0.102 **	0.461 **	0.176 **
Control	305.08	109.35	1.333	0.845	49.435	2.911	10.975	3.864
Rest	491.48	189.97	1.303	0.828	50.934	2.614	11.612	3.891
Control x Rest	**	**	NS	NS	*	*	*	NS
G. Mean	479.83	184.931	1.305	0.829	50.840	2.632	11.572	3.889

Table 11. Effect of levels of FYM and proportions of FYM and fertiliser on nutrient content of Curculigo *orchioides* rhizome at harvest

Treatment	N(%)	P (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
FYM (t ha^{-1})									
10	1.806	0.231	0.652	0.22	0.401	1452.2	110.90	77.78	69.00
20	1.853	0.219	0.756	0.231	0.118	1107.9	106.12	65.06	65.90
30	1.916	0.209	0.687	0.222	0.114	1016.4	97.52	95.50	108.32
Sem	0.019	0.001	0.008	0.003	0.006	123.16	2.096	7.493	14.17
CD (0.05)	0.057	0.005	0.025	NS	NS	NS	6.319	22.584	NS
FYM:Fert proportions									
100:0	1.845	0.236	0.698	0.245	0.101	1537.3	100.23	61.13	96.23
75:25	1.858	0.243	0.716	0.197	0.099	906.1	99.30	73.83	57.37
50:50	1.839	0.228	0.721	0.245	0.116	1071.7	108.43	121.80	68.11
25:75	1.792	0.212	0.662	0.220	0.112	1136.3	104.63	68.43	100.23
0:100	1.956	0.180	0.694	0.217	0.130	1309.3	11.63	73.03	83.40
Sem	0.024	0.002	0.011	0.004	0.007	153.18	2.706	9.673	19.43
CD (0.05)	0.074	0.006	0.033	0.012	0.022	NS	8.158	29.156	NS
Interaction	**	**	**	**	*	NS	NS	NS	NS
Control	1.756	0.172	0.657	0.190	0.138	1061.0	98.10	59.70	180.70
Rest	1.858	0.220	0.698	0.225	0.112	1192.2	104.85	79.45	81.07
Control x Rest	*	**	*	**	NS	NS	NS	*	**
Gen. Mean	1.852	0.217	0.696	0.223	0.113	1183.9	104.43	78.21	87.30

Conclusion: Application of FYM at 20 t/ha is optimum for realising highest yield of the drug with the best quality. Substitution of 25% of the above dose of organic manure with equivalent quantity of NPK fertilizer is not detrimental.

Exp. 7. Effect of mulching and sources of nutrients on yield and quality of *Curculigo orchioides*

Objective: To find out the effect of mulch and the best source of nutrients for optimum yield and quality of *Curculigo orchioides*

Design : RBD

Replications : 3

Treatments : 8x2 = 16

Sources of nutrients :

Control	FYM 10t/ha + *Azotobacter* 10 kg/ha
FYM 10t/ha	FYM 10t/ha + *Phosphobacter* 10 kg/ha
Vermicompost 1.3 t/ha	FYM 10t/ha + VAM 10 kg/ha
Poultry manure 2.7 t/ha	Fertiliser N, P_2O_5, K_2O: 40:30:20 kg/ha

Mulching: With and without mulching

Results: The effect of mulching and alternative nutrient sources on the growth of the plant is shown by data presented in table 12. Mulching significantly improved the plant height and canopy spread of the plant.

Table 12. Effect of mulching and nutrient sources on the growth of *Curculigo orchioides*

Treatments	Plant height (cm)	Canopy spread (cm)	No. of suckers /plant	No. of leaves /plant
Mulching				
Not mulched	14.26	14.18	1.76	5.16
Mulched	15.96	15.58	1.88	5.29
SEm	0.356	0.473	0.102	0.147
$CD_{(0.05)}$	**	*	NS	NS
Nutrient source				
Control	14.13	13.75	1.80	4.90
FYM	15.69	15.72	1.80	5.13
Vermicompost	15.15	17.54	1.57	5.50
Poultry Manure	16.18	15.75	2.00	5.13
FYM+Azotobacter	15.39	13.57	1.71	5.24
FYM+Phosphobacter	10.01	14.58	2.00	5.60
FYM+VAM	15.53	14.77	1.50	5.37
NPK	13.80	13.38	2.20	4.93
SEm	0.711	0.946	0.201	0.294
$CD_{(0.05)}$	NS	NS	NS	NS
Interaction	NS	NS	NS	NS
G. Mean	15.11	14.88	1.82	5.23

The effect of the treatments on the root yield and its quality is depicted by data in table 13 and 14. The yield was unaffected and the contents of fibre, starch, sucrose and several nutrients decreased, in general due to mulching.

Nutrient source had no significant influence on the growth of *Curculigo orchioides*. Among the sources, poultry manure had distinct superiority over other sources in terms of rhizome yield. It was followed by FYM + phosphobacter application. The protein content of rhizome was highest with the application of FYM+VAM. Ash, fibre and starch contents were higher in the control. Poultry manure increased the P content of rhizome. Micronutrient contents were not affected by the nutrient source.

Table 13. Effect of mulching and nutrient sources on the rhizome yield of *Curculigo orchioides* and its proximate composition

Treatment	Rhizome yield (kg ha^{-1})		Proximate analysis of dry rhizome at harvest (%)					
	Fresh	Dry	Glucose	Sucrose	Starch	Fibre	Protein	Ash
Mulchi ng								
No mulched	630.748	239.006	1.120	0.926	56.642	3.160	12.254	3.747
Mulched	612.095	238.985	1.173	0.882	53.621	3.009	12.023	3.745
Sem	18.036	7.455	0.017	0.010	0.302	0.014	0.086	0.020
$CD_{(0.05)}$	NS	Ns	*	**	**	**	NS	NS
Nutrient source								
Control	429.688	160.007	1.186	0.883	58.995	3.363	12.822	4.022
FYM	600.813	228.444	1.090	0.942	51.610	2.737	11.478	3.779
Vermicompost	454.410	168.906	1.161	0.872	52.338	3.253	11.981	4.108
Poultry Manure	982.721	382.625	1.84	0.803	57.898	3.040	11.756	3.868
FYM+Azotobacter	602.772	238.293	1.162	1.003	56.515	3.217	11.422	3.681
FYM+Phosphobacter	781.674	308.986	1.148	0.883	53.233	2.748	11.894	3.355
FYM+VAM	541.361	213.660	1.141	0.979	53.785	3.178	13.158	3.316
NPK	577.937	211.042	1.104	0.866	56.780	3.142	12.547	3.839
Sem	36.073	14.911	0.033	0.120	0.604	0.028	0.172	0.040
$CD_{(0.05)}$	104.187	43.065	0.101	0.054	1.820	0.085	0.517	0.120
Interaction	**	**	Ns	**	**	**	**	**
G. Mean	621.422	238.995	1.147	0.904	55.132	3.085	12.138	3.746

Table 14. Effect of mulching and nutrient sources on the nutrient content of *Curculigo orchioides* rhizome at harvest

Treatment	N (%)	P (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Mulching									
Not mulched	1.961	0.199	0.742	0.276	0.139	977.86	108.53	92.15	34.01
Mulched	1.924	0.198	0.675	0.256	0.127	804.70	101.55	81.55	29.25
Sem	0.014	0.002	0.014	0.003	0.002	52.597	1.874	7.756	2.213
$CD_{(0.05)}$	NS	NS	**	**	**	NS	*	NS	NS
Nutrient source									
Control	2.052	0.145	0.732	0.244	0.139	1171.5	124.10	125.50	44.55
FYM	1.837	0.216	0.691	0.272	0.146	367.40	101.35	118.55	29.65
Vermi compost	1.917	0.175	0.753	0.288	0.114	1166.1	111.75	109.75	35.50
Poultry Manure	1.881	0.233	0.739	0.268	0.123	772.30	97.40	57.20	37.30
FYM+Azotobacter	1.828	0.215	0.670	0.261	0.155	679.10	98.10	69.35	28.35
FYM+Phosphobacter	1.903	0.211	0.670	0.268	0.127	833.30	95.70	90.70	26.30
FYM+VAM	2.105	0.220	0.636	0.284	0.136	785.75	92.00	57.95	24.20
NPK	2.106	0.170	0.778	0.244	0.124	853.60	119.90	65.80	27.20
Sem	0.027	0.004	0.029	0.005	0.005	115.92	3.921	11.152	4.914
$CD_{(0.05)}$	0.083	0.012	0.086	0.016	0.014	347.76	11.763	33.456	NS
Interaction	**	**	**	**	**	NS	NS	NS	NS
G. Mean	1.942	0.198	0.090	0.266	0.133	891.13	105.04	86.85	31.63

Conclusion: Mulching though significantly improved growth did not influence the economic yield of *C. orchioides*. Different sources of organic manures had no significant influence on the growth of *Curculigo orchioides*. However, poultry manure had distinct superiority over other sources in terms of rhizome yield.

Expt. 8. Chlorophyll content of leaves of C*urculigo spp*.

Expt. 8.1 Chlorophyll content of leaves of two species of C*urculigo*.

Table 15. Chlorophyll content of leaves of two species of *Curculigo*

Sl No.	Species	Chlorphyll content		
		Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)
1	*Curculigo orchioides*	1.165	0.451	1.616
2	*Curculigo trichocarpa*	1.345	0.494	1.839

The experiment was intended to compare the photosynthetic efficiency of two species of Curculigo. The relative higher content of chlorophyll in *C. trichocarpa* reflects higher photosynthetic capacity. This species produce larger tubers when compared to *C. orchioides*.

Table 16. Chlorophyll content of leaves of *C. orchioides* at different stages of growth.

Age (months after planting)	Chlorophyll a		Chlorophyll b		Chlorophyll a+b	
	Panamkuzhi biotype	Vellanikkara biotype	Panamkuzhi biotype	Vellanikkara biotype	Panamkuzhi biotype	Vellanikkara biotype
1	1.17		0.45		1.62	
2	0.64	0.64	0.24	0.24	0.88	0.82
3	0.96	0.94	0.34	0.32	1.30	1.25
4	0.78	0.88	0.50	0.72	1.27	1.59

Up to 4 months of planting there is no significant variation in the chlorophyll content of the two ecotypes of *Curculigo orchioides.*

Expt. 9. Evaluation of the quality of market samples of *Curculigo orchioides*

Objective : To assess the quality variation in market samples of *C. orchioides.*

Table 17. Mineral content of wild, cultivated and market samples of *Curculigo orchioides roots.*

Location	N (%)	P (%)	Ca (%)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Wild									
Panamkuzhi	0.309	0.126	1.243	0.348	0.266	3355.0	18.40	81.30	24.40
Vellanikkara	1.380	0.099	1.744	0.278	1.561	3288.0	1899.5	92.50	21.05
Cultivated									
Panamkuzhi	2.796	0.218	0.706	0.234	0.115	1159.5	105.27	71.635	76.600
Vellanikkara	2.491	0.178	0.879	0.208	0.728	939.5	61.015	51.295	37.205
Market samples									
Trivandrum	1.021	0.075	0.975	0.233	0.076	6.411	0.418	0.548	0.060
Kollam	1.087	0.108	1.389	0.151	0.071	23.785	0.770	0.390	0.086
Cherthalai	0.824	0.052	1.068	0.299	0.196	16.105	0.412	0.540	0.178
Aluva	0.539	0.099	0.920	0.202	0.031	2.297	0.797	0.629	0.652
Kolappully	0.972	0.084	1.282	0.253	0.157	29.025	0.858	0.563	0.161
Manjeri	1.004	0.089	1.353	0.214	0.104	28.755	0.804	0.486	0.078
Kasargod	0.950	0.088	0.997	0.389	0.086	11.670	0.405	0.225	0.048
Idukki	0.806	0.048	1.342	0.204	0.277	29.645	0.820	0.924	0.236
Chennai	1.003	0.085	1.301	0.215	0.125	25.865	0.795	0.464	0.073
Mysore	0.956	0.093	1.246	0.272	0.081	25.020	0.770	0.374	0.111
Visakhapatnam	1.169	0.131	1.826	0.380	0.086	2.244	0.885	0.308	0.561
Pune	1.093	0.108	1.389	0.156	0.070	24.445	0.787	0.389	0.087
Sem	0.038	0.002	0.041	0.035	0.043	13.336	7.462	2.964	2.778
$CD_{(0.05)}$	0.115	0.007	0.125	0.106	0.129	40.200	22.495	8.934	8.373
G. Mean	1.152	0.105	1.241	0.252	0.252	560.454	244.64	18.911	10.099

Table 17. Proximate composition of wild, cultivated and market samples of *Curculigo orchioides* roots.

Location	Ash	Crude protein	Crude fat	Crude fibre	Starch	Glucose	Sucrose
Wild							
Panamkuzhi	5.110	1.928	4.006	7.217	49.83	4.120	2.103
Vellanikkara	7.985	8.626	2.724	5.560	43.97	1.130	0
Cultivated							
Panamkuzhi	5.282	17.472	2.049	4.075	52.02	0.569	0.769
Vellanikkara	4.520	15.568	1.367	3.050	50.01	0.767	0.391
Market samples							
Thiruvananthapuram	5.595	6.384	1.357	2.645	35.34	2.915	2.215
Kollam	8.373	6.828	1.712	2.828	52.54	0.815	0.019
Cherthalai	5.445	5.152	1.431	4.505	57.70	0.579	0
Aluva	5.024	3.367	1.399	2.468	57.43	0.833	0
Kolappully	12.022	6.073	1.759	3.178	48.38	0.710	0
Manjeri	7.814	6.273	1.761	3.563	52.95	1.035	0
Kasargod	5.231	5.936	1.471	2.742	55.66	1.090	1.510
Idukki	22.206	5.040	1.623	3.975	52.04	0.743	0
Chennai	8.026	6.127	1.742	3.082	52.81	1.115	0
Mysore	8.607	6.160	1.717	3.050	53.20	1.195	0
Visakhapatnam	12.299	7.304	1.855	3.675	35.16	0	0
Pune	8.545	6.832	1.727	2.851	52.54	0.810	0
SEm	0.265	0.233	0.161	0.128	0.936	0.491	0.170
$CD_{(0.05)}$	0.799	0.703	0.485	0.385	2.823	1.479	0.513
G. Mean	8.818	7.192	1.856	3.654	50.098	1.152	0.438

Results : Quality variations in the market samples and also due to domestication of the two biotypes of *Curculigo orchioides*, namely, Panamkuzhi biotype and Vellanikkara biotype were studied. N, P and Cu contents improved due to domestication in both the types. There was a notable decrease in the content of other nutrients due to domestication. There was a substantial increase in the crude protein content and a notable improvement in starch due to domestication, whereas, the crude fat, crude fibre and glucose contents showed a decrease.

Market samples of *Curculigo orchioides* showed much variation in the proximate and nutrient contents. The range of values recorded were ash 5.024-22.206%, crude protein 3.367-7.304%, Crude fat 1.357-1.855%, Crude fibre 2.468-4.505%, Starch 35.16-57.70%, glucose 0-2.915%, sucrose 0-2.215%, N 0.539-1.169%, P 0.052-0.131%, Ca 0.920-1.826%, Mg 0.156-0.389%, S 0.031-0.277%, Fe 2.244-29.025 ppm, Mn 0.405-0.885 ppm, Zn 0.225-0.924 ppm and Cu 060-0.652ppm.

Expt. 10. Development of TLC fingerprint of *C. orchiodes* crude drug

A thin layer chromatographic fingerprint was developed to characterise the crude drug of Curculigo. An acetone extract of the drug was subjected to alkali hydrolysis and the hydrolysate was partitioned into chloroform. TLC was developed on silica plate with a mixture of hexane, chloroform, acetone and diethylamine in the ratio 2.5 : 6 : 0.5 : 1. The spots were visualized by dipping in 1% alcoholic sulfuric acid followed by heating at 100°C for 5 minutes.

Fig. 5. TLC fingerprint of crude drug of *Curculigo orchioides*

PROFORMA FOR FINALISED AGRO-TECHNIQUES

1.	**Name of the Project Officer / Principal Investigator & Organization**	Dr. J. Thomas / Dr. Samuel Mathew Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala PIN 683 549 Tel. 0484-2658221 Fax: 0484-2659881 E-mail: amprs@satyam.net.in
2	**Name of allocated Medicinal Plants**	1. *Curculigo orchioides* 2. *Saraca asoca* 3. *Strychnos nux-vomica* 4. *Holostemma ada-kodien*

II. HOLOSTEMMA

Holostemma ada-kodien Schultes Family : Asclepiadaceae

a. Botanical Name: *Holostemma* ada-*kodien* Schultes

b. Vernacular names:
Sanskrit: Jivanti
Hindi: Chirvel
Marathi : Dudurli
Guj: Kharner, Khiravel
Tel: Palagurugu
Mal: Atapathiyan
Tam: Palaikkirai

c. Parts used for medicinal purpose: The roots of Holostemma are useful in ophthalmopathy, orchitis, cough, burning sensation, stomachalgia constipation, fever and *tridoshas*. It is used in preparations of *Vidaryadiganam*, *Dhanwandharam thaila*, *Manasamithravatakam*, *Balarishta and Anuthalia*. It is also useful in eye diseases and it imparts resistance to diseases.

3. Morphological characteristics including diagnostic characters for identification:

i. Habit : It is a large, glabrous, laticiferous twining shrub, much branched, shining stem, with large conspicuous flowers.

ii. Leaves: Simple, opposite, petiolate, glabrous and cordate. The young branches bear simple, opposite, decussate leaves.

	iii. Flowers:	Purple, large, pedicellate, complete, regular, bisexual and hypogynous, arranged in auxiliary umbellate cymes. Calyx: 5-partite, gamosepal ous, broadly ovate, obtuse, veined. Corolla: gamopetalous, thick, subrotate, deeply 5 lobed, the lobes overlapping to the right, ovate-oblong, obtuse, corona arising from the base of the staminal column. Androecium: stamens-5, epipetalous, alternating with the petals, adnate to the base of the corolla tube, the filaments connate in a 10-winged column, anthers large, horny, shining with membranous inflexed tips, pollen masses pendulous, clavate, elongate, compressed, attached by long caudicles to the hard brown linear pollen-carriers. Gynoecium: bycarpellary, apocarpous, ovary superior. The two free styles getting united towards the top or beneath the stigma. Stigma enlarged in to a thick, 5-cornered disc. Ovary one celled, ovules many, anatropous.
	iv. Fruits:	An aggregate of two follicles, linear, oblong, cylindric slightly tapering to a blund point, 9cm long.
	v. Seed:	Many, ovoid, flattened, winged with a coma of white silky hair at a micropylar end. Embryo large. Endosperm thin.
	vi. Roots:	Roots are long up to 1m or more, irregularly twisted, thick and cylindrical. When dry it is yellowish brown-to-brown black in colour with nearly smooth surface bearing white scars and small depressions. A mature root is about 1-2 cm thick when extracted for use.
b.	**Geo-climatic conditions**	*Holostemma* prefers a tropical climate.
	c) Best soil and land preparation	The planting is done at the starting of rainy season in May-June. For the better growth of the roots, the land should be ploughed properly. Ridges are to be taken 60 cm apart and planting done at a spacing of 30 cm on the ridges. Since the crop is twining in nature, pandals are to be provided.
	d) Recommended varieties, if any:	Nil

5. Best planting material(s) recommended for cultivation :

The plant can be propagated vegetatively by stem cuttings besides through seeds. The seeds are collected from the plant in November-December before being dispersed. Seeds are cleaned, dried and stored for sowing.

6. Best method & time of raising the plants:

Seeds are collected in December-January, cleaned, dried and stored in dry place. The stored seeds after soaking in water for 4-5 hours are sown in raised seedbeds. About 1 month old seedlings are then planted in poly bags of size 14x10 cm which are filled with soil, sand and dried cow dung in 1:1:1 ratio, respectively. Poly bags should be kept in shade and irrigated. In another 1- 1½ months, the seedlings are ready for transplanting. Pits of 30 cm cube size are taken at 1-1.2m distance and filled with 10 kg dried cow dung and sand. This is covered with surface soil and formed into a mound. Seedlings are transplanted on to the mounds from the polybags carefully. Regular irrigation is to be given till flowering. To aid in trailing, stakes are provided 1 month after planting.

7. Best period for sowing/planting seed/planting material

The best season is June.

8. Viability of seed/planting material:

Best germination is obtained when seeds are collected in November- December and sown in June.

9. Recommended seasonal practices and interculture operations

Practice	*Time*
Land preparation and planting	May- June
Providing stakes	July- August
Weeding and fertilisation	July- August
Harvesting	Jan - February

10. Maximum number of plants appropriate for plantation per acre through cropping/intercropping system

Pure plantation : 16000
Intercropping : 5000

11.	**Manure & Nutrient requirements including time & method of application**	The maximum yield was obtained by application of farmyard manure at the rate of 30 tons/ha. Application of NPK @ 100:50:50 kg/ha also is beneficial.
12.	**Recommended irrigation practices including quantity and frequency of application:**	The crop is grown as a rainfed crop during the monsoon period. It is to be irrigated with 5 cm water on alternate days after the cessation of monsoon for better yield.
13.	**Weed control method including chemical control:**	Hand weeding is usually adopted. Weeding twice at 2 and 4 months after planting is necessary to keep the crop weed free.
14.	**Diseases, insect, pests, nematodes, physiological disorders, if any and their control measures:**	Attack by aphid was observed during the rainy months (June - July). Spraying of 0.05% quinalphos could effectively control the pest.
15.	**Time of flowering & fruiting:**	Plants flowered in October and fruits harvested in November.
16.	**Period required for maturity:**	The roots are the parts used as drug and it takes two years for its harvest.
17.	**Best time for harvesting medicinally useful part (s) keeping in view the active chemical ingredients:**	Harvesting can be done at the end of first year when the vines start drying up.
18.	**Best harvesting, storage/packing techniques for useful part(s):**	Harvesting is done by digging the soil to collect the tubers. The tubers are cut in to pieces of 10 cm length and dried in sun before sale.

19.	**Concentration of active constituents in different parts of the plant:**	Holostemma tubers contains: Crude Protein : 11.28%, Crude Fat : 2.1% Crude fibre : 16.7%, Ash : 4.71% Starch : 52.79%
20.	**Per acre yield of useful part(s) :**	192 kg/acre
21.	**Per acre cost of cultivation :**	Rs. 10000/-
22.	**Whether the germplasm will be available for supply, if yes, name & address of the place where it will be available:**	Yes. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala – 683 549
23.	**Name & address of the gene bank where the germplasm is deposited and their accession number :**	The material is being deposited at the gene bank of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi

24. Photographs (plantation as a whole, single plant as a whole, only flowering & fruitinig parts, part used for medicinal value e.g. roots, stem , leaves etc.) :

Kindly see Page 35

25 Any other relevant information

a. TLC fingerprint of adapathiyan crude drug

A thin layer chromatographic fingerprint was developed to characterise the crude drug of adapathiyan. Methanol extract of the drug was subjected to TLC on silica plate with chloroform. The spots were visualized by dipping in 1% alcoholic sulfuric acid followed by heating at 100°C for 5 minutes.

b. Variation in the quality of Curculigo available in different markets in Kerala

A large variation was observed in the quality of adapathiyan crude drug collected from four important markets in Kerala.

Fig. 6. *Holostemma ada-kodien*

Field view

Habit

Plant at bloom

Root stock

Crude drug

II. *Holostemma ada-kodien*

Expt. 1. Phenotypical and development physiological studies on *Holostemma ada-kodien*

Description of the plant.

Habit: A large glabrous, twining shrub with much branched, glabrous, shining stem. The storage roots are long up to one meter or more, irregularly twisted and 1-2 cm thick. The plant flowers from June to December and bear fruits from 30to 45 days of flowering. The whole plant contains latex.

Leaves: Leaves are simple, opposite petiolate, cordate, and glabrous. The young branches bear simple opposite decussate leaves.

Inflorescence: Axillary umbellate cymes, many flowered.

Flowers: Flowers purple, large, pedicellate, compleat, regular, bisexual and hypogynous.

Calyx: 5-partite, gamosepalous, broadly ovate, obtuse, veined

Corolla: Gamopetalous, thick, subrotate, deeply 5 lobed, the lobes overlapping to the right, ovate-oblong, obtuse, corona arising from the base of the staminal column.

Androecium: Stamens – 5, epipetalous, alternating with the petals, adnate to the base of the corolla tube, the filaments connate in a 10-winged column, anthers large, horny, shining with membranous inflexed tips, pollen masses pendulous, clavate, elongate, compressed, attached by long caudicles to the hard brown linear pollen-carries.

Gynoecium: Bycarpellary, apocarpous, ovary superior. The two free styles getting united towards the top or beneath the stigma. Stigma enlarged into a thick, 5-cornered disc. Ovary one celled, ovules many, anatropous.

Fruit: An aggregate of two follicles, linear, oblong, cylindric slightly tapering to a blunt point.

Seed: Many, ovoid, flattened, winged with a coma of white silky hair at micropylar end. Embryo large. Endosperm thin

Expt. 2. Manurial requirements of *Holostemma ada-kodien*

Objective : To standardise the optimum level of fertiliser application for maximum tuber production in *Holostemma*

Design : RBD

Replication : 4

Treatments : 7

T1 Control
T2 NPK -100:50:50
T3 FYM -30 Kg/ pt
T4 Vermicompost- 10 Kg/pt
T5 Biofertiliser-20 gm/pt +K@ 50 Kg /ha
T6 NPK+FYM full dose
T7 NPK+FYM half dose

Results : The data on the effect of manural treatments on the crude drug yield of *H. ada-kodien* is given in table 1 8.

The maximum yield was obtained in farm yard manure applied plots. Biofertiliser application did not show any benefit. Though yield increased with NPK application over control, the yield was only 50% of that in farm yard manure treatment. Application of FYM along with NPK increased the yield substantially over NPK alone. This again confirms the beneficial effect of FYM. However, the yield from the combined application of NPK and FYM was significantly lower than that from FYM alone.

Table 18. Effect of manuraial treatments on the yield *Holostemma ada-kodien* of tubers at harvest.

Treatment	Fresh weight (g/plant)	Dry weight (g/plant)
Control	14.33	2.75
NPK @ 100:50:50 kg/ha	74.00	12.40
FYM 30 t/ha	150.00	47.75
Vermicompost 10 t/ha	54.00	13.00
Biofertiliser-20 kg/ha	21.00	3.25
NPK+FYM full dose	127.33	33.33
NPK+FYM half dose	85.00	25.75
Mean	75.09	34.55

Conclusion: The crude drug yield of *Holostemma ada-kodien* can be substantially improved by application of farmyard manure at 30 t/ha.

Exp.3. Optimum time of harvest in *Holostemma ada-kodien* (Poly bag experiment)

Objective : To standardise the best time of harvest for maximum yield in *Holostemma ada-kodien*.

Design : RBD

Replication : 4

Treatments : 4 times of harvest - 6, 12, 18, 24 months after planting.

The seedlings were raised in the nursery and planted in the polybags (9 x 18 inches) during June 1999. Plants flowered in October 2000 and the fruits harvested in November.

Results: Data on the time-of-harvest trial are given in table 19 and depicted graphically in Fig.7.

Table 19. Yield of tubers at different stages of harvest.

Age at harvest (months)	Fresh weight of tubers (g /plant)	Dry weight of tubers (g /plant)
6	220.05	99.65
12	178.62	126.42
18	103.01	25.5
24	46.04	11.37

The fresh weight of tubers was the maximum at the 6-months stage and thereafter it showed a decline. In contrast, the dry weight increased up to 12 months and declined thereafter. At six-month stage the tubers are succulent and there is more water content in tubers. By twelfth month, the tubers gained more dry matter due to accumulation of minerals, fibres etc. The18th month harvest showed a drastic reduction in fresh and dry weight but the fibre, ash and mineral contents continues to increase. This drastic reduction in tuber weight was due to the fact that after summer while the tubers remained in the soil, fresh shoots are produced and the plant started its active vegetative phase. May be that the stored food in the tubers were utilised for re-growth.

Fig.7. Dry yield of tubers of H. ada-kodien when harvested after different periods of growth

It is interesting to note that when the seedlings planting in the field, tuberisation was very poor even after twelve months where as, the tuber formation was fairly good in polybag grown plants. It is observed that when the plants are in the field, the roots continue to grow laterally. So it is presumed that the root has to strike an impervious layer / obstruction to start tuber thickening. It may so happen that the tubers may be formed few meters away from the base of the plant and they may not be harvested. The tuber formation in polybags is reasonably good and the entire tubers could be harvested. Hence polybag cultivation could be commercially adapted for *Holostemma* cultivation.

Conclusion: For realising maximum crude drug yield; H. ada-kodien crop shall be harvested at the end of the first year.

Expt. 4. Yield and quality of *Holostemma ada-kodien* at different stages of growth.

Holostemma ada-kodien was planted in plots as well as in poly-bags to find out the optimum stage of harvest. Plants were uprooted at the end of 6 months, 12 months, 18 months and 24 months. The rhizomes were cleaned, dried, powdered and stored in air-tight containers for the analysis of quality parameters. Nitrogen content was estimated by micro-Kjeldahl's method. Protein content was calculated by multiplication of nitrogen content with factor 6.25.Ash content was determined by dry ashing and crude fibre by alternate treatment with acid and alkali. Starch content of rhizomes was determined by estimation of monosaccharides after hydrolysis of starch with mineral acid. Crude fat was estimated by extraction in soxhlet apparatus using acetone.

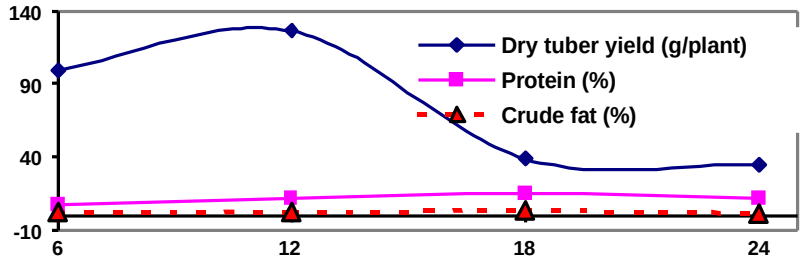
Results: Data on the yield of roots and the quality of crude drug harvested at 6,12,18 and 24 months of the crop are given in table 20. The trends are shown in Fig. 8.

The dry matter yield of the crude drug increased steadily up to the 12th month and showed reduced values thereafter up to the 24th month when the experiment was terminated. However, the contents of nitrogen, protein, ash, crude fibre and crude fat of rhizomes increased progressively with age up to 18 months with a concomitant decrease in the content of starch.

Table 20. Yield and quality of *Holostemma ada-kodien* at different stages of growth.

Stage of harvest (months)	Yield of Rhizomes (g/plant)		Nitrogen (%)	Protein (%)	Ash (%)	Crude fibre (%)	Starch (%)	Crude fat (%)
	Fresh wt.	Dry wt.						
6	220.05	99.65	1.13	7.56	3.01	7.69	56.43	1.98
12	178.62	126.42	1.81	11.28	4.71	16.70	52.79	2.10
18	103.01	25.5	2.43	15.18	6.20	23.30	42.38	3.23
24	46.04	11.37	1.87	11.68	4.97	22.05	35.82	1.76

Fig.8. Changes in rhizome yield, protein and crude fat content with progress of growth of *H. ada-kodien*



Conclusion: The best harvest stage would be the one when the rhizome yield as well as maximum content of protein, ash and crude fat. Even though the tuber quality is maximum at the 18 months stage, the crop may be harvested at the end of 12 months when the tuber yield is at its peak and quality is satisfactory.

Expt. 5. Evaluation of the quality of market samples of *Holostemma ada-kodien*

Objective : To asssess the quality variation in market samples of *Holostemma ada-kodien*.

Results: Market samples of *Holostemma* were collected from Muvattupuzha, Ernakulam, Trissur and Kottayam. On these samples percentage of ash, estimation of nitrogen, estimation of polysaccharide, estimation of crude fibre and NPK analysis were done. The results are presented in table 21.

Table 21. Quality parameters in the crude drug samples of *Holostemma ada-kodien* collected from different markets

Place of collection	Ash (%)	Protein (%)	Polysaccharides (%)	Crude fibre (%)	N %	P %	K %
Muvattupuzha	4.8	5.494	43.84	4.55	0.879	0.1289	0.61
Ernakulam	6.54	5.744	26.09	4.37	0.907	0.1321	0.599
Trissur	5.27	4.900	39.74	4.47	0.784	0.1185	0.621
Kottayam	6.81	4.585	39.85	3.51	0.734	0.1166	0.568

The ash content was the highest in Kottayam sample while it was lowest in Muvattupuzha sample. The contents of N and P was high in Ernakulam samples and potassium content was high in Trichur sample. Among the quality parameters studied, maximum variation was seen in the content of polysaccharides.

Conclusion: The results indicate that the quality of raw drug vary much in the market. The variation in quality may be due to the variation in maturity of the crop, season of collection and the type of soil and ecological variation in its natural state of growth in the wild.

Expt. 6. Development of TLC fringerprint of *Holostemma ada-kodien* crude drug.

A thin layer chromatographic fingerprint was developed to characterise the crude drug of adapathiyan. Methanol extract of the drug was subjected to TLC on silica plate with chloroform. The spots were visualized by dipping in 1% alcoholic sulfuric acid followed by heating at 100°C for 5 minutes.

Fig. 9. TLC fingerprint of crude drug of *Holostemma ada-kodien*

PROFORMA FOR FINALISED AGRO-TECHNIQUES

1.	**Name of the Project Officer / Principal Investigator & Organization**	Dr. J. Thomas / Dr. Samuel Mathew Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala PIN 683 549 Tel. 0484-2658221 Fax: 0484-2659881 E-mail: amprs@satyam.net.in
2	**Name of allocated Medicinal Plants**	1. *Curculigo orchioides* 2. *Holostemma ada-kodien* 3 *Saraca asoca* 4. *Strychnos nux-vomica*

III. ASOKA

Saraca asoca (Roxb.) de Wilde Family: Caesalpinaceae

a Botanical Name *Saraca asoca* (Roxb.) de Wilde

b Vernacular names

Sanskrit : Asoka, Gatasokah	Hindi: Asok, Asoka
Bengali : Ashok	Tel: Asokamu , Vanjalamu
Tamil : Asogam	Mal: Asokam

c Parts used for medicinal purpose: The bark is useful in dyspepsia, fever, dipsia, burning sensation, visceromegaly, colic, ulcers, menorrhagia, metropathy, leucorrhoea and pimples. The leaf juice mixed with cumin seeds are used for treating stomachalagia.

The flowers are considered to be uterine tonic and are used in vitiated conditions of *pitta*, syphilis, cervical adinitis, hyperdipsia, burning sensation, haemorrhoids, dysentery, scabies in children and inflammation. The aerial part is CNS depressant and diuretic.

3. Morphological characteristics including diagnostic characters for identification:

i. Habit : A medium sized handsome evergreen tree up to 9m in height with numerous spreading and drooping glabrous branches.

	ii. Leaves:	Leaves are alternate, paripinnate, copper red when young and green when mature. Leaflets usually 5-pairs; sometimes 4-6 pairs, short petioled, oblong lanceolate, glabrous and size of 20x7cm. Leaves are pinnate, 30-60 cm long having 2-3 pairs of lanceolate leaflets.
	iii. Infloresence:	Axillary, many flowered, pedunculate, corymbose cyme, about 10 cm long. Flowers are orange or orange yellow, arranged in dense corymbs and very fragrant.
	iv. Fruits:	Fruits are flat black pods, leathery and compressed with 4-8 seeds/pod, scimitar shaped, dehiscent, woody pod, tapering at both ends.
	v. Seed:	Seeds are ellipsoid oblong and compressed, 4-8 per pod, obovate, orbicular, compressed, smooth and exalbuminous.
4	**a) Habitat**	Asoka grows well in areas with well-distributed rainfall and in slightly shady areas.
	b) Geo-climatic conditions	The tree is grown throughout India except in north western part of the country upto an elevation of about 750 m.
	c) Best soil and land preparation	Asoka requires soil rich in organic mater and moisture. Square shaped pits of 60cm depth are taken at 3m spacing and filled with topsoil, sand and dried cow dung.
	d) Recommended varieties, if any:	Nil
5.	**Best planting material(s) recommended for cultivation :**	Seeds are collected when they ripen and fall down and are sown after soaking in water for 12 hours on raised beds. Seeds germinate within 20 days when they are planted in polybags. 2-month-old seedlings are transplanted to the main field at a spacing of 3m x 3m.
6.	**Best method & time of raising the plants:**	
7.	**Best period for sowing/planting seed/planting material**	The best season is June – July
8.	**Viability of seed/planting material:**	Seeds should be sown immediately after collection.

		Practice	***Time***
9.	**Recommended seasonal practices and interculture operations :**	Collection of seeds and sowing in nursery	May -June
		Land preparation and planting of seedlings	August - September
		Weeding and fertilization	October- November
		shade (during the initial years)	December to May
		Providing irrigation	December to May
10.	**Maximum number of plants appropriate for plantation per acre through cropping/intercroppin g system**	Pure plantation : 444 Intercropping : 100	
11.	**Manure & Nutrient requirements including time & method of application**	The basal portion of the trees are to be cleared of weeds and FYM at 2 kg/tree/ year may be applied twice; first in May- June and again in October-November. The dose is to be increased gradually to 10 kg from 5th year onwards. Chemical fertilizers are not usually applied. However its application during the initial years will help in better establishment of the plant. NPK at 90:45:45 g/tree/year is recommended.	
12.	**Recommended irrigation practices including quantity and frequency of application:**	The crop will receive adequate water during the rainy season. During summer months, the seedlings require to be irrigated on alternate days with about 5 litres of water/plant. Watering of grown up trees during summer months speeds up growth.	
13.	**Weed control method including chemical control:**	The area around the basin of the plant is to kept weed free by frequent weeding. The interspaces can be kept weed free either by hand weeding or protected spray of non-selective herbicides like paraquat (0.8% Gramaxone) or glyphosate (0.4%Glycel).	

14. Diseases, insect, pests, nematodes, physiological disorders, if any and their control measures:	No serious pest or disease is generally noted in this crop.
15. Time of flowering & fruiting:	Seeds are formed usually during February-April
16. Period required for maturity:	Seeds mature by April – May
17. Best time for harvesting medicinally useful part (s) keeping in view the active chemical ingredients:	Asoka can be cut after 20 years for collection of bark, the medicinally useful part. It is cut at a height of 15cm from the soil level. If given irrigation and fertilizers the stump will produce new shoots and it can be harvested again after 5 years. Alternatively, the bark can collected without cutting down the tree. The bark is peeled off first, vertically from one side of the main trunk. The excised area is renewed with fresh bark in 1-2 years. Then, the bark on the other side can be peeled off.
18. Best harvesting, storage/packing techniques for useful part(s):	
19. Concentration of active constituents in different parts of the plant:	Distribution of phytoconstituents in different parts of the tree a. Tannin Stem 0.57 - 7. 85% Root 0.84 - 4.72% Flower 5.71 - 11.51% Leaf 3.95 - 8.29% b. Ash : 2.43 - 6.69% c. Extractives : 5.74 – 14.07 %
20. Per acre yield of useful part(s) :	1000 kg of dry stem bark
21. Per acre cost of cultivation :	Rs. 4000/-

22.	**Whether the germplasm will be available for supply, if yes, name & address of the place where it will be available:**	Yes. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala – 683 549
23.	**Name & address of the gene bank where the germplasm is deposited and their accession number :**	The material is being deposited at the gene bank of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi

24. Photographs (plantation as a whole, single plant as a whole, only flowering & fruitinig parts, part used for medicinal value e.g. roots, stem , leaves etc.) : Kindly see Page 47

25 Any other relevant information

a. TLC fingerprint of asoka crude drug

A thin layer chromatographic fingerprint was developed to characterise the crude drug of asoka. One gram of the dried and powdered stem bark was immersed in 2M HCl and hydrolyzed on a water bath at 100ºC for 40 min. It was filtered and the filtrate partitioned with ethyl acetate. This extract that may contain aglycones of flavonoids was evaporated and taken in a few drops of ethanol. The ethanol fraction was analysed by TLC on silica gel with a mixture of dichloromethane, hexane and acetic acid in the ratio 9 : 0.5 : 0.5. The spots were visualized by dipping in 1% alcoholic sulfuric acid followed by heating at 100ºC for 5 minutes.

b. Variation in the quality of asoka available in different markets in Kerala

A large variation was observed in the quality of asoka crude drug collected from four important markets in Kerala

Fig. 10. *Saraca asoca*

Five-year old tree

Two-year old sapling

Infloresence

Crude drug (stem bark)

III. *Saraca asoca*

Exp. 1. Phenotypical and development physiological studies on *Saraca asoca* grown in hot humid tropics

The experiment was undertaken on a mature plant and observations on the growth and development of the plant were recorded. The salient observations are recorded below.

Leaves: leaves are alternate, paripinnate, copper red when young and green when mature. Leaflets usually 5 pairs, sometimes 4-6 pairs, short petioled, oblong lanceolate, glabrous and size of 22 x 7 cm.

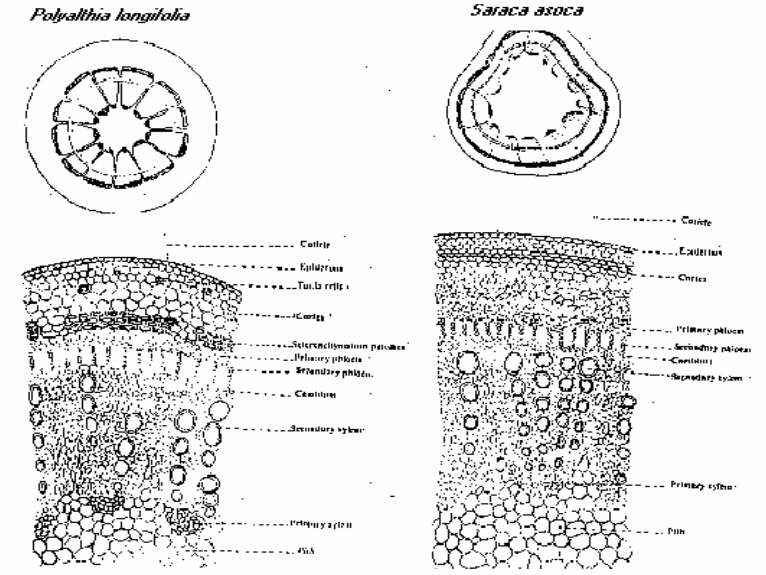
Inflorescence: Axillary, many flowered, pedunculate, corymbose cyme, about 10cm long.

Flowers: Orange red in corymbose cyme; bract small, deciduous, bracteoles reddish oblong-spathulate, sub-persistent and amplexicaul. Calyx petaloid, orange red, lobes 4, ovate or oblong, unequal and imbricate, calyx tube cylindric. P etals absent. Stamens usually 7, fiilaments long and filiform, anthers reniform-oblong, versatile and dehiscing longitudnally. Ovary superior, unilocolar, many ovuled, style long, filiform, ending in a minute capitate stigma. Fruit is a scimitar shaped, dehiscent, woody pod, tapering at both ends, seeds 4-8 per pod obovate orbicular, compressed, smooth and exailbuminous.

Expt. 2. Anatomical differences between *Saraca asoka* and *Polyalthia longifolia*.

Thin sections of stem of *Saraca asoka* and *Polyalthia longifolia* were prepared and examined under microscope to bring out the anatomical differences between them. The observations are shown in Figure 11.

Fig.11 Transverse sections of stems of *Polyalthia longifolia* and *Saraca asoca*



The findings are furnished in the table 18 also.

Table 18. Anatomical differences between *Saraca asoka* and *Polyalthia longifolia*.

Anatomical features	*Saraca asoka*	*Polyalthia longifolia*
Epidermis	Single layered, cells slightly larger than *Polyalthia*. Cuticle present amoung the epidermal cells.	Single layered cells, cuticle present. Cells smaller than *Saraca asoca*.
Cortex	Differentiated into three layers - outer, middle and inner. Cells are closely packed with less intercellular spaces.	Differentiated into three layers, outer, middle and inner. Outer and inner layers are smaller cells than the middle layer. Outer layers are chlorophilated cells containing tannin cells. Inner cortical cells are sclerenchymatous
Stele	Volume occupied by the stelar area is less compared to that of *Polyalthia*.	Stelar area is larger than that of *Saraca asoka*.
Pith	Large	Small

These features can serve as a guideline for identifying the original drug *Saraca asoka* from the adulterant, *Polyalthia longifolia*.

Expt. 3. Standardization of propagation techniques in *Saraca asoca*

Objective: To identify the best propagation techniques in *Saraca asoca*

Design : CRD

Replication : 2

Treatments : Seed treatment a) Soaking in water

b) Control (without soaking)

Table 19. Effect of seed treatments on the germination of *Saraca asoca* seeds and seedling viogour (on the 60th day of sowing)

Treatment	No. of days for germination	Germination (%)	No. of leaves per plant	Mean height of the plant
Soaking in water	13	100	4	19.98
Control	16	95	3	16.40

From the study, it has shown that fresh seeds of *Saraca* have a good germination and does not require any special seed treatment for obtaining good stand of the crop. However, it may be noted that pre-soaked (immersing in water overnight and draining of the water before planting) seeds germinated faster than the un-soaked seeds. Further, the mean no of leaves and average height of plants from pre-soaked seeds is higher than plants grown from seeds without any treatment.

Conclusion. Pre-soaking of seeds in water before sowing induced earliness in germination and boosted the seedling vigour.

Expt. 4. Manurial requriments of *Saraca asoca*

Objective: To find out the optimum requirements of various manure for the maximum yield.

Design : RBD

Replication : 4

Treatments : 7

1. Control
2. NPK - 100: 50: 50 kg/ha
3. FYM - 20 kg /plot
4. Vermicompost - 10 kg /plot
5. Biofertilizer - 20 gm/plot + 10 kg FYM
6. (Rhizobium + Phosphobacter)
7. *NPK + FYM -full dose*
8. NPK + FYM -half dose

Table 20. Influence of manorial treatments on the growth of *Saraca asoka*.

Treatment	Plant height	Number of branches	Number of leaves
Control	24.00	3	10
NPK	28.66	3	11
FYM	35.00	6	15
Vermi-compost	27.50	4	12
Bio-fertilizer	34.00	5	14
NPK+FYM Full dose	30.00	7	19
NPK+FYM Half dose	29.80	3	12
General Mean	29.85	4.42	13.28

The seedlings were raised in the nursery during the month of June 1998. The plants were transplanted to the main field during August 1998. The treatments were imposed as detailed. Mean plant height, no. of branches and no. of leaves after two years of growth was 29.85, 4.42 & 13.28 respectively. The plant responded well to organic manuring and also to biofertilisers. Further, there was advantage when fertilizers were applied along with farm yard manure.

Expt. 5. Development of a TLC finger print of *Saraca asoca* crude drug

A TLC fingerprint of the crude drug (stem bark) of asoka tree was developed. A thin layer chromatographic fingerprint was developed to characterise the crude drug of asoka.

One gram of the dried and powdered stem bark was immersed in 2M HCl and hydrolyzed on a water bath at 100 ºC for 40 min. It was filtered and the filtrate partitioned with ethyl acetate. This extract that may contain aglycones of flavonoids was evaporated and taken in a few drops of ethanol. The ethanol fraction was analysed by TLC on silica gel with a mixture of dichloromethane, hexane and acetic acid in the ratio 9 : 0.5 : 0.5. The spots were visualized by dipping in 1% alcoholic sulfuric acid followed by heating at 100ºC for 5 minutes.

The fingerprint will help to chemically identify the original drug material.

Expt.6. Quality variation in different plant parts, age and ecological groups of *Saraca asoca*

Saraca asoca trees of varying age and size were located from different agro-ecological situations in the state. Samples of bark as well as wood of both stem and root, flower and leaf were collected from these tress and analysed for tannin, one of the principal medicinal constituents of the tree. The results are given in table 21.

Table 21. Tannin content of different parts of *Saraca asoca*

No.	Place of collection	Age (years)	Tannin (%)					
			Stem		Root		Flower	Leaf
			Bark	Wood	Wood	Bark		
1	Chottanikara	35	4.037					
2	Kodungalloor	50	2.855					
3	Paravoor	65	7.851					
4	Odakkali	5	6.2452		0.8476	4.7284	7.4496	7.1374
5	Odakkali	5	7.182		1.5390	3.7918	7.3604	6.602
6	Vellanikkara	10	3.1002				9.5462	6.7358
7	Vellanikkara	15	4.7062				7.8288	8.2872
8	Vellanikkara	25	5.0408				7.5612	4.0148
9	Thodupuzha	15	3.7694	1.33824			7.0036	5.7098
10	Thodupuzha	20	4.3494	0.80294				6.2228
11	Thodupuzha	25	4.1486	1.0706			8.877	5.3752
12	Thodupuzha	30	3.1672	0.80294				4.7954
13	Thodupuzha	40	4.2154	0.63452				4.3048
14	Thodupuzha	60	2.1858	0.5799			11.063	3.9478
15	Thodupuzha	<60	2.855	0.98138				5.6652
16	Asamannoor	25	5.13	1.0152			11.509	6.5128

17	Asamannoor	30	5.8436	0.78064				4.3048
18	Asamannoor	45	4.4386	1.0296			5.7098	5.7068
19	Asamannoor	50	2.8996	1.126				5.4646
20	Asamannoor	70	4.4832	1.87354				5.7544
21	Asamannoor	100	3.814	1.13752			10.684	7.0482
22	Vazhakkulam	25	5.4422	0.62452				6.379
23	Alwa town	25	6.156	0.93678			6.156	4.9292
24	Aluva town	40	5.576	0.78064				9.0108
25	Aluva town	75	6.2674	0.67282			8.743	4.7284

The data obtained was re-tabulated on the basis of broad age groups of the trees and given in table 22.

Table 22. Tannin content of different plant parts in age groups of *Saraca asoka*

Sl. No.	Age Group	No. of samples	Average tannin % (as tannic acid)					
			Stem bark	Stem wood	Root bark	Root wood	Flower	Leaf
1	5-15	5	5.0006	1.3383	4.2601	1.1933	7.8377	6.894
2	16-25	6	5.0445	1.1236			8.5258	5.572
3	26-45	6	4.5463	0.8056			5.7098	5.625
4	46-65	5	3.7293	0.8960			11.063	5.026
5	66-100	3	4.8550	1.2280			9.7135	5.844
	Mean		4.6362	1.0783	4.2601	1.1933	8.5700	5.792
Polyalthia longifolia stem bark (adulterant)			2.4534	--	1.6728	1.1821	--	---

The data show that the total tannin content of the plant did not have any bearing on the age of the tree. This was true in case of tannin content of any part of the tree.

The average tannin content of the different tree parts is given in table 23.

Table 23. Comparison of tannin content of *asoka* with the common adulterant, *Polyalthia longifolia*

Sl.No	Plant Part	Tannin (%)	
	.	*Saraca asoka*	*Polyalthia longifolia*
1	Root Bark	4.260	1.673
2	Root Wood	1.193	1.182
3	Stem Bark	4.6312	2.453

Data show that the flowers had the highest tannin content followed by leaves. Stem bark and root bark had higher contents compared to their respective woods.

Stem & root bark of *Polyalthia* species is used widely to adulterate the crude drug of *Saraca asoka*. However, the stem bark of the adulterant contains only about half the tannin content of the

original drug. However, tannin contents of root wood of the original drug and of the adulterant were comparable, though low.

Expt. 7. Quality variation in market samples of *Saraca asoca*

Objective : To asssess the quality variation in market samples of *Saraca asoca* .

Market samples were collecte d from Muvattupuzha, Ernakulam, Trissur and Kottayam. Quality estimations such as ash, crude fibre and mineral nutrients (N,P and K) were undertaken. The data are given in table 24.

Table 24. Quality parameters in the crude drug samples of *Saraca asoca* collected from different markets

Place of collection	Ash (%)	Protein (%)	Starch (%)	Crude fibre (%)	N (%)	P (%)	K (%)	Tannin (%)
Muvattupuzha	2.43	3.36	15.20	34.54	0.538	0.0385	0.311	8.832
Ernakulam	6.69	6.93	10.83	28.35	1.109	0.0741	0.574	4.639
Thrissur	5.34	4.55	9.32	33.22	0.728	0.0973	0.487	3.212
Kottayam	5.84	4.13	10.00	28.45	0.661	0.0512	0.259	4.795

Results obtained: The data on quality of raw drugs of the samples of *Saraca asoca* showed wide variability in all the quality parameters evaluated. The greatest variation was observed with respect to the protein content (3.36 – 4.13%), Ash content (2.43 – 6.69%), Polysaccharides (9.32–15.2 %) and crude fibre (28.35 – 34.54%). Nitrogenand potassium contents were highest in Ernakulam sample, while phosphorus was highest in Trichur sample.

Conclusion: *Saraca* being a tree species, the raw drug available in the market vary widely due to variation in age, place of growth including soil and other ecological factors and extent of adulteration in the sample.

Expt. 8. Distribution of principal chemical constituents in the aerial parts of Asoka tree

A 25-year *Saraca asoca* tree was selected for studying the distribution of principal chemical constituents in the aerial parts. Samples of the stem bark were collected from various parts of the tree trunk varying in diameter as well as age. The samples were analysed for total tannin, ash and total extractives. The results are shown graphically in Fig. 12.

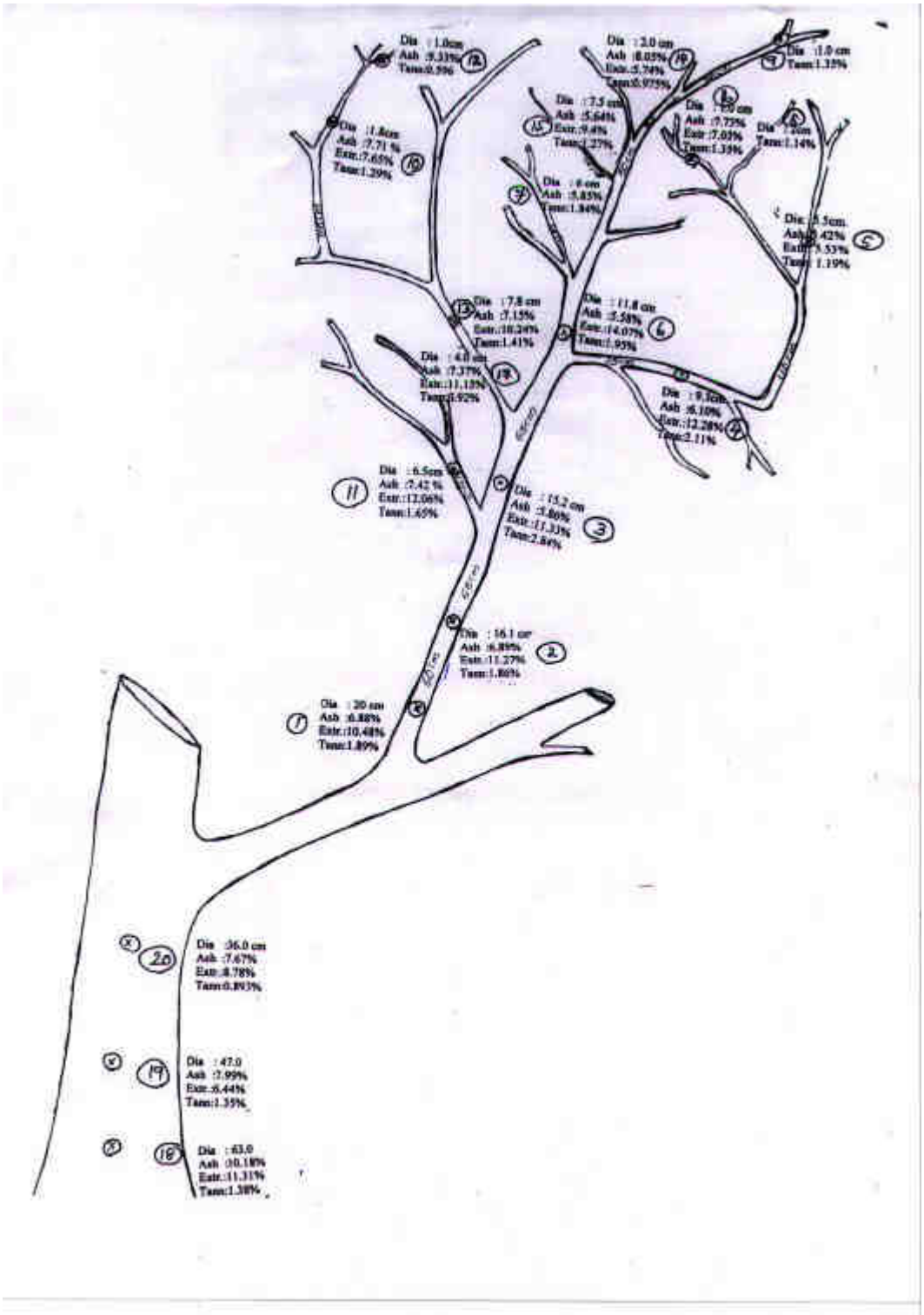


Fig.12. Distribution of principal chemical constituents in the aerial parts of Asoka tree

The parameters tested namely total tannin, ash and total extractives are considered to reflect the medicinal property of the drug. It is generally believed that the quality of the drug (stem bark) is positively correlated with its age. If this is true, the base of the tree trunk will yield the best quality material and the branch tips will yield the most inferior drug. Accordingly, the drug of asoka is recommended to be collected from the main trunk. Contrary to this, it is found in this analytical study that there is no strict relationship between the location on the stem and the quality of the drug sample collected from there. Then the results indicate that the drug (stem bark) can be collected not only from the mature trunk, but also from the young branches and twigs.

This finding has far reaching effect on the pattern of collection and utilization of this drug plant. As a general practice, more than 50 year old asoka trees are mercilessly cut to collect the small amount of bark that it bears. With unscrupulous felling of the trees together with the manifold increase in the demand for this rare drug, there is a great shortage for the asoka drug. Further it is expected that in a short perid of time, the natural stock of asoka tress is going to be exhausted. If it is that the young bark also yields equally good drug material as observed in this study, methods can be devised to utilize the existing stock of trees in a sustainable manner. The branches on the tree can be harvested in a phased manner without felling the tree.

PROFORMA FOR FINALISED AGRO-TECHNIQUES

1.	**Name of the Project Officer / Principal Investigator & Organization**	Dr. J. Thomas / Dr. Samuel Mathew Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala PIN 683 549 Tel. 0484-2658221 Fax: 0484-2659881 E-mail: amprs@satyam.net.in
2	**Name of allocated Medicinal Plants**	1. *Curculigo orchioides* 2. *Holostemma ada-kodien* 3. *Saraca asoca* 4. *Strychnos nux-vomica*

IV. STRYCHNINE TREE

Strychnos nux-vomica Linn. Family : Loganiaceae

a Botanical Name *Strychnos nux-vomica* Linn.

b Vernacular names

Sanskrit : Karaskara	Hindi: Kajra, Kuchila
Marathi : Jharkhatchura	Kannada : Hemmushti, Ittangi
Oriya : Kora, Kachila	Telugu: Mushti, Mushidi
Tamil : Itti, Kagodi, Kanjiram	Mal: Kaanjiram

c Parts used for medicinal purpose: Strychnos is highly toxic to man and animals producing stiffness of muscles and convulsions, ultimately leading to death. In small doses it can serve as efficacious cure forms of paralysis and other nervous disorder. The seeds are used as a remedy in intermittent fever, dyspepsia, chronic dysentery, paralytic and neuralgic affections. It is also useful in impotence, neuralgia of face, heart diseases. Leaves are applied as poultice in the treatment of chronic wounds and ulcers and the leaf decoction is useful in paralytic complaints. Root and root bark used in fever and dysentery.

3. Morphological characteristics including diagnostic characters for identification:

i. Habit : A large deciduous tree with a fairly straight and cylindrical bole having dark grey or yellowish grey bark and minute tubercles.

	ii. Leaves:	Simple, opposite, orbicular, to ovate, 6-11.5 x 6-9.5cm, coriaceous, glabrous, 5 nerved, apex obtuse, acute or apiculate, transverse nerves irregular and inconspicuous.
	iii. Infloresence:	Many flowered terminal cymes, 2.5-5 cm across. Bracts (5mm) and bracteols (1.5mm) small.
	iv. Flower	White or greenish white and fragrant. Calyx 5 lobed, pubescent and small (2mm). Corolla salver shaped, tube cylindrical slightly hairy near the base within the greenish white, tube much elongate than the lobes. Tube 7mm and lobes 2.5mm long. Lobes 5 and valvate. Stamens 5, filaments short, 0.1mm long. Anthers 1.5mm sub exserted, linear oblong. Ovary 1.5mm, pubscent, 2 celled, ovules 1-many. Style 9mm, stigma capitate.
	iv. Fruit:	Berry, 5-6 cm diameter, globose, indehiscent, thick shelled, orange red when ripe with fleshy pulp enclosing the seeds.
	v. Seed:	Seeds many, discoid, compressed, coin like, concave on one side and convex on the other, covered with fine grey silky hairs.
4	**a) Habitat**	The plant is found throughout India in deciduous forests up to 1200m. It is also found in Sri Lanka, Siam , Indochina and Malaysia.
	b) Geoclimatic conditions	Tropical and subtropical climate
	c) Best soil and land preparation	It is grown in different soil types such as laterite, sandy and alluvial. Seeds are sown in poly bags. The saplings are later transplanted to the main field on to pits of about 1mx1m taken at a spacing of 6mx6m; filled with top soil and organic manure.
	d) Recommended varieties, if any:	Different ecotytpes are seen.
5.	**Best planting material(s) recommended for cultivation :**	Seeds

6. Best method & time of raising the plants:

Dec-January

Fresh and dry seeds of *Strychnos nux-vomica* has poor germination. Germination can be substantially increased treating the seeds with hot water (50° C) for a period of six to twelve hours prior to sowing.

7. Best period for sowing/planting seed/planting material:

Seedlings can be planted in main field with the onset of South-West monsoon in May-June.

8. Viability of seed/planting material:

Viability of seeds decreased on storage. Fresh seeds are sown after hot water treatment.

9. Recommended seasonal practices and interculture operations:

Practice	*Time*
Collection of seeds and planting in poly bags	December- January
Land preparation and planting	May- June
Weeding and fertilization	July- August
Weeding and fertilization	October- November
Providing shade (during initial years)	December-May
Irrigation	December to May

10. Maximum number of plants appropriate for plantation per acre through cropping/intercropping system

Pure plantation : 110

Intercropping : 25

11. Manure & Nutrient requirements including time & method of application

The basins of the trees are cleared of weeds and after application of manures and fertilizers covered with soil. FYM at 2kg per tree is to be applied during early stages and the dose is gradually increased to 20 kg from 5th year onwards. The plant responded very well to organic manuring, fertilizer application is generally detrimental to the growth of *Strychnos.* However, when inorganic fertilizer (NPK 100:50:50 kg/ha) is applied along with FYM the negative effect of fertilizer could be neutralized to some extent.

12. Recommended irrigation practices including quantity and frequency of application:

The crop will receive adequate water during the rainy season. In the absence of rain, the saplings are to irrigated on alternate days with about 5 litres of water/plant. For grown up trees, irrigation during summer months is beneficial.

13. Weed control method including chemical control:

The area around the basin of the plant is to kept weed free by frequent weeding. The interspaces can be kept weed free either hand weeding or protected spray of non selective herbicides like paraquat (0.8% Gramaxone) or glyphosate (0.4%Glycel).

14. Diseases, insect, pests, nematodes, physiological disorders, if any and their control measures:

No serous pest or disease is observed on the crop.

15. Time of flowering & fruiting:

Flowering is during March-April and fruiting during May-December.

16. Period required for maturity:

Fruits take about 8 -9 months to mature

17. Best time for harvesting medicinallyuseful part (s) keeping in view the active chemical ingredients:

Mature fruits are to be harvested from time to time.

18. Best harvesting, storage/packing techniques for useful part(s):

Mature pods are collected and seeds from them are extracted, washed and dried and stored for later use.

Roots, bark and leaves can also be used medicinally.

19. Concentration of active constituents in different parts of the plant:

Strychnine and brucine are two important and toxic alkaloids present in all parts of the plant the distribution of which is as follows.

Plant part	Strychnine (%)	Brucine (%)
Root bark	1.775	2.887
Root wood	0.316	0.440
Stem bark	0.963	2.140
Stem wood	0.586	0.015
Leaves	0.235	0.474
Seed	0.379	0.591

20.	**Per acre yield of useful part(s) :**	50-75 dry dry seed per tree per year
21.	**Per acre cost of cultivation :**	First year : Rs. 4000 Subsequent years : Rs. 2500
22.	**Whether the germplasm will be available for supply, if yes, name & address of the place where it will be available:**	Yes. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor Post, Ernakulam Dist. Kerala – 683 549
23.	**Name & address of the gene bank where the germplasm is deposited and their accession number :**	The material is being deposited at the gene bank of the National Bureau of Plant Genetic Resources (NBPGR), New Delhi
24.	**Photographs (plantation as a whole, single plant as a whole, only flowering & fruitinig parts, part used for medicinal value e.g. roots, stem , leaves etc.) :**	Kindly see Page 61

25 Any other relevant information

Development of TLC fingerprint of Nuxvom crude drug.

A thin layer chromatographic fingerprint was developed to characterise the crude drug of this plant. The drug was extracted with methanol and TLC was developed on silica plate with a mixture of hexane, chloroform and diethyl amine in the ratio 7:2:1. The spots were visualized by dipping in Wagner's reagent.

Fig. 13. Strychnos nux-vomica

Five-year old tree

Two-year old sapling

Fruit

Crude drug (seeds)

IV. *Strychnos nux-vomica*

Exp. 1. Phenotypical and development physiological studies on *Strychnos nux-vomica* grown in hot humid tropics

The experiment was undertaken on a mature plant and observations on the growth and development of the plant were recorded. The salient observations are recorded below.

Leaves: simple, opposite, orbicular to ovate, 6-11.5x6-9.5cm, coriaceous, glabrous, 5 nerved, apex obtuse, acute or apiculate, transverse nerves irregular and inconspicuous.

Inflorescence: many flowered terminal cymes, 2.5-5cm across. Bracts (5mm) and bracteoles(1.5mm) small.

Flower: white or greenish white and fragrant. Calyx 5 lobed, pubescent and small (2mm). Corolla salver shaped, tube cylindrical slightly hairy near the base within and greenish white, tube much elongate than the lobes. Tube 7mm and lobes 2.5mm long. Lobes 5 and valvate. Stamens 5, filaments short, 0.1mm long. Anthers 1.5mm sub exserted, linear oblong. Ovary 1.5 mm, pubscent, 2 celled, ovules 1-many. Style 9mm, stigma capitate.

Fruit: is a berry, 5-6cm diameter, globose, indehiscent, thick shelled, orange red when ripe with fleshy pulp enclosing the seeds. Seeds 1-many, discoid, compressed, coin like, concave on one side and convex on the other , covered with fine grey silky hairs.

General Features

Leaf fall during December (Do not shed all the leaves at a time).

New foliage - February

Flowering - March – April

Fruiting - May – December.

Fruits takes about 8 – 9 months to mature.

Expt. 2. Standardisation of propagation techniques in *Strychnos nux-vomica*

Objective: To standardise the best propagation technique in *Strychnos nux-vomica*

Design : CRD

Replication : 3

Treatments : Seed treatment on freshly collected seeds

Control -
Water at 65 ^{0} C -
Mechanical scarification - (seed coat were broken with hammer)
H_2SO_4 treatment - (seed were soaked in conc. sulphuric acid for one minute)
HNO_3 treatment - (seed were soaked in conc. nitric acid for 1 minute)

All the seeds were soaked in water overnight and then the treatments were imposed. The treated seeds were sown in pots. The observation on germination was recorded weekly. The germination recorded on 43rd day after sowing is tabulated below.

Another set of experiment was conducted with dried seeds stored for one year. Here again, the seeds were soaked overnight in water and then treatment was applied.

Table 25. Effect of seed treatments on germination of seeds of *Strychnos nux-vomica*

Treatment	Germination (%)	
	Fresh seeds	1-year old seeds
Control	10	15
Hot water	55	40
Mechanical scarification	5	0
H_2SO_4 treatment	10	5
HNO_3 treatment	0	5

Results obtained: The data indicated that fresh seeds gave better germination percentage than one-year-old seeds. Hot water treatment substantially increased the germination.

Expt. 3. Standardisation of hot water seed treatment in *Strychnos nux-vomica*

Design : CRD

Replication - 3

Treatments – Factorial combination of two types of seeds, four hot water temperatures and four durations of hot water treatment (2 x 4 x 4 factorial)

Seed types	s1	Fresh seeds
	S2	Dry seeds

Temperatures	t_1	50°C
	t_2	60°C
	t_3	70°C
	t_4	80°C

Duration of soaking	d_1	6 hrs
	d_2	12 hrs
	d_3	24 hrs
	d_4	48 hrs

Table 26. Effect of hot water seed treatment on the germination percentage of ***Strychnos nux-vomica*** seeds.

Treatment Combination	No. of days for first seedling emergence	Germination at the end of 60 days (%)
S1t1d1	37	93
S1t1d2	40	87
S1t1d3	60	7
S2t1d1	25	70
S2t1d2	25	67
S2t1d3	33	40
S2t1d4	46	3
S2t2d1	25	20
S2t2d2	40	27
Control (fresh)	43	53
Control (1-year old)	39	33

Results : Maximum germination of 93 percent was obtained when fresh seeds were treated with hot water at 50 °C for six hours. This was followed by 87% germination obtained when the treatment time was increased to 12 hours. The germination was 53% without treatment in fresh seeds. All other treatments were inferior to untreated control. In dry previous season seeds, the germination percent was only 33 percent when seeds are sown directly. The germination could be substantially increased to 70 % when the seeds were treated in warm water (50 °C) for 6 – 12 hours.

Conclusion: It can be concluded that fresh and dry seeds of *Strychnos nux-vomica* had poor germination and the germination could be substantially increased with hot water treatment at 50 °C for a period of six to twelve hours.

Exp 4. Manurial requirements of *Strychnos nux-vomica*

Objective: To find out the optimum level of manure or fertiliser application for maximum growth of Strychnine tree.

Design : RBD

Replication : 4

Treatments : 8

1. Control
2. NPK - 100: 50: 50 kg/ha
3. FYM - 20 kg /pt
4. Vermicompost - 10 kg /pt
5. Biofertilizer - 20gm/pt + 10 kg FYM
6. (Azatobacter + Phosphobacter)
7. *NPK + FYM -full dose*
8. NPK + FYM -half dose

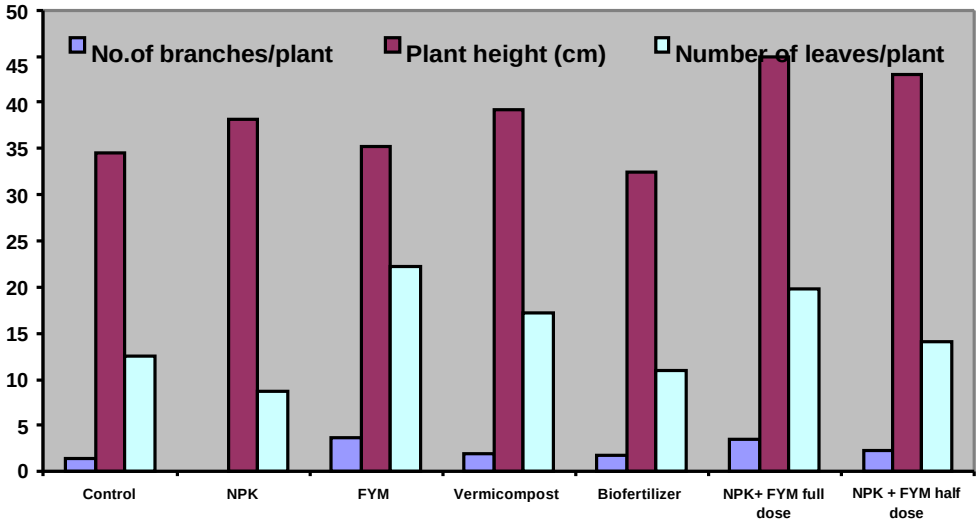
Manuring was done as per treatment two times in a year during the months of June and September.

Table 27. Effect of manurial treatments on growth of *Strychnos nux-vomica*

Treatment	Plant height (cm)	Number of branches/plant	Number of leaves/plant
1. Control	34.50	1.50	12.50
2. NPK- 100:50:50 kg/ha	38.25	0.00	8.75
3. FYM- 20 kg/pt	35.25	3.75	22.25
4. Vermicompost 10kg/pt	39.25	2.00	17.25
5. Biofertilizer – 20gm each/pt (Rhizobium + Phosphobacter)	32.50	1.75	11.00
6. NPK+ FYM full dose	45.00	3.50	19.75
7. NPK + FYM half dose	43.00	2.25	14.00
$CD_{0.05}$	8.278	1.781	NS

Results. Data on the growth parameters are given in table 27 and presented graphically in Fig.14. Mean plant height, no. of branches and no. of leaves after one and half years of growth was 33.07, 5.74 & 22.01 respectively. It can be seen that the plant responded very well to organic manuring as

Fig. 14. Growth parameters of *Strychnos nux-vomica* as influenced by manurial treatments



evident from the treatments T_3&T_4. An interesting observation is that fertilizer application is generally detrimental to the growth of *Strychnos*. However, when farmyard manure is applied mixed with fertilizer, the adverse effect of fertilizer could be alleviated to some extent.

Expt. 5. Development of a TLC fingerprint of *Strychnos nux-vomica*. From the methanol extract of the plant (seed or bark or leaves), alkaloids were separated by sequential acid and alkali treatment. The extract was spotted on pre-coated silica F_{254} plate and developed with Hexane:Chloroform: Diethyl amine in the ratio 7:2:1. The spots were located by viewing under UV light. Spots of strychnine and brucine on the TLC plate were identified by comparing their Rf values with those of authentic standards. The TLC fingerprint is shown in the figure 14.

Fig. 15. TLC finger print of *Saraca asoca* seed

Expt. 6. Standardisation of method for estimation of major alkaloids in *Strychnos nux-vomica*

The broad outline of extraction and clean up of Nux-vomica samples were based on the method suggested by Manske (1950). Modifications were made to suit the requirements of estimation of the alkaloids by HPLC. A 0.5 g of the finely powdered plant material was taken in a 250 ml R.B. flask and refluxed with 30 ml redistilled methanol for 1 h on a water bath. The methanol extract was decanted and filtered through Whatman No. 1 filter paper. Extraction was repeated thrice till the extract tested negative for alkaloids by TLC. The solvent was evaporated and the residue was dissolved in water. Two milli litres of 10% lead acetate solution was added and the contents warmed on a water bath for coagulating the impurities. Two milli litres of 10% oxalic acid was added to remove the excess lead acetate and the solution was filtered. The pH of the filtrate was adjusted to 8.0 by adding 5% Na_2CO_3 solution and allowed to stand for 30minutes. Alkaloids in the extractives were then separated 4 times into 30ml portions of chloroform. The combined chloroform extract was washed twice with 25ml each of 5% NaCl solution and dried over anhydrous sodium sulphate. Chloroform was evaporated and the residue was analysed for alkaloids by HPLC.

Note:- Seeds of *Strychnos nux-vomica* were found to contain about 2.5% fixed oil. Seed was de-oiled before extraction of alkaloids. For this, the powdered seed material was packed in a 1.5cm diameter glass column and eluted with 100ml petroleum ether till all the fat was removed.

The alkaloids in *S. nux-vomica* extract were analysed by HPLC. The instrumental parametrs are given below.

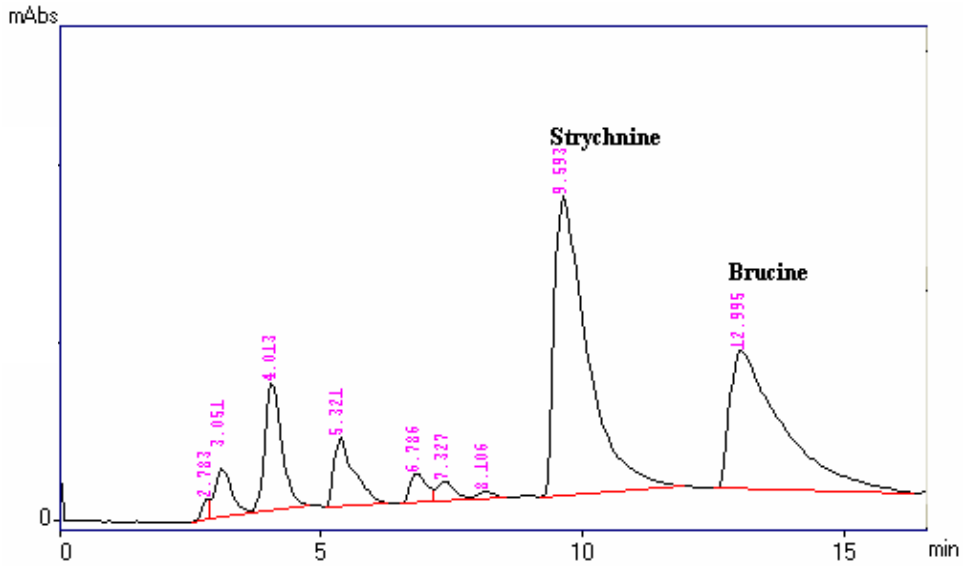
Column : 250 x 4 mm SS column packed with 5µ Lichrosorb DIOL material

Mobile phase : Hexane (50) + DCM (50) + Methanol (1) + Triethylamine (1) at a flow rate of 1 ml min^{-1}

Detector : Photometric (256 nm)

Injection volume : 100 µl

A typical chromatogram is presented in figure 15. Peaks of strychnine and brucine were identified by co-chromatography with authentic standards.



Retention time

Fig.16. High Performance Liquid Chromatogram of Nux-vomica root bark extract.

Expt. 7. Alkaloid content of different plant parts of different age groups of *Strychnos nux-vomica* trees from various localities

Data on strychnine content of different parts of *Strychnos nux-vomica* is given in table 28. Out of root, stem, leaf and seed, the root proved to be the best source of strychnine. This was followed immediately by stem. In the root and stem, strychnine was concentrated in the bark than in wood. The difference was most conspicuous in case of the root. The average strychnine content of bark was 1.775% where as that in wood was only 0.316%. Almost the same trend was observed in case of stem, though the difference was less. The strychnine content of bark was 0.963% and that of wood was 0.586%. The study has revealed that in *Strychnos nux-vomica,* root bark is the most concentrated source of strychnine (1.775%) followed by stem bark (0.963%). Although strychnine was present in leaf and seed to the extent of 0.235% and 0.379% respectively, the levels were much less than those in root and stem bark.

Table 28. Strychnine content of different parts of *Strychnos nux-vomica*

Sl No	Place of collection	Age of tree (y)	Strychnine content (%) of plant part					
			Root		Stem		Leaf	Seed
			Bark	Wood	Bark	Wood		
1	Muvattupuzha market, Eranakulam Dist		1.244	0.325	-	-	-	
2	Pala, Kottayam Dist.	3	-	-	0.398	-	0.046	
3	Thattekkadu,	5	3.244	0.366	0.694	0.457	0.105	
4	Pala, Kottayam Dist.	10	-	-	1.322	-	-	
5	Chenkara	10	2.083	0.302	0.881	-	0.146	
6	Thattekkadu	15	1.911	0.458	1.022	0.741	0.155	
7	Chenkara	18	2.168	0.278	-	-	0.179	
8	Koottala, Thrissur Dist.	20	0.653	0.205	1.213	-	-	
9	Pala, Kottayam Dist.	25	-	-	0.915	-	0.268	
	Edamalayar,Idukki Dist.	25	1.338	0.304	0.932	-	0.076	
10								
11	Bhuthathankettu	25	1.089	0.141	0.560	0.560	0.107	
12	Pala, Kottayam Dist.	45	2.260	0.381	1.091	-	0.422	
13	Pala, Kottayam Dist.	>50	3.919	0.416	1.787	-	0.664	
14	Pala, Kottayam Dist.	>50	0.797	0.255	-	-	0.353	
15	Pala, Kottayam Dist.	>50	0.591	0.360	0.741	-	0.278	
16	Kuruppampady, Eranakulam Dist							0.350
17	Manjapra, Eranakulam Dist							0.388
18	Perumbavoor, Eranakulam Dist							0.398
Range			0.591-3.919	0.141-0.458	0.398-1.787	0.457-0.741	0.046-0.664	0.358-0.398
Mean			1.775	0.316	0.963	0.586	0.233	0.379

Data on brucine content of different parts of *Strychnos nux-vomica* is given in table 29. More or less similar trend as in case of strychnine was observed in case of this alkaloid. Mean brucine content of *Strychnos nux-vomica* was highest in root bark (2.887%) followed by stem bark (2.140%). Root wood and stem wood also contained brucine but at very low levels, 0.440% and 0.015% respectively.

Small amounts of brucine were also available in leaf and seed (0.474% and 0.591% respectively). In summary, root bark is the richest source of the alkaloid brucine followed by stem bark.

Table 29. Brucine content of different parts of *Strychnos nux-vomica*

Sl. No	Place of collection	Age of tree (y)	Brucine content (%) of plant part					
			Root		Stem		Leaf	Seed
			Bark	Wood	Bark	Wood		
1	Muvattupuzha		0.279	0.362	-	-	-	
2	Pala	3	-	-	1.292	-	0.206	
3	Thattekkadu	5	4.192	0.519	1.530	0.007	0.133	
4	Pala	10	-	-	1.820	-	-	
5	Chenkara	10	3.390	0.444	1.544	-	0.137	
6	Thattekkadu	15	1.837	0.526	1.106	0.014	0.034	
7	Chenkara	18	4.816	0.505	-	-	0.485	
8	Koottala	20	0.965	0.345	3.884	-	-	
9	Pala	25	-	-	2.087	-	0.335	
10	Edamalayar	25	2.758	0.614	2.665	-	0.088	
11	Bhuthathankettu	25	2.985	0.357	3.395	0.024	0.351	
12	Pala	45	3.591	0.474	1.834	-	1.474	
13	Pala	60	4.193	0.419	2.512	-	1.121	
14	Pala	80	3.765	0.268	-	-	0.438	
15	Pala	100	1.869	0.451	2.006	-	0.889	
16	Kuruppampady							0.604
16	Manjapra							0.671
17	Perumbavoor							0.497
Range			0.279 – 4.816	0.268 – 0.614	1.106 – 3.884	0.007 – 0.024	0.133-1.474	0.497-0.671
Mean			2.887	0.440	2.140	0.015	0.474	0.591

Expt. 8. Quality evaluation of *Strychnos nux-vomica and Strychnos wallichiana (syn. S. cinnamomifolia / S. colubrina)*

Data on strychnine content of different parts of *Strychnos wallichiana* is given in table 30. *Strychnos wallichiana* is a species of *Strychnos* with twining habit. These plants are seen only in the wild. Estimation of strychnine content of different parts of this plant showed that this is a less important source of strychnine when compared to *Strychnos nux-vomica*. But for root bark, which contained on an average 1.073% strychnine, other parts examined yielded low levels of this alkaloid. Above all, *Strychnos wallichiana* is shy flowering and hence they rarely produce seed. Seed is more or less a pure source of alkaloid strychnine and is preferentially used for the extraction of this alkaloid in the preparation of homeopathic drugs. Taking all these factors into consideration, *Strychnos wallichiana* can be considered less important from the point of view of utilisation as a source plant in various indigenous systems of medicines.

Table 30. Strychnine content of different parts of *Strychnos*

Sl No	Place of collection	Age of tree (y)	Strychnine content (%) of plant part				
			Root		Stem		Leaf
			Bark	Wood	Bark	Wood	
1	Bhuthathankettu	5	0.682	0.214	0.212	0.384	0.435
2	Vadattupara	10	1.314	0.104	0.094	0.287	0.093
3	Bhuthathankettu	15	0.969	0.117	0.096	0.315	0.184
4	Bhuthathankettu	>50	1.328	0.152	0.038	0.224	0.872
Range			0.682 – 1.328	0.104 – 0.214	0.038 – 0.212	0.224 – 0.384	0.093 - 0.872
Mean			1.073	0.147	0.11	0.303	0.396

Table 31. Brucine content of different parts of *Strychnos wallichiana*

Sl. No	Place of collection	Age of tree (y)	Brucine content (%) of plant part				
			Root		Stem		Leaf
			Bark	Wood	Bark	Wood	
1	Bhuthathankettu	5	1.036	0.332	1.247	0.026	0.013
2	Vadattupara, Ernakulam	10	0.418	0.249	0.889	0.079	0.028
3	Bhuthathankettu	15	0.119	0.235	0.364	0.005	0.002
4	Bhuthathankettu	>50	0.068	0.417	0.698	0.047	0.002
Range			0.068-1.036	0.235-0.417	0.364-1.247	0.005-0.079	0.002-0.028
Mean			0.410	0.308	0.800	0.039	0.011

Data on brucine content of different parts of *Strychnos wallichiana* is given in table 31. Brucine content of stem bark ranged from 0.364% to 1.247% with an average of 0.800% and it was the best source of the alkaloid when compared to other plant parts. The alkaloid contents of other parts were too low to be considered as important.

Conclusion. In an overall, root bark and stem bark were found to be the richest source of both the alkaloids in *Strychnos nux-vomica* where as in *Strychnos wallichiana* root bark was the best source of strychnine and stem bark the best source of brucine. No relationship was apparently noticed between the alkaloid content of different parts and age of the plant. On the other hand, plants having same age collected from different places of the state showed wide variations in the total alkaloid content (strychnine + brucine). This variation can be attributed to the genetics, age and ecological factors. The genetic difference if any among the plants studied and the influence of eco-climatic factors on alkaloid content require detailed examination.

Expt.9. Distribution of principal chemical constituents in the aerial parts of Strychnine tree

A 30-year Strychnine tree was selected for studying the distribution of principal chemical constituents in the aerial parts. Samples of the stem bark were collected from various parts of the tree trunk varying in diameter as well as age. The samples were analysed for ash, total extractives, strychnine and brucine content. The results are shown graphically in Fig. 16.

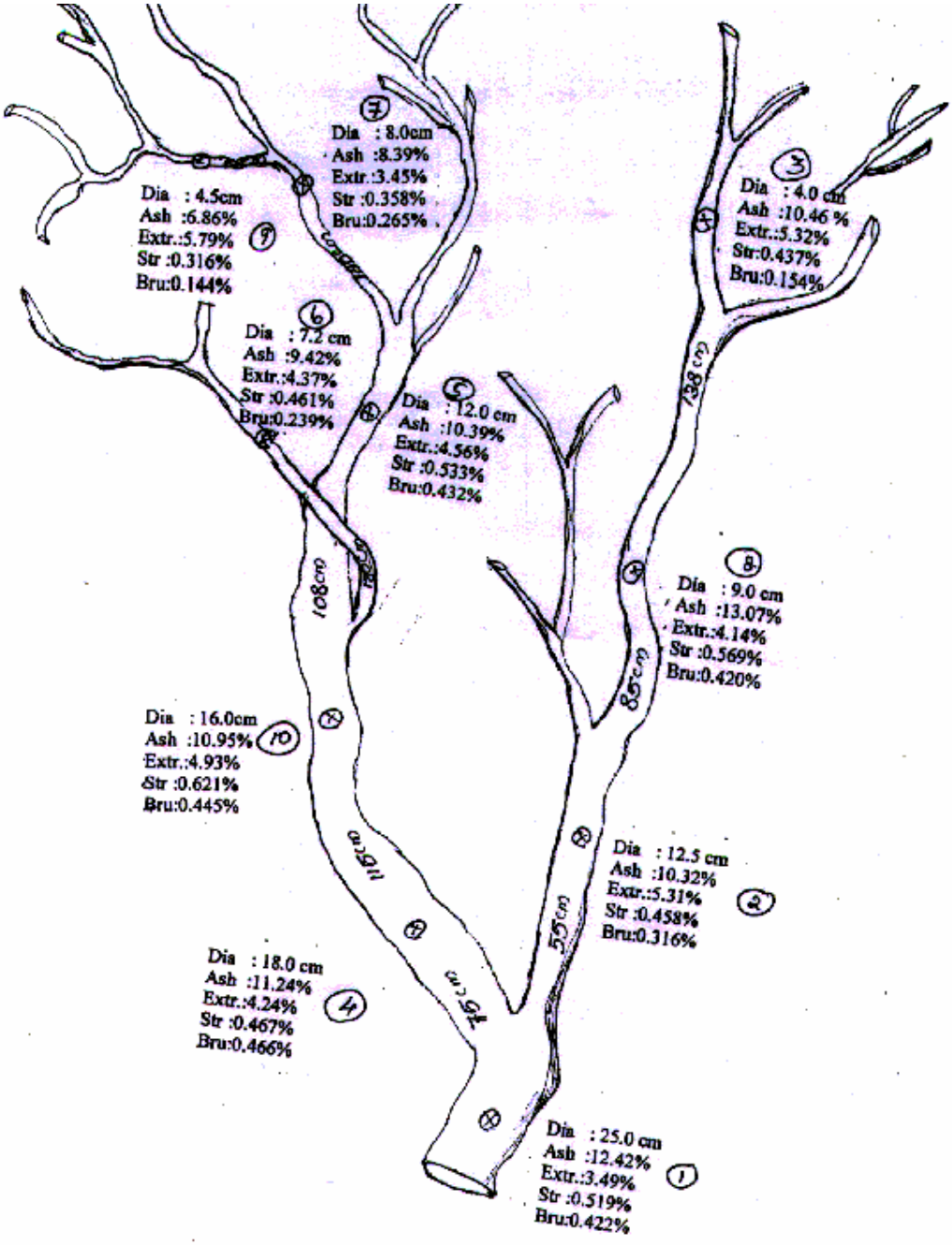


Fig. 17 . Distribution of principal chemical constituents in the aerial parts of Strychnine tree

The parameters tested namely ash, total extractives, strychnine and brucine content are considered to reflect the medicinal property of the drug. It is generally believed that in trees, the quality of the drug (stem bark) is positively correlated with its age. If this is true, the base of the tree

trunk will yield the best quality material and the branch tips will yield the most inferior drug. Accordingly, the drug of such trees is recommended to be collected from the main trunk.

Contrary to this, it is found in this analytical study that there is no strict relationship between the location on the stem and the quality of the bark at that point. Then the results indicate that the drug (stem bark) can be collected not only from the mature trunk, but also from the young branches and twigs.

This finding has far reaching effect on the pattern of collection and utilization bark of trees. As a general practice, in several cases, more than 50 year old trees are mercilessly cut to collect the small amount of bark that it bears. This may lead to extinction of such valuable trees. If it is that the young bark also yields equally good drug material as in observed in this study, methods can be devised to utilize the existing stock of trees in a sustainable manner. The branches on the tree can be harvested in a phased manner without felling the tree.

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