STANDARDISATION OF PROPAGATION AND STAGE OF HARVEST IN ADAKODIEN

(*Holostemma annulare* K. Schum.)

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**Standardisation of propagation and stage of harvest in adakodien (*Holostemma annulare* K. Schum.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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We, the undersigned members of the Advisory Committee of **Ms.Meera, N.** a candidate for the degree of **Master of Science in Horticulture** agree that the thesis "**Standardisation of propagation and stage of harvest in adakodien (*Holostemma annulare* K. Schum.)**" may be submitted by Ms.Meera, N. in partial fulfilment of the requirement for the degree.

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To my parents

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INTRODUCTION

The Indian flora is endowed with abounding medicinal wealth. Kerala bordered by Western Ghats is an important resource of many of the valuable medicinal plants. Until recently, these plants from the natural habitat catered to the requirements of the indigenous systems of medicine such as Ayurveda, Unani and Sidha. Change in the ecosystem due to denudation of forests poses problems for natural existence and indescriminate collection depletes the natural source and many species have been either endangered or on the verge of extinction. Ayurveda with its roots in Kerala require more than 1000 medicinal plants for its various formulations and nearly 60 plants are in high demand. The resurgence of interest in phytopharmaceuticals and the resulting demand for the natural herbs have warranted concentrated efforts for the domestication and cultivation of these plants on a commercial scale.

Holostemma annulare K. Schum. belonging to the family Asclepiadaceae is an endangered species which is renowned for its pharmacological properties. Known as Jeevanti in Sanskrit, the plant is popular as adakodien or adapathiyan in our vernacular language. The plant is a handsome, extensive, laticiferous, twining shrub found in most parts of India, ascending upto an altitude of 6000 ft. It is grown in gardens for its pretty flowers and are trained over trellis work. Leaves are opposite, ovate or triangular acute or acuminate, deeply cordate and the midrib glandular at the base. Flowers appear in axillary umbellate cymes, purplish inside and silvery white or pinkish outside, fragrant and fleshy (Plate 1). Fruit is a follicle, thick, cylindrical and bluntly pointed. Seeds usually flat, ovoid, winged and surmounted with a dense brush of long hairs (Plate 2). The plant is having a tuberous root system (Plate 3).

As the name Jeevanti indicates the drug maintains the juvenility and health and is classified by Charaka as a rasayana drug. The root forms the officinal part which is reported to possess cooling, alterative, tonic and lactative properties and is very useful in ophthalmia. The root preparations are used for relief in gonorrhoea, diabetes, cough and stomach ache. The leaves are rich in vitamin A and leaves fried in ghee is considered as a potent remedy for night blindness. The synonyms like sietavirya, madhurarasa snigdha are attributed to the plant and it cures tridosha (Nair *et al.*, 1982).

The drug forms the main component in commercial ayurvedic preparations like anuthailam, jeevantiadighritham, balarishtam and chyavanaprasam. The enormous uses, the high demand and the attractive price (Rs.200/kg of dried roots) have created enthusiasm among the farmers for taking up the cultivation of this plant. Moreover, being an endangered species commercial cultivation can go a long way in conserving the specie. Domestication and standardisation of horto techniques deserves utmost importance at this juncture. The availability of planting materials is a major limitation for domestication. Further there is no standard stage fixed for harvesting the crop. Standardisation of optimum stage of harvest assumes great

Plate 1.   Infloresence of *Holostemma annulare*

140535

Plate 2. Dry fruits and seeds of *Holostemma annulare*

Plate 3. Dry roots of *Holostemma annulare*

170535

significance since harvesting the plant without any criteria will lead to reduction in quantity and quality of the produce and also its curative properties. Hence as a first step towards domestication of adakodien the present study was undertaken to identify and to standardise the ideal and most economic method of propagation and optimum stage of harvest. The economics of cultivation will also be worked out to study the possibility of grooming this as a commercial crop.

Review of Literature

REVIEW OF LITERATURE

Holestemma annulare popularly known as adapathiyan or adakodien is a renowned medicinal plant in the ayurvedic system of medicine. The need for domestication of this crop is badly felt, only recently since the plant has become endangered due to unscrupulous collection and depletion of natural source. As such, literature available on the cultivation aspects of this crop is scanty. Informations pertinent to this study on other plants which are valued for secondary metabolites are also gathered and presented in this chapter.

2.1 Methods of propagation

2.1.1 Propagation through seeds

In nature higher plants reproduce primarily by seeds, excepting crops in which seed set is naturally prevented. The characteristic genetic variability that occurs among seedling progenies are advantageous to allow continued adaptation of a particular species to possible changes in the environment.

Sreedevi (1989) made an elaborate study on the pollination, fertilization and seed set in Asclepiads. An efficient pollinating mechanism was reported where in the pollen grains of an anther locule adhere to form a pollinial bag. Two such pollinial bags from two adjacent half anthers are attached to form a common corpusculum seen closer to the stigmatic corner by two caudicles. Mature pollinial bags are loosely placed within the locule, so that they can be easily pulled out during the visit of an insect and facilitate cross pollination. It is also reported that such a complex system for insect pollination had not been seen in any of the insect pollinated crops. Apis and Helictus are the frequent pollinators of Asclepiads. Honeybees, flies and wasps are also efficient pollinators. With all these adaptations for cross pollination, the general level of fruitset is low ranging from 5-10 per cent. This can be attributed to the low degree of removal and insertion of pollinia into the stigmatic chamber properly (Willson *et al.*, 1979). This is supported by field observation that considerable number of pollinia was misplaced on petals, stigmatic lobes and nectaries and the pollinia being eaten away by ants. In species of Asclepias, Calotropis and Daemia, it is found that pollinial furrows faces the line of anther dehiscence, so that pollen tubes from pollinia can enter into the stigmatic chamber and facilitates in situ germination. The fruit is a follicle with two fruitlets each containing 100 to 400 flat compactly packed tufted seeds which can be carried by wind to a great distance. Though some of the members of Asclepiads are vegetatively propagated by stem cuttings, in nature seeds are the chief means of propagation. Hence production of large number of seeds and efficient means of seed distribution are of great significance.

In *Rauvolfia serpentina* seed propagation is commonly practised. The germination percentage was found to vary from place to place and it ranged from 58 to 74 per cent in seeds which were sown fresh. The seed rate recommended is @ 5.5 kg ha^{-1}. The root yield was reported to vary depending on the age of the plant and the planting material. Badhwar *et al.* (1957 and 1963) recommended propagation of *Rauvolfia serpentina* by seeds for better economic yield and observed 25-50 per cent germination. On an average irrigated two year old plantation yielded 2,200 kg and a three year old plantation 3,300 kg air dried root per hectare.

Bhaskaran (1964) reported that seed propagated plants had maximum height, maximum root yield (346.90 kg ha^{-1}) and maximum root alkaloid content. Sahu (1979) also found that yield contributing characters like length, diameter and weight of fresh and air dried roots were higher from seed propagated plants compared to vegetative methods by using root and stem cuttings.

Hegde (1988) reported that in *Catharanthus roseus* plants propagated through cuttings flowered 3 to 4 months earlier than from seed. It is advocated that for dry matter production the plant should be propagated from seed and for seed production from cuttings. The productivity in terms of root, leaf and stem was not affected by the method of propagation but the leaf yield in the first year was significantly higher in plants propagated through cuttings than those from seeds. He also found that method of propagation had no influence on the harvest index. Root shoot ratio was considerably lower in plants propagated through cuttings than those from seeds.

Palmarosa and lemongrass are generally propagated through seeds and citronella by transplanting rooted slips. Rooted slips could also be used as a planting material in lemongrass depending on the availability. Direct sowing in palmarosa was reported to be less expensive with the added advantage of early vegetative growth, while transplanting of seedlings ensured uniform plant population (Gupta, 1972). A higher economic yield and increase in lifespan of transplanted lemongrass has been reported by Nair *et al.* (1980). Similarly, in palmarosa transplanting of seedling was observed to be better than transplanting rooted slips as the former gave higher percentage of geraniol.

2.2 Vegetative propagation

Vegetative or asexual propagation is used to produce a plant identical in genotype with the source plant. New roots and or shoots are regenerated on stems, leaves or roots. Vegetative propagation is not a natural phenomenon for most plant species, special techniques and facilities have been developed to facilitate propagation.

2.1.2.1 Propagation through stem cuttings

In propagation by stem cuttings, segments of shoots containing lateral or terminal buds develop adventitious roots under proper condition and thus produce independent plants. The type of wood, the stage of growth, the time of the year in which the cuttings are taken and several other factors influence the rooting of cuttings in plants (Hartman and Kester, 1978).

2.1.2.1.1 Influence on biometric characters

In *Rauvolfia serpentina*, Chandra (1956) reported that the hard wood cuttings of size 5" to 8" length produced roots within 15 days after planting with hormonal treatment. Sahu (1979) also reported that 7.5 cm long stem cuttings with two buds was the best material for vegetative propagation and noticed 66 per cent germination. According to him 100-125 kg of stem cuttings were required for planting one hectare. Gauniyal *et al.* (1988) suggested that the stem cuttings of 6-7 cm with 2 buds is best suited for propagation of *Rauvolfia serpentina* and the hard wood cuttings performed better than soft wood cuttings.

In pepper, single node cuttings or primary wood gave 90-95 per cent rooting (Hughes, 1966). Shanthamalliah *et al.* (1974) reported that in black pepper semiherbaceous cuttings taken from the middle portion of the stem rooted better than the herbaceous cuttings from soft terminal or hardwood cuttings. According to Nambiar *et al.* (1977) for rapid multiplication of the hybrid pepper cv. Panniyur-1, two noded cuttings could be used. Nambiar *et al.* (1978) compared the rooting behaviour of two, four and six noded cuttings and found that two noded cutting were the best planting material resulting in maximum rooting and field establishment.

Bavappa and Gurusinghe (1980) showed that pepper cuttings with even one node could be successfully propagated similar to cuttings with several nodes. Hegde (1983) found that three noded cuttings of Panniyur-1 pepper rooted better than one or two noded cuttings.

Swamy *et al.* (1960) reported that geranium could be successfully propagated through stem cuttings. The highest percentage of rooting with maximum number and length of roots in geranium was registered at high pH (5.5). The rooting percentage was found to be high in middle cuttings than in basal cuttings (Duraiswamy and Arumugam, 1980). Arumugam and Kumar (1980) studied the effect of leaves on rooting of stem cuttings of bergamot mint and reported that stem cuttings could be used for large scale cultivation. El-keltawi and Croteau (1985) reported the use of single node cuttings to propagate several species of mint.

Mitra and Kushari (1985) observed that in *Solanum khasianum* the rooting percentage was highest with four noded cuttings and lowest with one noded cuttings. The rooting was better under partial shade. In *Solanum hispidum*, cuttings were moderately easy to root and treatment with indole butyric acid increased the per cent of rooting as well as root growth. In the case of *Rosa damacena* mist propagation under plastic resulted in high percentage of rooted cuttings. They further observed that two node cuttings with lower leaves removed was the best suited for propagation (Ivanova and Gladun, 1986).

Coffman and Gentner (1979) found that *Cannabis sativa* could be successfully propagatd by vegetative cuttings but morphological and biochemical differences were observed between the seed derived plants and their vegetative propagules. In lavender, vegetative reproduction was found to be slower and expensive

Dioscorea, the diosgenin content and sprouting of tubers were not affected by planting materials. Rybacek (1984) made a comparison of growth, yield and quality of hops (cv. Osvald's Clone No.72) propagated through rooted shoots, suckers, sprout cuttings and seedlings. The results showed that in one or two year old gardens the yield and 100 cone weight were in the order of seedling > sprouts > shoots > suckers.

In liquorice, Badalov (1973) reported that cuttings with 10-15 cm lengtl and 1-1.5 cm diameter produced the best growth and the most productive plant: whereas smaller cuttings adversely affected the productivity of the plants. Dhar *et al* (1986) made a comparison of vegetative and seed propagation in Cymbopogon anc found that clonally propagated plants yielded higher berbage but lesser oil than seec established plants. Subha (1990) did not observe any significant difference betweer two noded and three noded cuttings for root yield as well as for root characters.

2.1.2.2 Propagation through root cuttings

Plants that naturally produce suckers freely can be propagated easily by root cuttings. Stoutmeyer (1968) reported that over 150 species were successfully propagated in this way. Vegetative propagation of clonal material through root cuttings has been recognised as a rapid method to produce genetically identical material. But it is also opined that the difficulties in securing cuttings from the ground and the damage inflicted on stock plant make this method less frequently used (Flemer, initial strain (Staikova *et al.*, 1980). Selvarajan and Madhava Rao (1982) reported that herbaceous cuttings of *Pogostemon cablin* were found to be the ideal planting material with 100 per cent rooting and survival. In *Rosmarinus officinalis* rooting per cent was highest when 9-12 cm long cuttings taken from one year old shoots were used.

Rooted cuttings of *Artemesia rutifolia* covered with or without plastic film showed a survival rate of 78.30 per cent (Shi *et al.*, 1990). Vegetative propagation studies in medicinal plants by Philip *et al.* (1991) revealed that hardwood cuttings of 20 cm length in *Sida retusa*, semi hardwood cuttings of 15 to 20 cm length in *Vitex negundo* and six noded semi hardwood cuttings in *Piper longum* showed better response to rooting. Higher the number of nodes greater will be the rooting percentage and survival rate.

Sudhadevi (1992) compared the rooting behaviour of softwood, semi hardwood and hardwood cuttings of *Alstonia venenata* with and without leaves. The results revealed that softwood cuttings failed to root. Semi hardwood cuttings recorded maximum sprouting compared to hardwood cuttings. Retention or removal of leaves did not have remarkable influence on the rooting of cuttings. She also reported that in *Coscinium fenestratum* the percentage of sprouting was only 40 per cent in stem cuttings on 60th day and they failed to root within this period. Pal *et al.* (1993) studied the rooting of shoot cuttings of Datura and found that semi hardwood cuttings were difficult to root while the leafy softwood cuttings rooted easily.

2.1.2.1.2 Influence on yield attributes

Singh *et al.* (1979) compared different planting materials in Japanese mint under normal time of planting (January-February) and they could not observe any

1961). An important characteristic associated with the use of root cutting is the restoration of more juvenile condition which aid in rooting.

In *Rauvolfia serpentina*, Badhwar *et al.* (1956) reported that cuttings from fresh or green roots are the best material for propagation. Chandra (1956) reported the highest percentage of success by using root cuttings of 2" having a thickness of 0.2"-0.3" supplemented with normal treatment. The success percentage increased with increase in size of the root cutting (Badhwar *et al.*, 1963). Sahu (1969) reported that 7.5 cm long root cuttings gave 72 per cent germination and about 100 kg of root cuttings was required for planting one hectare. Biswas (1988) compared the performance of root cuttings, stem cuttings and seeds for propagating diploid and tetraploid plants of *Rauvolfia serpentina*. The highest root yield was obtained from plants raised by root cuttings. Sudhadevi (1992) reported that in *Coscinium fenestratum* the root cuttings recorded a sprouting percentage of 70 but no rooting was observed.

2.1.3 Propagation by root stumps

In Rauvolfia 5 cm of the root with a portion of the stem above the collar planted in May-June in irrigated fields gave 90-95 per cent success (Sahu, 1969).

2.2 Stage of harvest

Harvest management is one of the most important factor which markedly influences the yield and quality.

2.2.1 Influence on biometric characters

Kolammal (1979) reported that the roots of *Holostemma annulare* collected for medicinal use were usually one centimetre thick was also occassionally met with. Samuel *et al.* (1993) observed that the roots in 17 month old crop reached 50 cm length and 5-10 cm girth.

Sobti *et al.* (1978) reported that *Ocimum gratissimum* harvested just after 10-15 days of flowering had a plant height varying from 150-300 cm with a mean height of 240 cm. The size of the leaf blade varied from 5.5 x 2.5 cm^2 to 14.5 x 6.3 cm^2 with a mean value of 9.8 x 4.4 cm^2. Choudhury *et al.* (1986) reported that in *O. gratissimum* the average height and corresponding number of branches showed an increasing trend from the first harvest to the fourth harvest and number of branches of *O. gratissimum* harvested at 60 days interval four times in a year varied from 63.3 to 98.2 cm, 18.2 to 57.3 respectively in the first year. Both the parameters were the highest in the fourth cutting (Choudhury and Bordoloi, 1984). Pillai (1990) reported that there was no significant difference in the height of the plants due to variation in the intervals of harvest. However the relative increase in height was greater for 75 days harvest interval. There was also not much variation in the total number of branches due to different intervals of harvest. The maximum number of branches was observed at 75 days harvest interval. With further increase in the maturity of the plant the leaf area decreased.

In Palmarosa, Chinnamma (1985) reported that increasing the interval of harvest from 40 to 65 days significantly increased the height of the plant in the first and second year. The crop harvested at 65 days interval recorded the maximum height for the first two years.

2.2.2 Influence of stage of harvest on yield attributes

Samuel *et al.* (1993) tried trial cultivation of adapathiyan and reported that the yield of dry roots after 11 months of planting comes to 1450 kg. The receipt and net profit worked out from a hectare was Rs.3.6 lakhs and Rs.2.5 lakhs respectively.

Jogi *et al.* (1972) recommended the harvesting of ginger 6.5 months after planting when the crude fibre content was low. Nybe (1978) obtained the maximum yield of green ginger 180 days after planting, but the minimum yield of dry ginger was obtained during the period between 210 and 225 days after planting. He also observed that the drying percentage continued to increase with the maturity of the crop and maximum drying percentage was obtained during the last stage (270 days after planting).

Sarin *et al.* (1977) obtained the maximum yield of green rhizomes in Costus from a six month old crop. However, Singh *et al.* (1979) reported that harvesting Costus at 21 months duration gave higher yield of fresh and dry rhizome per unit area. Joseph (1983) found that a crop of 9 months duration gave highest yield of green rhizome and diosgenin.

In *Kaempferia galanga*, Rajagopalan (1983) found that 6 month old crop gave maximum yield of fresh rhizome. But the dry rhizome yield was maximum in 7 month old crop. He also reported that fresh and dry rhizome yield per hectare and drying percentage were significantly influenced by the planting time, harvesting time and propagation method.

In all the cultivated species of Cymbopogons the yield of grass was the lowest in the first year and highest in the second and third year after planting (Gupta and Jain, 1978). Chinnamma (1985) found that in palmarosa herbage yield in the first year, second year and total yield for the two years were significantly influenced by different intervals of harvest and the minimum value was recorded by 65 days interval in both the years.

Kurien *et al.* (1984) found that clocimum harvested at full flowering stage gave herbage yields of 9.1, 40.5 and 15.9 kg ha^{-1} in the first, second and third harvest respectively. Pillai (1990) found that in clocimum the highest herbage yield was registered at the shortest interval of harvest (60 days) and a decreasing trend was observed with increase in the interval of harvest. The least herbage yield was recorded at the last harvesting interval (120 days) wherein the plants possessed only a few leaves and only the stalks persisted.

In *Catharanthus roseus* harvesting at 200 days after planting gave better yield of leaves, stem and roots (Pareek *et al.*, 1981). In *Glycyrrhiza glabra*, Shah *et al.* (1976) recorded 3843 kg ha^{-1} of dry roots in two harvests (at 15 and 27 months of planting) and 4545 kg ha^{-1} in a single harvest at 27 months of planting. In *Panax ginseng* the major development in root weight occurs between the 4th and 5th year of life. So the best time for harvesting the root is at the end of the summer of 5th year (Soldati and Tanaka, 1984).

2.2.3 Influence on secondary metabolites

Plant secondary products are compounds present in plants which are believed to have no role in the basic life process, but have secondary non-essential role. They are characterised by their extreme chemical diversity. The commercial importance of secondary products is that they are more active in man. The most important groups are those which are pharmacologically active and find use as drugs. The production of these secondary metabolites are confined to special cells or organs and synthesised at certain stages of maturity which are accumulated in the system or not, necessitating the harvest of the crop at optimum stage (Mizrahi, 1988).

Samuel *et al.* (1993) studied the influence of harvesting stage ranging from 8 to 11 months on the chemical components of *Holostemma annulare*. It is reported that the percentage of carbohydrate showed a decreasing trend as the age of the plant increased and the content was lowest (56 per cent) in 11 month old crop. The protein and alkaloid content was highest in 11 month old crop compared to 8 month and 9 month old crop.

In *Costus speciosus*, Sarin *et al.* (1977) obtained the highest diosgenin content with the onset of flowering in July. Gupta *et al.* (1981) also observed that the diosgenin content increased from the dormant stage of the plant to a maximum at the stage of flower bud appearance. The diosgenin content then declined till the plants became dormant again. In contrast, Joseph (1983) observed a steady decrease in the percentage of diosgenin with delay in harvest, the maximum being during the 6th month (November) and minimum during the 12th month (May). However, minimum yield of diosgenin per unit area was obtained at 9 months after planting showing a steep decline further.

Abrol and Kapoor (1962) reported that the diosgenin content in *Dioscorea deltoidea* tubers increased to a limited extent with the age of the plant. In the case of *Dioscorea caucasia* harvesting at the end of fourth growing season was found to produce highest yield of diosgenin per unit area (Kodash *et al.*, 1977). According to Hegde *et al.* (1981) harvesting of tubers earlier than February 15 reduced the tuber and diosgenin yield in *Dioscorea floribunda*. In *Dioscorea deltoidea* high tuber yield (200.8 q ha^{-1}) and the highest diosgenin content (2.08%) were obtained with 75 g tubers as planting material and harvesting done after 32 months.

Helmi *et al.* (1975) found that oil and oleoresin yield in ginger increased with the age of rhizome when harvested at 8, 9.5, 11 and 12 months age. Mathai (1976) studied the seasonal variation in the chemical constituents of spice cultivars and found that oleoresin, starch and essential oil content increased with increase in maturity.

Nybe (1978) observed that oleoresin and oil content was maximum at 165 days after planting and minimum at 270 days in four types of ginger, viz. Rio-de-Janeiro, Maran, Kuruppampady and Wynad Local. The maximum yield per hectare of oleoresin, oil and dry ginger was recorded at 270, 195, 225 and 225 days after planting respectively in four types. But the drying percentage continued to increase with the maturity of the crop. Based on these results Nybe (1978) recommended the above said periods as the optimum stage for harvest of the four types at which the maximum yield of oleoresin, oil and dry ginger could be obtained.

Mehta *et al.* (1980) reported that in turmeric curcumin content of leaves decreased and that of rhizome increased with increased maturity. Govind (1987) observed that for turmeric planted in April the optimum harvesting time was between middle and late December. The curcumin content was found to be the highest in late November and thereafter it slightly declined.

In *Rauvolfia*, yield of root, per cent of total alkaloid and reserpine cor ent increased with the age of the plant (Sobti *et al.*, 1959). Banerjee (1974) four that in Ipecac the yield of alkaloid increased with the age of the plant which was dı to an increase in total dry weight of roots and not due to an increase in percentage (alkaloid in the tissues. In *Panax ginseng* the ginsengosides increased with maturi (Soldati and Tanaka, 1984).

In *Datura metel*, hyoscine usually predominated in the preflowerii stage and the hyoscyamine increased in the later stages (Gupta *et al.*, 1972). Shah *al.* (1976) observed that the diosgenin content was lowest (0.34 per cent) in the leaves of *Balanites roxburghii* at the flowering stage in February. Then it gradually increased reaching the maximum content of 0.8 per cent in December at the fruit maturation stage. Gulati *et al.* (1978) found that in *Mentha piperita* first harvest of the crop taken during June and the second harvest on September gave the best yield and quality of oil. In Senna, the highest concentration of sennosides was reached on the 50th day after planting but maximum yield of leaflets as well as total sennosides were obtained when stripping was initiated on 70th day after planting (Shah *et al.*, 1979). In *Digitalis lanata* it was found that harvesting the crop from June to August gave the maximum glycoside concentration as well as foliage yield (Rajukannu *et al.*, 1981).

Krishevetskaya (1940) reported that in *Ocimum gratissimum* the time of mass blossoming is the optimum time of harvest as the volatile oil is principally contained in the leaves and blossom. Balyan *et al.* (1982) found that the oil content in clocimum was maximum at flower initiation and seed setting stages (0.66% and 0.65% respectively) and the oil content went down to 0.53 per cent at full flowering stage. Choudhury and Bordoloi (1984) reported that the highest oil content in *Ocimum gratissimum* was obtained for the second cutting among the four cuttings taken in the first and second year. The oil recovery in clocimum grown under Odakkali condition was found to be maximum for the first harvest taken in July (0.7%) and the content went down in the next harvest. Pillai (1990) found that in clocimum the highest oil recovery of 0.9 per cent on dry weight basis was recorded at 60 days interval and with further increase in growth the oil content decreased. The eugenol content was significantly influenced by different intervals of harvest. The crop harvested at 60 days possessed maximum eugenol (82.5%) in the essential oil. The eugenol content was found to decrease with increase in the harvest interval.

Kumar and Hegde (1983) found that in Java strain of patchouli longer cutting interval of 90 days recorded higher herbage and oil yield than shorter cutting interval of 60 days. The optimum time for the premonsoon harvest of the leaves of *Eucalyptus citriodora* was found to be middle of May and middle of November. The physiochemical properties of oil remain unaffected at different times of harvest (Nair *et al.*, 1983).

In Palmarosa, Lal (1935) recommended the period from the fourth week of October to the third week of November as the ideal maturity period best suited for harvest and distillation. According to Subba Rao *et al.* (1948) the best time of harvest in palmarosa to get maximum yield was 7 to 10 days after flowering. Ghosh and Chatterjee (1976) reported that the essential oil content of palmarosa was highest during the reproductive stage of growth and declined thereafter. Pareek *et al.* (1981) suggested harvesting of palmarosa at early seeding stage for the production of perfumary grade oil. They also observed higher percentage of free geraniol with superior odour value of oil at early seeding stage as compared to oil at maximum flower open stage.The Kerala Agricultural University has recommended that palmarosa grass should be harvested one week after flowering when the essential oil content reaches a maximum (KAU, 1982). Chinnamma (1985) reported that oil yield was influenced significantly by the different intervals of harvest in the first two years. The increase in oil yield was almost linear as interval of harvest increased and the crop harvested at 65 days interval recorded the highest oil yield. The geraniol content of the oil decreased as the interval of harvest increased from 40 to 55 days and thereafter it increased. The highest content of geraniol (64.60%) was recorded at 65 days interval. The acetate content of the oil increased upto 50th day interval (27%). The lowest value (14.9%) was recorded at 65 days interval. Maheshwari *et al.* (1992) reported that in Palmarosa harvesting the crop at early seeding (112-115 days after planting) gave 25 per cent and 51 per cent more herbage and oil yield over harvesting at the vegetative stage.

2.3 Chemical composition of *Holostemma annulare*

CSIR (1959) has published the economic importance of *Holostemma annulare* with the uses and chemical composition. Analysis of the root powder revealed moisture (10.08%), protein (4.07%), sugar (24.00%), starch (35.40%), fibre (12.20%) and ash (3.07%). The ash contained calcium and phosphate (2.50%). The medicinal properties are attributed to the sugars present in the roots. Ramiah *et al.* (1981) isolated and identified different sugars α-amyrin, lupeol and β-sitosterol and six aminoacids such as alanine, aspartic acid, glycine, valine, serine and threonine from the root extracts and considered these chemicals as components of *Holostemma annulare*.

Materials and Methods

MATERIALS AND METHODS

The present study was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Trichur during July 1992 to January 1994. The place is situated 10° 31'N latitude and 76° 13'E longitude, with an elevation of about 40 M above the Mean Sea Level. The soil of the experimental plot was deep laterite with clay loam texture and of moderate fertility. The area is characterised with heavy rains during June-September (South West Monsoon) and October-November (North East Monsoon) months followed by a summer season from March to May. The meteorological data for the experimental period as recorded by the Agrometeorological observatory at the College of Horticulture, Vellanikkara are presented (Fig.1 and Appendix I).

The details of the materials used and methodology followed for the investigation are described in this session.

3.1 Preparation of planting materials and nursery

The planting materials for the nursery screening comprised of

i) Seedlings

ii) Vine cuttings 2 noded

iii) Vine cuttings 3 noded

iv) Root stumps 5 cm

v) Root stumps 10 cm

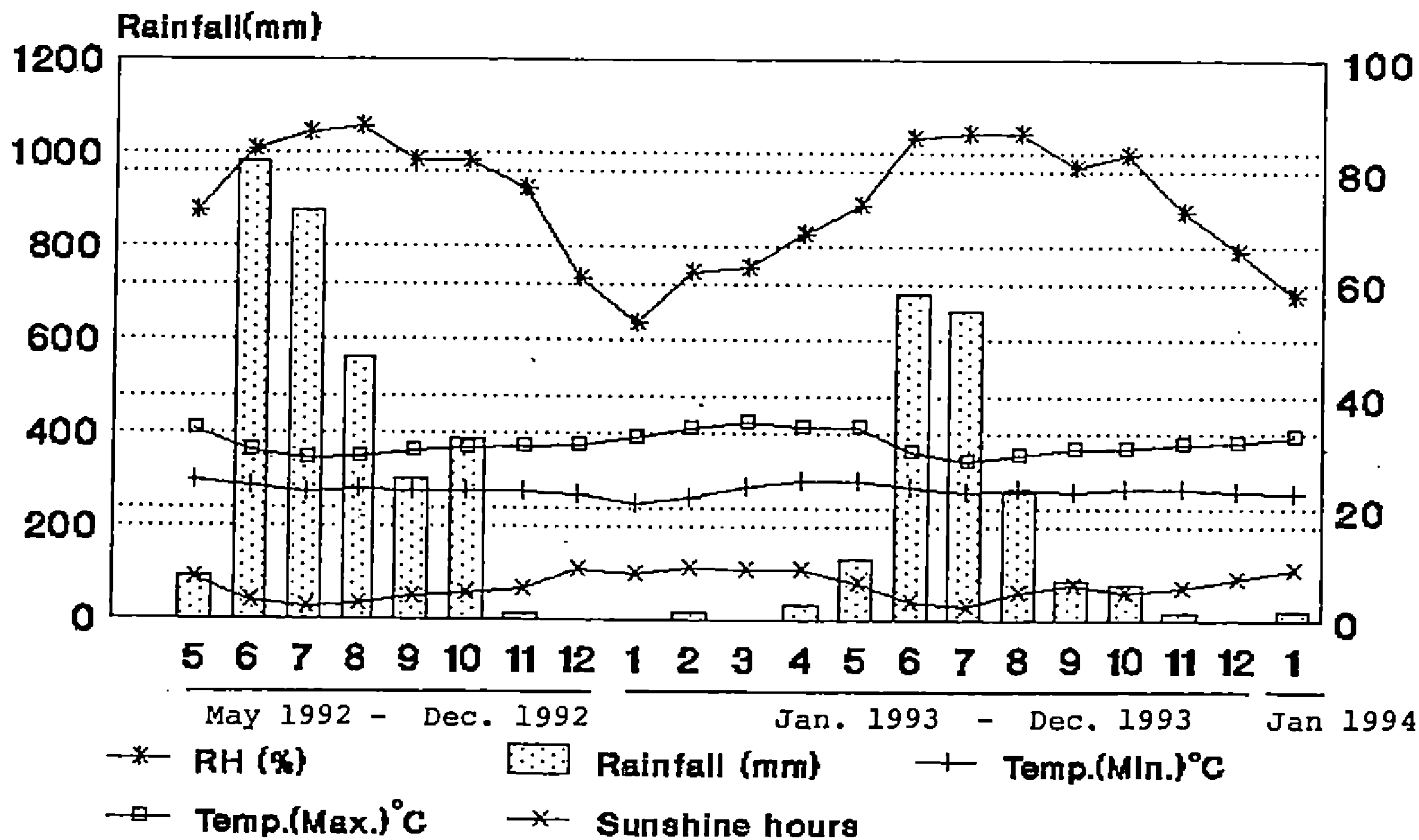
vi) Main root cuttings 5 cm

vii) Main root cuttings 10 cm

viii) Secondary root cuttings 5

ix) Secondary root cuttings 1(

Fig.1. Meteorological data (Monthly average) for the crop period (May 1992 - Jan. 1994)



The plants for selecting the vegetative propagules were collected in their juvenile growth phase from the forests of Vellanikkara (Trichur District) during April 1992. For preparing the vine cuttings the very soft apical portions and leaves were excised and the shoots were made into binodal and three nodal cuttings. Root stumps of 5 cm and 10 cm were prepared by retaining 2.5 cm and 5 cm root with a stem portion of 2.5 and 5 cm respectively above the collar region. Main root cuttings and secondary root cuttings of 5 cm and 10 cm were also prepared.

The vine cuttings and root stumps were planted in polythene bags filled with potting mixture (sand, soil, and farmyard manure in 1:1:1 ratio). They were kept under partial shade and irrigated frequently to avoid desiccation.

For raising root cuttings, raised beds of 2 m x 1 m size and 20 cm height was prepared. Cattle manure was applied at the rate of 10 kg per bed and incorporated into the soil. Sand was spread over the bed to a thickness of about 5 cm. Root cuttings were planted horizontally at a spacing of 10 cm x 10 cm. The bed was shaded and watered twice daily. The planting materials were maintained in the nursery for two months.

Seedlings of two months age were procured from Herbal garden of Kottakkal Aryavaidyasala at Kottappuram, Palghat District.

3.2 Preliminary nursery screening

Nine propagules raised as outlined in 3.1 were screened based on the initial establishment and vigour. Vigour of the propagule was measured in terms of

1. Days to sprout and sprouting percentage
2. Length and girth of shoot (cm)

3. Length and girth of root (cm)

4. Number of leaves

In the case of seedlings the days to sprout and sprouting percentage were noted by sowing the seeds during the next season.

Based on this preliminary screening five propagules were selected and advanced for the field trial.

3.3 Field experiment

3.3.1 Experimental details

The field trial was laid out in a 5 x 3 factorial Randomised Block Design with three replications.

Treatments included combinations of five propagules and three stages of harvest as outlined below.

Propagules - 5	Stage of harvest - 3
P_1 - Seedlings	S_1 - 9 months
P_2 - Vine cuttings 2 noded	S_2 - 12 months
P_3 - Vine cuttings 3 noded	S_3 - 18 months
P_4 - Root stumps 10 cm	
P_5 - Main root cuttings 5 cm	

3.3.2 Land preparation and planting

The area was cleared off weeds and ploughed well to bring the soil to a fine tilth and then levelled. Raised beds of 1 m x 1 m size and with a height of 25 cm were formed with an interchannel of 25 cm between the beds. Farmyard manure was applied at the rate of 10 t ha^{-1} and incorporated into the soil prior to planting. The propagules were transplanted at a spacing of 50 cm x 25 cm during July.

3.3.3 Crop management

The fertilizers were applied at the rate of 150:60:60 N, P_2O_5 and K_2O Kg ha^{-1}. Full P_2O_5, ½N and ½K_2O was given as basal dose and the remaining was top dressed in two equal splits at 2 months and 4 months after planting. Staking was also given. The plants were mulched and irrigation was given on alternate days during summer months. Weeding and earthing up was carried out 6 times during the cropping period. Occurrence of leaf spot disease was controlled by spraying 0.3 per cent captafol.

3.3.4 Observations

3.3.4.1 Percentage of field establishment

The number of plants established in the field was counted separately for each treatment and their percentage was worked out.

3.3.4.2 Pre-harvest observations

Four plants in the centre of the plot were selected from each treatment for recording observations on various biometric characters and the observations were taken at three months interval.

3.3.4.2.1 Length of vines

The length of the plant was measured from the ground level to the tip of the top most leaf and expressed in centimetre.

3.3.4.2.2 Number of branches

The total number of branches in each plant was counted.

3.3.4.2.3 Diameter of the main vine

The diameter of the main vine was recorded from each observational plant at 2 cm from the ground level using a non elastic twine, measured in scale and recorded in centimetre.

3.3.4.2.4 Internodal length

Internodal length was obtained by dividing the vine length by number of nodes present in the observational plant and expressed in centimetre.

3.3.4.2.5 Leaf area

To estimate the leaf area 100 leaves of different maturity were collected randomly covering all the treatments. The length and breadth of each leaf was measured and the area was found out graphically. A regression equation was derived from the data based on the leaf length and breadth measurements. The equation is $Y = -0.16 + 0.995\ LB$, where Y is the leaf area, L and B are the length and breadth of the individual leaf.

The total leaf area per plant was then calculated by multiplying the average leaf area with number of leaves per plant.

3.3.4.2.6 Observations on flowering

For taking observations five seedling plants were tagged, the number of inflorescence per plant and the number of flowers per inflorescence were counted and the average was worked out. The bud primordia of 10 flowers were tagged after visible emergence and the number of days taken for flower opening was counted and recorded. The number of days for which the flowers remain opened was also recorded for 10 flowers.

From five observational plants, the fruit setting percentage out of total number of flowers per plant was recorded. Period for fruit maturation, number of seeds per fruit and 100 seed weight (mean of five fruits) were also recorded.

3.3.4.3 Post-harvest observations

Harvesting was carried out at three stages 9 months, 12 months and 18 months after transplanting. Plants were dug out separately taking care to collect the entire roots and cleaned with water to remove the adhering soil particles. Four plants in the centre of the bed was selected and the following observations were taken.

3.3.4.3.1 Length of root

The length of root was measured separately for each observational plant and expressed in centimetre.

3.3.4.3.2 Diameter of the main root

Diameter of the main root was measured using a nonelastic twine, measured in scale and recorded in centimetre.

3.3.4.3.3 Volume of roots

The entire roots from each observational plant was dipped in water taken in a measuring cylinder. The original level and the final level of water was noted and the rise in water level was expressed in milli litre.

3.3.4.3.4 Fresh weight of aerial parts (Stem and leaf)

Fresh weight of stem and leaves were recorded separately for each observational plant and expressed in gram.

3.3.4.3.5 Fresh weight of roots

After cleaning the roots fresh weight was recorded separately for each observational plant and expressed in gram. Fresh root yield ha^{-1} was arrived at based on the gross area of the plots and taking into consideration the space for inter-channel and expressed in tonnes.

3.3.4.3.6 Dry weight of aerial parts (Stem and leaf)

The stem and leaf samples, after recording the fresh weight were transferred to an oven maintained at 70°C and dried to a constant weight on test weighing and expressed in gram.

3.3.4.3.7 Dry weight of roots

After taking the fresh weight, the root samples were dried in an oven at 70°C to a constant weight on test weighing and expressed in gram. The total yield of dry roots ha^{-1} was worked out based on the gross area of the plots and taking into consideration the space for interchannel and expressed in tonnes.

3.3.4.3.8 Driage percentage

Based on the fresh weight and dry weight of roots the driage percentage was worked out.

3.3.4.3.9 Harvest index

Harvest index was worked out by using the formula

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}}$$

3.4 Chemical Analysis

3.4.1 Estimation of free amino acids

The separation of free amino acid was done by ion exchange chromatography (Malik *et al.*, 1980).

3.4.1.1 Preparation of ion exchange chromatographic column

For this a cation exchange resin Dowex 50 (sulphonated polystyrene cation exchange resin) of 200-400 mesh size was used. This was made in the acid form by suspending the resin in 4 N HCl for 15 minutes. The suspension was then filtered through a buchner funnel and was repeatedly washed with distilled water till the filtrate became neutral. Ten grams of the resin was suspended in water, stirred and allowed to settle. The supernatant was decanted. To this citrate buffer of pH 2.8 was added to cover the resin, stirred well and allowed to settle for one hour. This suspension was used for filling the glass column (size of 30 cm x 5 cm). Citrate buffer (pH 2.8), approximately two column volume was run through the column and the flow rate was adjusted to 1 ml per 3 minutes by using the peristaltic pump and LKB automatic fraction collector.

3.4.1.2 Preparation of root sample, separation and identification of free amino acids

One gram of fresh root was taken after cleaning. The extract was prepared by grinding the roots with 80 per cent ethanol using a mortar and pestle. The extract was centrifuged at 2000 rpm and clear supernatant was taken and 1 ml sample was allowed to percolate through the column. After loading the column with the sample, pH gradient was applied by gradient applicator and the amino acids bound to the resin was eluted. Since the isoelectric point (pI value) of each amino acid is different they were eluted at different pH values. Thus the separation of amino acid was achieved. Fractions were collected as 1 ml aliquotes in serially numbered tubes placed in an LKB automatic fraction collector. The presence of amino acid was tested with ninhydrin reagent.

3.4.1.3 Ninhydrin test

Ninhydrin reagent was prepared by dissolving 2 g ninhydrin in 25 ml methyl cellosolve. To this 25 ml of 0.2 M acetate buffer (pH 5.5) was added. The sample (0.1 ml) was tested with 1 ml ninhydrin reagent. Fractions having amino acid which developed the pink colour was noted. The amino acid corresponding to particular pI value was identified by comparing with the standard table (Whitaker, 1972).

3.4.1.4 Estimation of total amino acid

The sample was prepared as in 3.4.1.2. One ml sample was applied to the column and allowed to adsorb on the resin. Elution was proceeded by applying citrate buffer of high pH. Fractions were collected till it showed no positive test for amino acid (tested with ninhydrin reagent as outlined in 3.4.1.3). The collected fractions were pooled and 1 ml was taken in a test tube, diluted to 4 ml and was used for estimation. To this 1 ml ninhydrin reagent was added and mixed well. A blank was also prepared with distilled water. The tubes were kept in boiling water bath for 15 minutes, cooled and 1 ml of 50 per cent ethanol was added. The pink colour developed was measured at 550 nm in a spectrophotometer. The percentage of total amino acid was calculated from the standard curve.

3.4.1.5 Preparation of standard curve

The stock solution was prepared by dissolving a mixture of five amino acids at 10 mg each in 50 ml distilled water. 10 ml of this stock solution was diluted to 100 ml as working standard. Pippetted 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml into test tubes and made up the volume to 4 ml with distilled water. To each tube 1 ml ninhydrin reagent was added and kept in boiling water bath for 15 minutes. The tubes were cooled and 1 ml of 50 per cent ethanol was added. The pink colour developed was measured at 550 nm in a spectrophotometer. A standard curve was prepared based on the absorbance and concentration. From the standard curve the percentage of amino acid in the sample was worked out.

3.4.2 Estimation of soluble carbohydrate

The soluble carbohydrate in the root was estimated by phenol sulphuric acid method (Dubois, 1956; Sadasivam and Manikam, 1992).

One gram of dried root powder was extracted with water and the extract was centrifuged at 2200 rpm for 15 minutes. One ml supernatant was taken and 0.1 ml lead acetate was added to remove the polyphenolic material. Excess lead acetate was precipitated by adding 2 ml of 5 per cent potassium oxalate. This solution was centrifuged again and the clear supernatant was taken for estimation of soluble carbohydrate.

3.4.3 Preparation of glucose standard and working out of soluble carbohydrate (%)

Stock solution of glucose was prepared by dissolving 100 mg glucose in

100 ml distilled water. Ten ml of the stock solution was made upto 100 ml as working standard.

Pipetted out 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 ml working standards into a series of test tubes. Made up the volume to 1 ml with distilled water. A blank was also prepared with 1 ml distilled water. To each tube 1 ml phenol (5%) and 5 ml sulphuric acid (96%) was added and shaken well. After 20 minutes the colour was read at 490 nm in a spectrophotometer. Based on the absorbance and concentration a standard curve was prepared.

Pipetted out 0.1 ml sample solution and reagents were added and the colour read at 490 nm similar to that of glucose standard. From the standard curve the soluble carbohydrate was calculated and expressed as percentage.

3.5 Correlation studies

Simple correlation of nine morphological characters with dry root yield per plant at 18 month old stage was worked out using the method outlined by Snedecor and Cochran (1967).

3.6 Economics of cultivation

The economics of cultivation and cost benefit analysis for the best treatment combination was worked out and expressed on per hectare basis as detailed in Table 10.

3.7 Statistical Analysis

The data was statistically analysed as per the procedure outlined by Panse and Sukhatme (1978). Level of significance used in F and t values was $P = 0.05$ and $P = 0.01$.

Results

RESULTS

The results of the investigation on standardisation of propagation and stage of harvest in adakodien (*Holostemma annulare* K. Schum.) are presented in this chapter.

4.1 Nursery screening

A preliminary nursery screening was carried out using nine planting materials to select the best propagules for field trial. The selection was based on the following parameters.

4.1.1 Days to sprout and sprouting percentage

The influence of planting material on sprouting was noted (Table 1) and no significant difference was observed with respect to days to sprout. It was observed that vine cuttings sprouted earlier within 3 days after planting and root stumps within 4 days after planting. The main root cuttings (5 cm and 10 cm) started sprouting 12 days after planting. The secondary root cuttings (5 cm and 10 cm) were sprouted 14 days after planting. The maximum sprouting percentage was observed within one week after sprouting. In the case of seeds they started germination 4 days after sowing and 82 per cent germination was obtained within one week.

The sprouting percentage was also recorded. The three noded vine cuttings recorded maximum sprouting percentage of 87.69 closely followed by two noded vine cuttings (86.92%). Root stumps 5 cm and 10 cm recorded 83.46 and 84.23 per cent of sprouting respectively whereas seeds recorded 82.00 per cent germination. The root cuttings recorded lowest sprouting and secondary root cuttings

5 cm showed the least sprouting of 59.23 per cent. Large sized vegetative propagules recorded higher sprouting percentage when compared to small sized ones but the difference was significant only in the case of secondary root cuttings 5 cm and 10 cm.

4.1.2 Length and girth of shoot

Significant difference was observed between planting materials on the length of shoot (Table 1). Seedlings recorded the maximum shoot length of 11.44 cm which was significantly superior to other treatments. Minimum shoot length of 5.82 cm was recorded by secondary root cuttings 5 cm. The treatments from T_2 to T_8 were found to be on par.

4.1.3 Number of leaves

The difference between planting materials for leaf production was found to be significant (Table 1). Seedlings had maximum number of leaves (9.2) and minimum leaf number (5.0) was producted by secondary root cuttings 5 cm.

4.1.4 Length and girth of root

The effect of treatments on root length was found to be significant (Table 1). Maximum root length of 8.68 cm was recorded by seedlings which was significantly superior to other treatments. Minimum root length of 5.6 cm was recorded by secondary root cuttings 5 cm. The root girth was not significantly influenced by the type of planting materials.

Based on sprouting and vigour of sprouts five planting materials such as seedlings, 2 noded vine cuttings, 3 noded vine cuttings, root stumps 10 cm and main root cuttings 5 cm were selected for the field trial (Plate 4).

Table 1. Effect of planting materials at nursery stage on biometric characters

Treatments	Days to sprout	Sprouting (%)	Shoot length (cm)	Shoot girth (cm)	Number of leaves	Root length (cm)	Root girth (cm)
T_1 Seedlings	4	82.00	11.44	1.00	9.2	8.68	0.54
T_2 Vine cuttings 2 noded	3	86.92	7.52	0.70	7.2	7.28	0.43
T_3 Vine cuttings 3 noded	3	87.69	7.54	0.72	7.4	7.30	0.42
T_4 Root stumps 5 cm	4	83.46	6.86	0.70	6.4	6.36	0.36
T_5 Root stumps 10 cm	4	84.23	8.20	0.80	7.8	7.38	0.42
T_6 Main root cuttings 5 cm	12	72.69	7.14	0.67	6.6	6.40	0.40
T_7 Main root cuttings 10 cm	12	74.62	8.00	0.70	6.8	6.80	0.42
T_8 Secondary root cuttings 5 cm	14	59.23	5.82	0.51	5.0	5.60	0.32
T_9 Secondary root cuttings 10 cm	14	62.31	6.00	0.55	5.2	5.80	0.34
SEm±		NS	1.2135	NS	1.3481	1.3440	NS
C.D.(0.05)			2.234		1.198	2.726	

Plate 4. Planting materials selected for field trial

P_1 - Seedlings

P_2 - Vine cuttings - 2 nodes

P_3 - Vine cuttings - 3 nodes

P_4 - Root stumps - 10 cm

P_5 - Main root cuttings - 5 cm

4.2 Field trial

The field trial was laid out in a 5 x 3 factorial R.B.D with 3 replications. The treatments included 15 combinations of the above five planting materials and three stages of harvest (9, 12 and 18 months after planting). A general view of the experimental field is shown in Plate 5.

4.2.1 Percentage of establishment in the field

Among the planting materials seedlings recorded the highest field establishment (91.67%) followed by root stumps (87.50%) and root cuttings (84.70%). Whereas the vine cuttings with two and three nodes had the lowest field establishment (83.30%).

4.2.2 Biometric characters

4.2.2.1 Length of vines

The data relating to the length of vine as influenced by the treatments are presented in Table 2. Planting materials did not significantly influence the vine length but the age of the plant showed a significant influence. A progressive increase in vine length was observed as the age of the plant increased. Maximum vine length (482.63 cm) was recorded by 18 month old plants and minimum (142.95 cm) by 3 month old plants. Among the planting materials seedlings recorded the maximum vine length (362.74 cm).

However the interaction effect was found to be insignificant (Table 2a).

4.2.2.2 Number of branches

Only the age of the plant showed significant influence on this character

Plate 5. A general view of the experimental plot

(Table 2). Eighteen month old plants produced the highest number of branches (10.95) and lowest branch number (1.27) was recorded by 3 months old plants. However, this character was not altered significantly by the type of planting materials and seedlings recorded maximum number of branches (6.73). The interaction effect was also insignificant (Table 2a).

4.2.2.3 Number of leaves

Leaf number was found to be significantly influenced by the treatments (Table 2). Among the planting materials root stumps produced maximum number of leaves (42.87) which was on par with P_1, P_2 and P_3. The lowest number of leaves (38.25) was recorded by main root cuttings. The number of leaves showed an increasing trend upto 12 months after planting (52.51) and thereafter reduced to 40.87 at 18 months after planting.

The interaction between planting materials and age of the plant was found to be insignificant (Table 2a).

4.2.2.4 Total leaf area

The data presented in Table 2 showed that planting material and stage of harvest significantly influenced the total leaf area per plant. Maximum leaf area of 1339.91 cm^2 was recorded by root stumps and minimum by root cuttings (1190.01 cm^2). The treatments P_1, P_2, P_3 were found to be on par with P_4.

The total leaf area per plant also showed an increasing trend upto 12 months after planting (2010.2 cm^2) and thereafter decreased to 1353.44 cm^2 in 18 months old plants.

The interaction between the planting materials and age of the plant was

Table 2. Effect of planting materials and age of plant on the length of vine, number of branches, number of leaves and total leaf area

Treatments	Length of vine (cm)	Number of branches	Number of leaves	Total leaf area/plant (cm^2)
PLANTING MATERIALS				
P_1 Seedlings	362.72	6.73	42.43	1263.01
P_2 Vine cuttings 2 noded	344.64	6.44	41.48	1325.11
P_3 Vine cuttings 3 noded	345.19	6.43	41.47	1299.05
P_4 Root stumps 10 cm	352.32	6.52	42.87	1339.91
P_5 Main root cuttings 5 cm	336.56	5.65	38.25	1190.01
SEm±			0.9479	32.5648
CD (0.05)	NS	NS	2.684	92.191
AGE OF PLANT				
S_1 3 MAP	142.95	1.27	32.75	759.15
S_2 6 MAP	248.99	2.84	39.88	963.32
S_3 9 MAP	330.02	4.75	33.74	819.64
S_4 12 MAP	425.31	8.53	52.51	2010.20
S_5 15 MAP	459.85	9.79	48.06	1786.77
S_6 18 MAP	482.63	10.95	40.87	1353.44
SEm±	9.2204	0.3292	1.0384	35.6730
CD (0.05)	26.102	0.932	0.984	100.99

MAP - Months After Planting

Table 2(a). The interaction effect between planting materials and age of the plant

Treatment combinations				
P_1S_1	52.43	1.23	33.07	743.31
P_2S_1	40.10	1.30	33.23	841.51
P_3S_1	40.90	1.33	33.43	751.58
P_4S_1	43.60	1.30	34.33	783.83
P_5S_1	37.70	1.17	29.67	675.51
P_1S_2	60.43	3.03	38.67	921.81
P_2S_2	46.50	2.93	40.90	989.78
P_3S_2	47.10	2.80	41.23	983.00
P_4S_2	94.00	3.00	40.93	1010.01
P_5S_2	41.50	2.43	31.67	904.00
P_1S_3	38.67	4.90	34.37	804.06
P_2S_3	28.67	4.00	34.17	849.73
P_3S_3	30.43	5.13	35.27	840.76
P_4S_3	32.83	5.23	33.57	824.40
P_5S_3	19.50	4.47	31.33	779.26
P_1S_4	42.30	9.17	53.00	2007.13
P_2S_4	419.00	8.77	53.17	2054.36
P_3S_4	422.17	8.40	53.00	2048.45
P_4S_4	432.97	9.03	54.07	2089.14
P_5S_4	409.73	7.30	49.33	1891.93
P_1S_5	478.93	10.60	49.50	1751.31
P_2S_5	453.67	10.17	48.73	1845.32
P_3S_5	449.37	9.93	48.57	1839.22
P_4S_5	465.30	9.77	49.33	1868.25
P_5S_5	451.93	8.50	44.17	1629.75
P_1S_6	503.67	11.47	46.00	1350.47
P_2S_6	479.50	11.50	38.67	1369.96
P_3S_6	481.17	11.00	37.33	1331.31
P_4S_6	489.80	10.77	45.00	1455.83
P_5S_6	459.00	10.03	37.33	1259.63
SEm ±				
CD (0.05)	NS	NS	NS	NS

found to be insignificant (Table 2a).

4.2.2.5 Diameter of the main vine

Diameter of the vine was found to be significantly influenced by the planting materials (Table 3). Seedlings which recorded the maximum diameter of 1.84 cm was significantly superior to all other propagules. Minimum diameter (1.57 cm) was recorded by main root cuttings. Age of the plants also imparted a significant influence on diameter of the main vine. The diameter of the vine showed a progressive increase with the advance in age of the plant. Maximum diameter (2.25 cm) was recorded at 18 months after planting and lowest diameter (1.04 cm) at 3 months after planting.

The interaction between the treatments was found to be insignificant (Table 3a).

4.2.2.6 Internodal length

Planting materials and age of the plant exhibited significant influence on the internodal length of vine (Table 3). The internodal length ranged from 7.87 cm to 9.26 cm due to the effect of planting materials. Maximum internodal length (9.26 cm) was recorded by seedlings. The internodal length increased significantly with increase in crop duration. Highest internodal length (13.53 cm) was recorded by 18 month old crop and lowest (4.55 cm) was recorded by 3 month old plants.

The interaction between treatments was found to be insignificant (Table 3a).

4.2.2.7 Flowering characters

Flowering characteristics were studied in seedling plants and the results

Table 3. Effect of planting materials and age of plant on diameter of vine and internodal length

Treatment	Diameter of the main vine (cm)	Internodal length (cm)
PLATNING MATERIALS		
P_1 Seedlings	1.84 (0.25)	9.26 (0.93)
P_2 Vine cuttings 2 noded	1.67 (0.21)	8.69 (0.90)
P_3 Vine cuttings 3 noded	1.66 (0.20)	8.98 (0.91)
P_4 Root stumps 10 cm	1.66 (0.22)	9.17 (0.93)
P_5 Main root cuttings 5 cm	1.57 (0.18)	7.87 (0.86)
SEm±	0.0077	0.0097
CD (0.05)	0.022	0.025
AGE OF PLANT		
S_1 3 MAP	1.04 (0.01)	4.55 (0.65)
S_2 6 MAP	1.24 (0.11)	5.46 (0.74)
S_3 9 MAP	1.56 (0.19)	6.77 (0.83)
S_4 12 MAP	1.93 (0.28)	10.35 (1.01)
S_5 15 MAP	2.05 (0.31)	12.10 (1.08)
S_6 18 MAP	2.25 (0.35)	13.53 (1.13)
SEm±	0.0084	0.0028
CD (0.05)	0.023	0.028

MAP - Months After Planting
Values in parenthesis indicate transformed values

Table 3a. The interaction effect of planting materials and age of the plant on diameter of the vine and internodal length

Treatment combinations		
P_1S_1	1.17 (0.07)	4.90 (0.69)
P_2S_1	1.03 (0.01)	4.07 (0.61)
P_3S_1	0.93 (0.03)	4.80 (0.67)
P_4S_1	1.03 (0.01)	5.03 (0.70)
P_5S_1	1.00 (0.01)	3.93 (0.60)
P_1S_2	1.50 (0.18)	5.73 (0.76)
P_2S_2	1.27 (0.10)	5.33 (0.73)
P_3S_2	1.30 (0.11)	5.37 (0.73)
P_4S_2	1.03 (0.14)	5.73 (0.76)
P_5S_2	1.10 (0.04)	5.13 (0.78)
P_1S_3	1.73 (0.24)	7.03 (0.84)
P_2S_3	1.53 (0.19)	6.70 (0.82)
P_3S_3	1.57 (0.19)	6.97 (0.84)
P_4S_3	1.53 (0.18)	7.07 (0.84)
P_5S_3	1.43 (0.16)	6.07 (0.78)
P_1S_4	2.06 (0.31)	10.90 (1.03)
P_2S_4	1.90 (0.28)	10.03 (1.00)
P_3S_4	1.90 (0.28)	10.63 (1.02)
P_4S_4	2.00 (0.29)	10.93 (1.04)
P_5S_4	1.80 (0.25)	9.27 (0.97)
P_1S_5	2.17 (0.34)	12.90 (1.11)
P_2S_5	2.07 (0.31)	12.33 (1.09)
P_3S_5	2.00 (0.30)	12.30 (1.09)
P_4S_5	2.08 (0.32)	12.40 (1.09)
P_5S_5	1.93 (0.29)	10.57 (1.02)
P_1S_6	2.43 (0.39)	14.07 (1.15)
P_2S_6	2.23 (0.34)	13.67 (1.13)
P_3S_6	2.23 (0.35)	13.80 (1.14)
P_4S_6	2.20 (0.34)	13.83 (1.14)
P_5S_6	2.17 (0.33)	12.27 (1.09)
SEm+		
CD (0.05)	NS	NS

Values in parenthesis indicate log transformed values are presented in Table 4. In adapathiyan the flowering season is July-October. The number of inflorescence per plant ranged from 14-20 with a mean count of 17.2. Each inflorescence produced 7.3 flowers on an average. From visible emergence of the bud to flower opening 25 days were needed. The fruit set was found to be very low (12.93%). The fruits matured in about 4-5 months. The number of seeds per fruit varied from 386-502. The mean 100 seed weight was found to be 0.38 g with a range of 0.3 to 0.5 g.

4.2.2.8 Fresh weight of stem

The data presented in Table 5 revealed that the fresh weight of stem was significantly influenced only by the harvesting intervals. The fresh weight of stem registered an increase from 27.43 g at S_1 to 71.23 g at S_2 and then decreased to 59.45 g at S_3. Among the planting materials, root stumps recorded maximum fresh weight (56.36 g) though it was statistically not significant.

The interaction between planting materials and stage of harvest was found to be insignificant (Table 5a).

4.2.2.9 Dry weight of stem

The effect of treatments on dry weight of stem was found to be significant (Table 5). Among the planting materials, the maximum dry weight of 17.82 g was recorded by root stumps (P_4) which was significantly superior to all other treatments. The treatments P_1, P_2 and P_3 were found to be on par. The main root cuttings recordced the minimum dry weight (13.31 g).

Dry weight of stem was significantly influenced by stage of harvest as shown in Table 5. The trend was similar to that of fresh weight which showed an increase at S_2 (20.28 g) and then declined at S_3 (16.95 g). However, the treatments

Table 4. Observations on flowering and fruit set

Characters	Range	Mean
1. Number of inflorescence per plant	14-20	17.2
2. Number of flowers per infloresence	5-10	7.3
3. Days from emergence to flower opening	24-26	25.0
4. Number of days the flowers remain opened	4-5	4.0
5. Fruit set	11.8-14	12.93
6. Period of fruit maturity (months)	4-5	4.5
7. Number of seeds per fruit	386-502	414.5
8. 100 seed weight (g)	0.3-0.5	0.38

were found to be not interacting (Table 5 a).

4.2.2.10 Fresh weight of leaf

The planting materials and stage of harvest significantly influenced the fresh weight of leaf (Table 5). Two noded vine cuttings recorded the maximum leaf weight of 26.29 g though it was on par with P_1, P_3 and P_4. The minimum fresh weight was recorded by main root cuttings (22.66 g). The fresh weight of leaf was influenced by the stage of harvest (Table 5). The fresh weight of leaf showed an increasing trend up to 12 months (31.67 g) which was then decreased to 28.13 g by 18 months after planting.

The treatment combinations were found to be not interacting (Table 5 a).

4.2.2.11 Dry weight of leaf

Data presented in Table 5 indicated that dry weight of leaf was significantly influenced by planting materials and stage of harvest. Among the planting materials maximum dry weight was recorded by two noded vine cuttings (6.98 g) and minimum by main root cuttings (5.88 g). The treatments P_2, P_3 and P_4 as well as P_1, P_3 and P_4 were found to be on par. The dry weight of leaf showed an increasing trend upto 12 months (8.51 g) and thereafter decreased to 7.39 g at S_3 (18 months after planting).

The interaction between planting materials and stage of harvest was not significant (Table 5 a).

4.2.3 Yield characters

4.2.3.1 Length of root

Data relating to the length of root as influenced by the treatments are

Table 5. Effect of planting materials and stage of harvest on fresh and dry weight of stem and leaf

Treatments	Fresh weight of stem (g)	Dry weight of stem (g)	Fresh weight of leaf (g)	Dry weight of leaf (g)
PLANTING MATERIALS				
P_1 Seedlings	52.92	15.36	24.79	6.47
P_2 Vine cuttings 2 noded	52.91	15.07	26.29	6.98
P_3 Vine cuttings 3 noded	50.40	14.94	24.86	6.63
P_4 Root stumps 10 cm	56.36	17.82	25.09	6.67
P_5 Main root cuttings 5 cm	50.31	13.31	22.66	5.88
SEm±		0.4786	0.6039	0.1290
CD (0.05)	NS	0.136	1.749	0.374
STAGE OF HARVEST				
S_1 9 MAP	27.43	8.67	14.41	3.67
S_2 12 MAP	71.23	20.28	31.67	8.51
S_3 18 MAP	59.45	16.95	28.13	7.39
SEm±	1.4140	0.3707	0.4678	0.0999
CD (0.05)	4.096	1.074	1.355	0.289

MAP - Months After Planting

Table 5a. The interaction effect between planting materials and stage of harvest

Treatment combinations				
P_2S_1	28.63	8.87	15.37	3.50
P_2S_1	28.13	8.50	15.37	4.03
P_3S_1	26.33	8.53	13.23	3.63
P_4S_1	29.03	10.53	14.67	3.70
P_5S_1	25.00	6.93	13.40	3.47
P_1S_2	71.97	20.50	32.27	8.40
P_2S_2	71.20	19.80	33.50	9.03
P_3S_2	69.03	19.73	32.00	8.70
P_4S_2	77.97	24.07	32.07	8.67
P_5S_2	66.77	17.30	28.50	7.77
P_1S_3	61.17	16.70	26.73	7.50
P_2S_3	58.20	16.90	30.00	7.87
P_3S_3	55.83	16.57	29.33	7.57
P_4S_3	62.87	18.87	28.53	7.63
P_5S_3	59.17	15.70	26.07	6.40
SEm±				
CD (0.05)	NS	NS	NS	NS

presented in Table 6. The root length was significantly influenced by the type of planting materials and stage of harvest. Among the planting materials maximum root length (71.18 cm) was recorded by seedlings which was significantly superior to other treatments. The minimum root length (64.86 cm) was recorded by main root cuttings 5 cm. The length of the root was found to increase with the advance in age of the plant (Plate 6). The roots harvested after 18 months recorded the maximum root length (83.27 cm), followed by 12 months old plants (66.37 cm) and 9 month old plants (56.88 cm) which was the lowest.

The interaction between the treatments was found to be insignificant (Table 6).

4.2.3.2 Diameter of the main root

The different intervals of harvest exhibited significant influence on root diameter (Table 6). Diameter of the root was found to increase as the age of the plant increased. Harvesting after 18 months (S_3) recorded the maximum diameter of 3.70 cm, followed by S_2 (2.14 cm) and S_1 (1.59 cm). Planting materials did not exert any significant influence on this character and the interaction effect was also insignificant.

4.2.3.3 Volume of roots

The planting materials imparted a significant influence on root volume (Table 6) and it ranged from 45.33 ml for main root cuttings to 62.38 ml in the case of seedlings. The root volume showed significant increase from 29.51 ml at 9 months after planting to 74.77 ml at 18 months after planting.

Table 6. Effect of planting materials and stage and harvest on root characters

Treatments	Root characters		
	Length of root (cm)	Diameter of root (cm)	Volume of root (cm)
PLANTING MATERIALS			
P_1 Seedlings	71.18	2.48	62.38
P_2 Vine cuttings 2 noded	69.33	2.62	48.61
P_3 Vine cuttings 3 noded	67.93	2.30	48.49
P_4 Root stumps 10 cm	70.90	2.52	51.81
P_5 Main root cuttings 5 cm	64.86	2.47	45.33
SEm $\pm$	1.2382		0.6243
CD (0.05)	3.586	NS	1.808
STAGE OF HARVEST			
S_1 9 MAP	56.88	1.59	29.51
S_2 12 MAP	66.37	2.14	49.70
S_3 18 MAP	83.27	3.70	74.77
SEm $\pm$	0.9591	0.0837	0.4836
CD (0.05)	2.778	0.242	1.401
INTERACTION			
P_1S_1	57.97	1.60	37.63
P_2S_1	57.50	1.67	26.77
P_3S_1	55.60	1.53	27.40
P_4S_1	58.33	1.70	29.57
P_5S_1	55.00	1.47	26.17
P_1S_2	68.83	2.17	57.50
P_2S_2	66.17	2.27	48.40
P_3S_2	65.30	1.97	48.23
P_4S_2	69.00	2.17	50.03
P_5S_2	62.53	2.13	44.33
P_1S_3	86.73	3.67	92.00
P_2S_3	84.33	3.93	70.67
P_3S_3	82.90	3.40	69.83
P_4S_3	85.37	3.70	75.83
P_5S_3	77.03	3.80	65.50
SEM $\pm$			1.0814
CD (0.05)	NS	NS	3.132

MAP - Months After Planting

Plate 6. Roots of *Holostemma annulare* at 18 months after planting

A

Left: Vine cuttings- 2 nodes

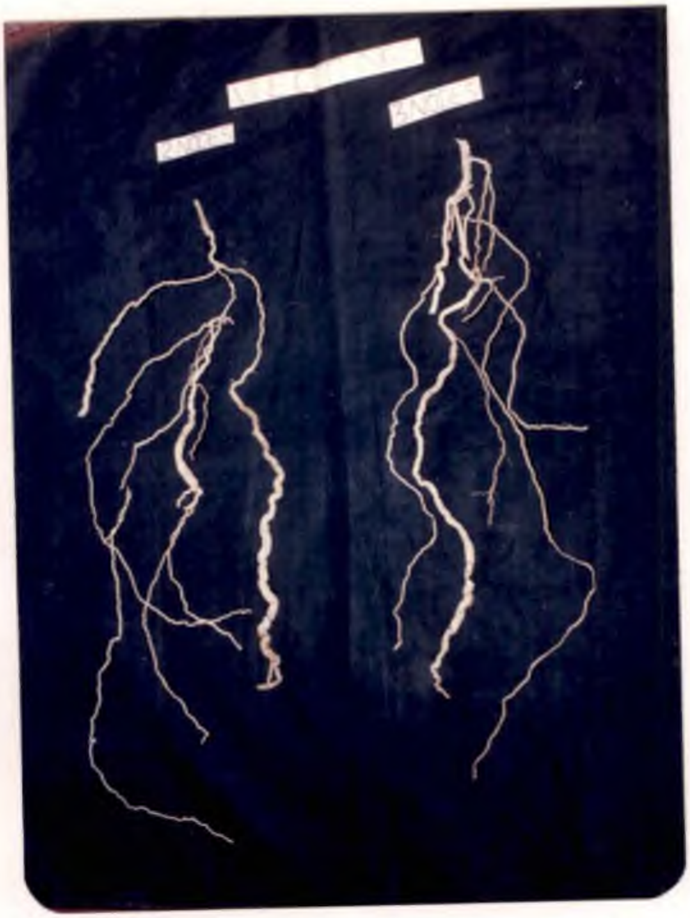
Right: Vine cuttings - 3 nodes

B

Left : Root stumps - 10 cm

Middle: Seedlings

Right : Main root cuttings - 5 cm



B

Significant interaction effect was observed for the treatment combinations (Table 6). The volume of root was maximum (92.00 ml) for seedlings harvested at 18 months after planting.

4.2.3.4 Fresh root yield per plant

The influence of planting materials and stage of harvest on fresh weight of root was found to be significant (Table 7). Seedlings recorded the highest fresh weight of 68.2 g per plant which was significantly superior to other treatments. Two and three noded vine cuttings were found to be on par with regard to this character. The fresh weight of root was found to increase with the age of the plant. Harvesting the plants at 18 months after planting resulted in maximum fresh weight (80.80 g) and lowest (32.83 g) in the case of harvesting at 9 months after planting.

The interaction between planting materials and stage of harvest was found to be significant (Table 7a). The most significant interaction was observed in the treatment combination P_1S_3.

4.2.3.5 Dry root yield per plant

The effect of treatments on dry weight of roots was significant (Table 7), the trend was similar to those of fresh weight of roots. The values ranged from 16.64 g (main root cuttings) to 25.36 g (seedlings) and 9.49 g (9 month old plants) to 31.7 g (18 month old plants) for planting materials and stage of harvest respectively.

Significant interaction was also observed between the treatment combinations (Table 7a). The most significant interaction was observed for the treatment combination P_1S_3 (40.07 g).

4.2.3.6 Fresh root yield per hectare

Planting materials and harvesting intervals exerted a significant influence on the per hectare yield of fresh roots (Table 7). Among the planting materials seedlings (P_1) was significantly superior to all the other treatments and recorded the highest root yield of 3.49 t ha^{-1} on fresh weight basis. The fresh root yield per hectare showed an increasing trend with the advance in age of the plant and it ranged from 1.68 tonnes at 9 months after planting (S_1) to 4.14 tonnes at 18 months after planting.

The interaction between the planting materials and stage of harvest was also significant (Table 7a). The most significant interaction was observed for the treatment combination P_1S_3. Seedlings harvested after 18 months recorded the highest fresh root yield of 5.25 t ha^{-1}.

4.2.3.7 Dry root yield per hectare

The results presented in Table 7 revealed that the planting materials and stage of harvest significantly influenced the yield of dry roots per hectare. Highest root yield of 1.19 tonnes was recorded by seedlings (P_1) followed by root stumps (1.05 t ha^{-1}), 2 noded vine cuttings (0.99 t ha^{-1}) and 3 noded vine cuttings (0.98 t ha^{-1}). Main root cuttings recorded a dry root yield of 0.85 tonnes per hectare which was found to be the lowest. The dry root yield was found to be increased as the age of the plant increased. Highest root yield of 1.56 tonnes was obtained from 18 month old plants followed by 12 month old plants (0.99 tonnes) and 9 month old plants (0.49 tonnes).

Interaction effefct was not significant (Table 7a).

4.2.3.8 Driage (%)

The data presented in Table 7 showed that the planting materials and stage of harvest had significant influence on the driage. The maximum driage was obtained from seedlings (35.99%) and minimum from main root cuttings (33.17%). The treatments P_1 and P_2 as well as P_2, P_3 and P_4 were found to be on par. Maximum driage of 39.30% was obtained from the roots harvested at 18 months after planting followed by 12 month old plants (35.39%) and lowest by 9 month old plants (28.83%).

However the planting materials and stage of harvest was found to be not interacting for this character (Table 7a).

4.2.3.9 Harvest index

Harvest index was found to be significantly influenced by the planting materials and stage of harvest (Table 7). Highest harvest index of 0.52 was shown by seedlings (P_1) which was significantly superior to other treatments. Main root cuttings gave the lowest index value of 0.45. The harvest index decreased from 0.44 at S_1 (9 months after planting) to 0.40 at S_2 (12 months after planting) and thereafter increased to 0.57 at S_3 (18 months after planting). Significant difference was also observed between S_1, S_2 and S_3.

The interaction between planting materials and stage of harvest was found to be in significant (Table 7a).

4.2.4 Chemical components

4.2.4.1 Free amino acids

The analysis of the root sample by ion exchange chromatography showed

Table 7. Effect of planting materials and stage of harvest on root yield, driage and harvest index

Treatments	Fresh root yield per plant (g)	Dry root yield per plant (g)	Fresh root yield (t ha^{-1})	Dry root yield (t ha^{-1})	Driage (%)	Harvest index
PLANTING MATERIALS						
P_1 Seedlings	68.20	25.36	3.49	1.19	35.99	0.52
P_2 Vine cuttings 2 noded	53.99	19.30	2.76	0.99	34.09	0.46
P_3 Vine cuttings 3 noded	52.36	19.09	2.68	0.98	34.95	0.47
P_4 Root stumps 10 cm	57.84	20.58	2.96	1.05	34.33	0.45
P_5 Main root cuttings 5 cm	47.89	16.64	2.45	0.85	33.17	0.45
SEm±	0.6769	0.2718	0.0447	0.0526	0.5856	0.0054
CD (0.05)	2.531	0.787	0.129	0.152	1.695	0.016
STAGE OF HARVEST						
S_1 9 MAP	32.83	9.49	1.68	0.49	28.83	0.44
S_2 12 MAP	54.54	19.37	2.79	0.99	35.39	0.40
S_3 18 MAP	80.80	31.71	4.14	1.56	39.30	0.57
SEm±	0.8738	0.2105	0.0346	0.0407	0.4536	0.0042
CD (0.05)	1.96	0.609	0.100	0.118	1.314	0.012

MAP - Months After Planting

Table 7a. Interaction between planting materials and stage of harvest

Treatment combinations						
P_1S_1	39.13	11.93	2.00	0.61	30.55	0.49
P_2S_1	32.33	9.07	1.65	0.47	28.03	0.42
P_3S_1	30.33	8.97	1.55	0.46	29.63	0.45
P_4S_1	34.00	9.97	1.74	0.51	29.37	0.42
P_5S_1	28.33	7.53	1.45	0.38	26.57	0.42
P_1S_2	62.93	24.07	3.22	1.23	38.18	0.46
P_2S_2	54.47	18.50	2.79	0.95	33.92	0.39
P_3S_2	52.80	18.30	2.70	0.94	34.64	0.40
P_4S_2	55.43	19.60	2.84	1.00	35.36	0.38
P_5S_2	47.07	16.40	2.41	0.84	34.86	0.40
P_1S_3	102.53	40.07	5.25	1.71	39.24	0.63
P_2S_3	75.17	30.33	3.85	1.55	40.33	0.55
P_3S_3	73.93	30.00	3.79	1.54	40.58	0.56
P_4S_3	84.10	32.17	4.31	1.65	38.26	0.55
P_5S_3	68.27	26.00	3.50	1.33	38.09	0.54
SEm±	1.5135	0.4708	0.0774			
CD (0.05)	4.383	1.363	0.224	NS	NS	NS

MAP - Months After Planting

that the roots contain six free amino acids and they were identified as alanine, aspartic acid, glycine, valine, serine and threonine. The number of free aminoacids were not influenced by planting materials and stage of harvest.

4.2.4.2 Total amino acids

The analysis of root samples for total amino acids showed that planting materials had no significant influence on the amino acid content (Table 8, Fig.2). However, root stumps recorded the highest amino acid content among the planting materials. Stage of harvest exhibited significant influence on this character. The amino acid content showed an increasing trend with advance in age. Total amino acid content was found to be maximum in the roots harvested at 18 months after planting (0.014), followed by 12 month old plants (0.004) and lowest from 9 month old plant (0.002).

No significant interaction was observed between planting materials and stage of harvest (Table 8).

4.2.4.3 Soluble carbohydrates

Soluble carbohydrate content was significantly influenced by planting materials and maximum content (4.92%) was observed in seedlings followed by root stumps (4.86%) and 2 noded vine cuttings (4.81%) which were found to be on par (Table 8, Fig.3).

Similar to aminoacid content the soluble carbohydrate also increased with advance in age of the plants and maximum content (7.48%) was observed in plants harvested at 18 months after planting. The interaction between planting materials and stage of harvest was found to be insignificant. However maximum percentage of chemical components

Treatments		Total aminoacids (%)	Soluble carbohydrate (%)
PLANTING MATERIALS			
P_1	Seedlings	0.006 (0.067)	4.92
P_2	Vine cuttings 2 noded	0.007 (0.075)	4.81
P_3	Vine cuttings 3 noded	0.006 (0.067)	3.97
P_4	Root stumps 10 cm	0.007 (0.076)	4.86
P_5	Main root cuttings 5 cm	0.006 (0.067)	3.89
SEm ±			0.2438
CD (0.05)		NS	0.706
STAGE OF HARVEST			
S_1	9 MAP	0.002 (0.039)	2.36
S_2	12 MAP	0.004 (0.059)	3.59
S_3	18 MAP	0.014 (0.116)	7.48
SEm ±		0.0055	0.1888
CD (0.05)		0.016	0.547
INTERACTIONS			
P_1S_1		0.002 (0.042)	2.73
P_2S_1		0.001 (0.041)	2.30
P_3S_1		0.002 (0.037)	2.20
P_4S_1		0.001 (0.042)	2.50
P_5S_1		0.002 (0.035)	2.09
P_1S_2		0.004 (0.054)	3.81
P_2S_2		0.005 (0.068)	4.12
P_3S_2		0.003 (0.054)	3.09
P_4S_2		0.005 (0.068)	3.95
P_5S_2		0.004 (0.054)	2.98
P_2S_3		0.013 (0.104)	8.22
P_2S_3		0.014 (0.117)	8.02
P_3S_3		0.013 (0.111)	6.63
P_4S_3		0.015 (0.119)	8.14
P_5S_3		0.013 (0.111)	6.39
SEm ±			
CD (0.05)		NS	NS

MAP - Months After Planting

Values in parenthesis indicate square root transformed values

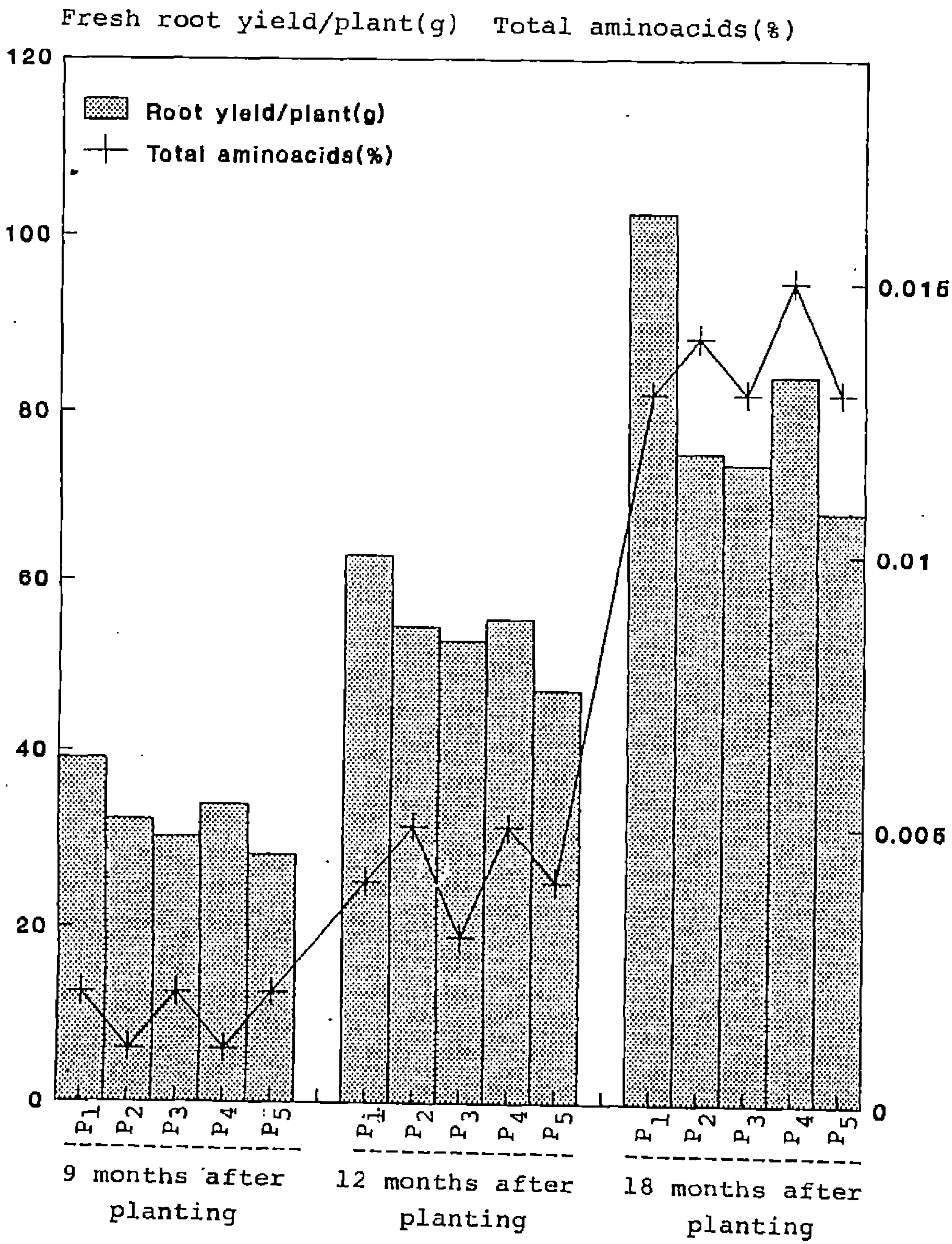


Fig.2 Relationship of total aminoacids with fresh root yield as influenced by planting materials and stage of harvest.

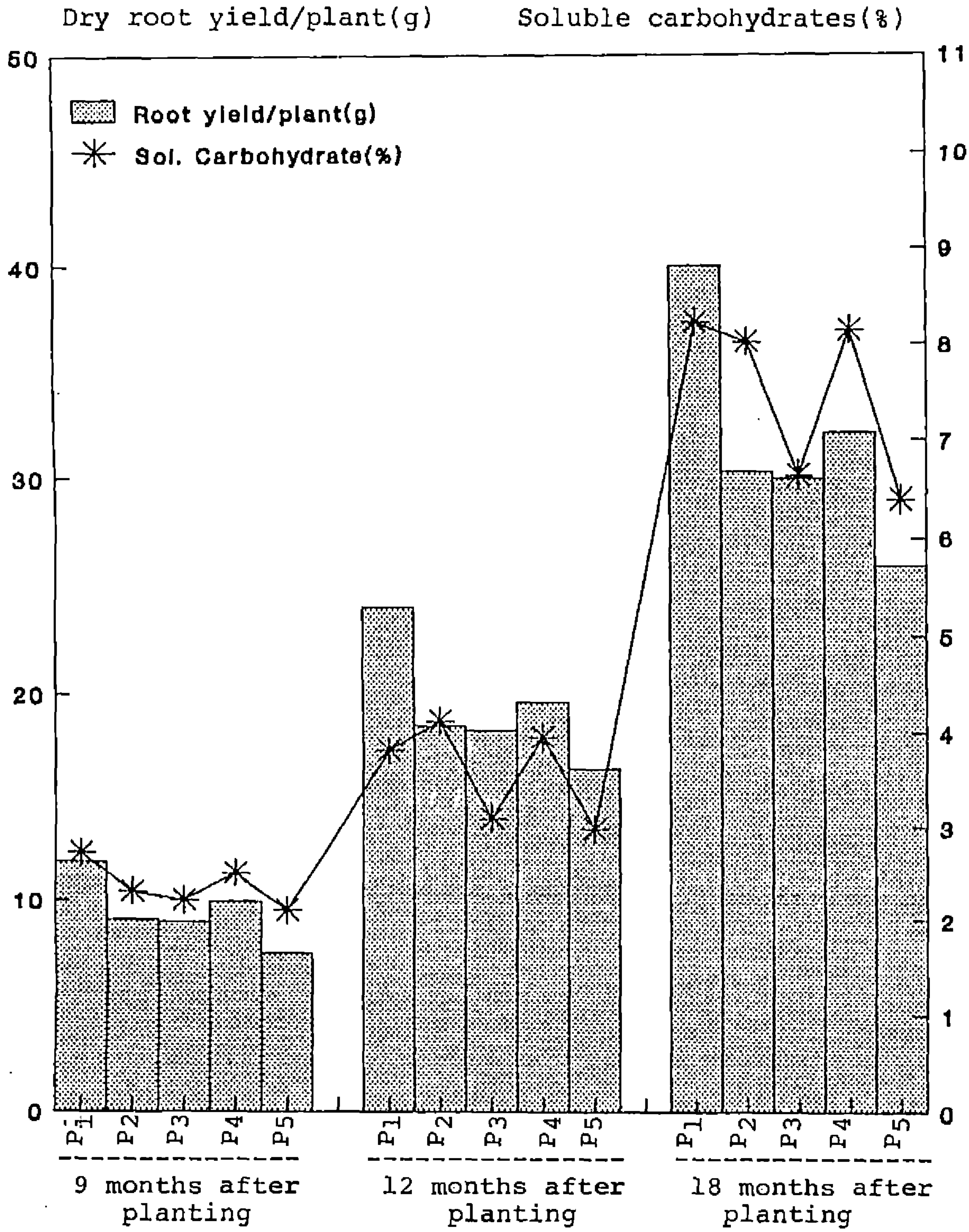


Fig.3 Relationship of soluble carbohydrates with dry root yield as influenced by planting materials and stage of harvest.

soluble carbohydrate (8.22%) was obtained from seedlings harvested after 18 months.

4.3 Correlation studies

In order to assess the nature and degree of association of the plant morphological characters with dry root yield correlation was worked out with nine characters. The correlation coefficients presented in Table 9 indicated that significant positive correlation exist. between internodal length, diameter of vine, number of branches and root volume with dry root yield per plant (Fig.4). The studies thus indicated the scope for selecting plants based on these four characters for augmenting the root yield.

4.4 Economics of cultivation

Economics of cultivation was worked out for the best treatment combination ie. seedlings harvested at 18 months after planting (Table 10). The yield of dry roots from one hectare was worked out to be 1.71 tonnes. At the current market price of Rs.200/- kg for dried roots, the gross return from one hectare was Rs.3,42,000. The total cost of cultivation for 18 month old crop on hectare basis was Rs.93,170/-. With a net return of Rs.2,48,830/- the benefit cost ratio was worked out to be 3.67:1

Table 9. Correlation between morphological characters and dry root yield

Characters	Correlation coefficient
1. Length of vine (cm)	0.442
2. Number of branches	0.554*
3. Diameter of the vine	0.699**
4. Internodal length	0.527*
5. Total leaf area (cm^2)	0.369
6. Fresh weight of stem (g)	0.375
7. Length of root (cm)	0.488
8. Diameter of root (cm)	0.209
9. Volume of root (ml)	0.962**

F value 1% 0.6411 * significant at 5% level
5% 0.5131 ** significant at 1% level

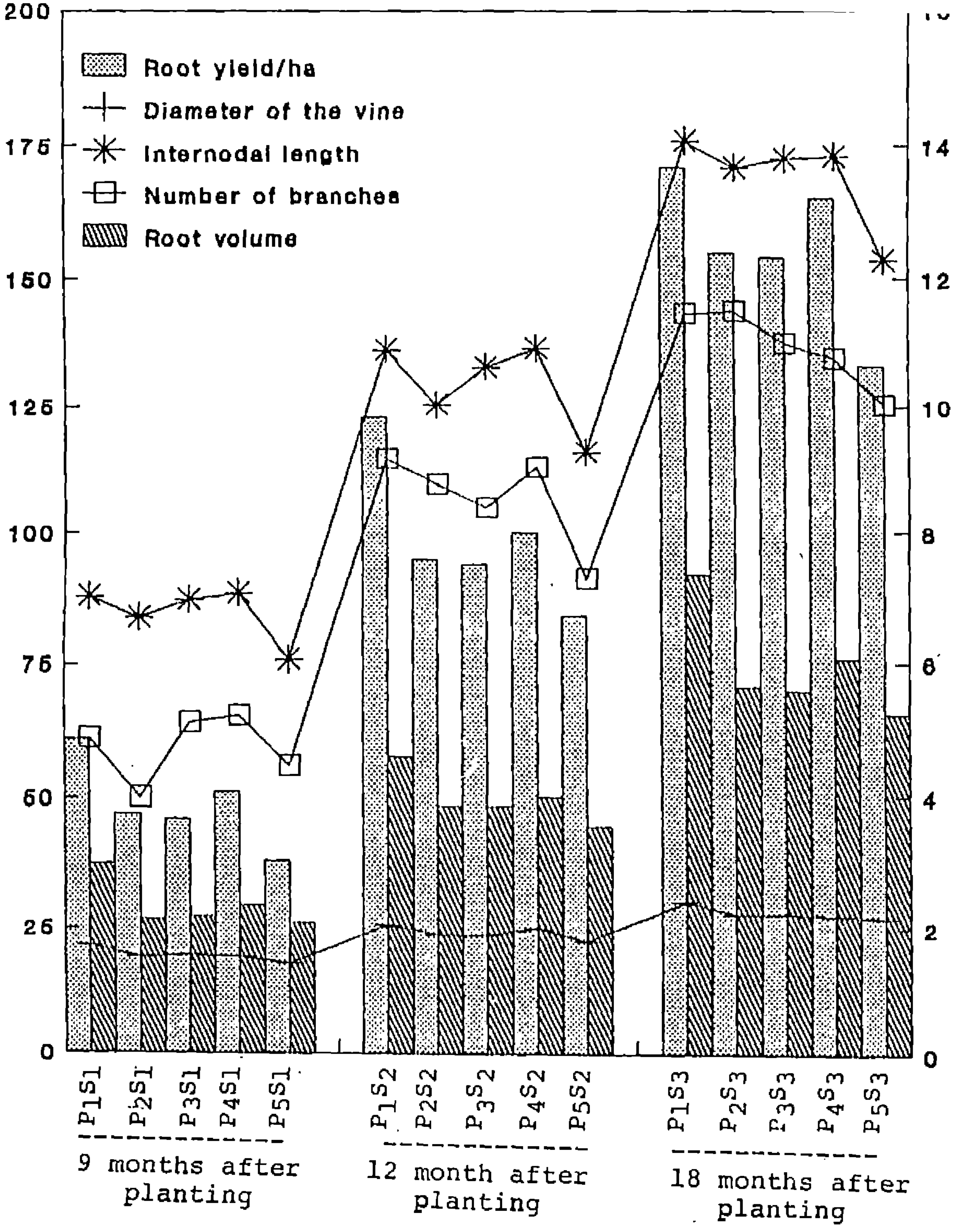


Fig.4 Relationship of biometric characters with dry root yield as influenced by planting materials and stage of harvest.

Table 10. Economics of cultivation for adakodien (one hectare)

	Men @ Rs.70/-	Women @ Rs.70/-	Total
1. Cost of seedlings 51200 Nos. @ Rs.1/-			51200.00
2. FIELD PREPARATION			
Weeding and digging	25	30	3850.00
Taking beds	30		2100.00
Planting	4	30	2380.00
3. MANURES AND FERTILIZERS			
FYM (@ 10 tonnes/ha)			3000.00
Fertilizers @ 150:60:60 NPK kg/ha			
Urea 200 kg @ Rs.3.25/kg			650.00
Factomphos 300 kg @ Rs.5.9/kg			1770.00
MOP 100 kg @ Rs.3.6/kg			360.00
Transportation and application	5	15	1400.00
4. INTERCULTURAL OPERATIONS			
Weeding and mulching	10	175	12950.00
Staking and earthing up	25		1750.00
Spraying PP chemicals	8		560.00
Irrigation	60		4200.00
5. Harvesting and processing of roots	30	70	7000.00
	197	320	93170.00

Total cost of cultivation for 18 months	- Rs. 93,170.00
Gross return from 1.71 tonnes of dry roots @ Rs.200/kg	- Rs. 3,42,000.00
Net return	- Rs. 2,48,830.00
Benefit cost ratio	- 3.67: 1

Discussion

DISCUSSION

Adakodien (*Holostemma annulare* K. Schum.) is one among the many medicinally important plants which needs domestication for its survival and to meet the rising demands for pharmaceutical purpose. The present investigation on standardisation of planting materials and stage of harvest in adakodian (*Holostemma annulare* K. Schum.) is the first step towards its domestication and to groom this as a commercial crop under Kerala conditions. This study was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1992-94. The results generated from the study is discussed in this chapter.

5.1 Nursery screening

A preliminary nursery screening was carried out using nine planting materials to select the best five for field trial. Seedlings showed its superiority in the nursery stage for the vigour of the sprouts measured in terms of shoot length and girth, leaf number and root length and girth (Table 1). The increased vigour of the seedlings in the nursery stage as observed in the present study may be a reason for the reported rating of seedlings as the best planting material under field conditions, giving higher economic yield in *Ravoulfia serpentina* (Bhaskaran, 1964; Sahu, 1979).

Early sprouting and highest sprouting percentage was observed in vine cuttings which can be attributed to the accelaration of axillary buds when apical dominance is disturbed by removal of terminal portion. Root stumps also share this advantage and showed similar performance as that of vine cuttings. Notable difference was observed between small and large sized vegetative propagules with respect to sprouting and vigour and latter showed better sprouting and vigour which may be due to greater food reserves. The difference was very much pronouced between main and secondary root cuttings and secondary root cuttings produced least vigorous sprouts and was less acceptable. In support, it may be stated that large sized root cuttings contain more amount of nutrients causing better sprouting in main root cuttings compared to secondary root cuttings. Differential response to sprouting between mother and finger rhizome was reported by Rajagopalan (1983) in *Kaempferia galanga.*

Main root being the bulk portion of the officinal part of the plant and since no significant difference was observed between 5 cm and 10 cm pieces, 5 cm piece was selected for field trial. The propagules selected for the field trial was in the order Seedlings < Root stumps 10 cm < 3 noded Vine cuttings < 2 noded Vine cuttings < Main root cuttings 5 cm.

5.2 Field trial

Based on the nursery screening five planting materials such as seedlings, 2 noded vine cuttings, 3 noded vine cuttings, root stumps 10 cm and main root cuttings 5 cm were selected and advanced for field trial. The treatments included combination of planting materials and three stages of harvest.

5.2.1. Field establishment

More than 80 per cent field establishment was shown by all the planting materials and the maximum establishment (91.67%) was recorded by seedlings and lowest by vine cuttings (83.30%). The well developed taproot system in seedling plants may be a reason for better establishment compared to vegetative propagules wherein lateral root system predominates. Among the vegetative propagules root stumps recorded greater establishment in the field. Similar result was also reported in *Rauvolfia serpentina* (Sahu, 1969) where root stumps planted in May - June in irrigated field gave 90 - 95 per cent success.

5.2.2. Influence of planting materials and stage of harvest on biometric characters

Influence of planting materials and stage of harvest on ten biometric characters such as length of vine, number of branches, diameter of the vine, internodal length, number of leaves and total leaf area per plant, fresh and dry weight of stem and leaf were studied. The planting materials had significant influence on all the characters studied except in vine length, number of branches and fresh weight of stem (Table 2 to 3a, Table 5).

Seedlings were found to be superior with respect to diameter of the vine and internodal length and showed significant difference from other vegetative propagules. Even though the difference was not significant between planting materials for vine length and number of branches, seedlings showed slight increase for these two characters which is in conformation with the report of Bhaskaran (1964) in *Rauvolfia serpentina*.

Among the planting materials highest number of leaves, total leaf area per plant, fresh weight and dry weight of stem was recorded by root stumps whereas the fresh and dry weight of leaf was found to be maximum in the case of vine cuttings followed by root stumps. In conformity with the present findings higher leaf yield from stem cuttings compared to seedlings were reported by Hegde (1988) in *Catharanthus roseus*.

Age of the plant showed significant influence on all the biometric characters studied (Table 2, 3 and 4). The vine length, branches per vine, diameter of the vine and internodal length showed a progressive increase with increase in age of the crop. Increase in biometric characters with increase in harvest interval was reported by serveral workers (Sobti *et al.*, 1978; Choudhury and Bordoloi, 1984 and Pillai, 1990 in *Ociumum gratissimum* and by Chinnamma, 1985 in Palmarosa).

The number of leaves, total leaf area per plant, fresh and dry weight of stem and leaf first showed an increasing trend upto 12 months and thereafter declined. The second harvesting conducted (12 months after planting) falls during July which coincides with the monsoon season. Under natural conditions *Holostemma annulare* behaves as a perennial with the withering of the vegetative parts during summer and rejuvenating during monsoon season which resulted in increased vegetative growth. The higher leaf area per plant might have resulted from higher leaf production and increased size of the leaf after rejuvenation. This result is in conformity with the results reported by Pillai (1990) in *Ocimum gratissimum*. The decreased leaf number, leaf area and leaf weight for the third harvest (18 months after planting) can be attributed to the leaf fall due to senescence and reduced moisture status of the soil. The observed reduction in the fresh weight of stem is quite rational in view of the initiation of the senescent stage prior to withering in summer.

The interaction between planting materials and stage of harvest was found to be insginficant for all the biometric characters studied.

The plant bears large number of flowers ranging from 70 to 200 with a mean of 125.56 but the fruit set percentage was found to be low (12.9%). In concurrence with the finding Sreedevi (1989) also reported that the fruit set in Asclepiads is low (5 to 10%), though the members of Asclepiadaceae family is well adapted for cross pollination. This low level of fruit set can be attributed to the low degree of removal and insertion of pollinia into the stigmatic chamber properly by the insect pollinators as observed by Willson *et al.* (1979). Each fruit carries large number of seeds ranging from 386 to 512. Having large number of seeds per fruit with high germination per cent (82.00) fruit set level seems to be adequate for successful propagation as observed by Sreedevi (1989) in Asclepiads.

5.3 Yield characters

The results revealed that the type of planting material exerted significant influence on the yield characters such as root length, root volume, fresh and dry root yield per plant and fresh and dry root yield per hectare (Table 6 and 7). However the root diameter was found to be unaffected by the type of planting material. Seedlings showed its superiority over vegetative propagules with respect to all the root characters studied, and recorded the maximum fresh weight of root (68.20 g), dry weight of root (25.36 g) and fresh and dry root yield per hectare (3.49 t ha^{-1} and 1.19 t ha^{-1} respectively). Harvest index and drying percentage was also highest in plants raised from seedlings (Table 7). Higher root yield from seed derived plants have been reported by Badhwar *et al.* (1957 and 1963); Bhaskaran (1964) and Sahu (1969) in *Rauvolfia serpentina*; Nybe (1978) in ginger, Rybacek (1984) in hops and Hegde (1988) in *Catharanthus roseus*.

Root stumps stood next to seedlings and recorded a fresh and dry root yield of 2.96 t ha^{-1} and 1.05 t ha^{-1} respectively. But no significant difference was observed in root yield between two noded and three noded vine cuttings. In the nursery stage also no significant difference was observed between these planting materials on root characters, and this was not stimulated under field condition. This may be due to the similar growth pattern of the plants though raised from different size of cuttings. Similar observations were also reported in *Plumbago rosea* (Subha, 1990). Main root cuttings were found to be inferior with respect to all the root characters and root yield and appear to be the least acceptable commercial planting material. Sahu (1969) reported that one year old plants of *Rauvolfia* raised from root cuttings gave higher root yield than those from stem cuttings. The rating of the propagules in the nursery stage holds true with regard to the yield under field condition.

Stage of harvest also exhibited a significant influence on root characters. Increasing the crop duration brought about a progressive increase in all the root characters such as root length, diameter, volume, fresh and dry root yield per hectare (Table 6 and 7). The maximum root yield on fresh weight and dry weight basis 4.14 t ha^{-1} and 1.56 t ha^{-1} respectively was obtained from the last harvesting interval (18 months after planting). All the planting materials exhibited their maximum yield potential at this stage. Similar trend of higher yield with increase in the harvesting stage was reported in ginger (Nybe, 1978); in *Costus speciosus* (Singh *et al.* 1979) and in *Glycyrrhiza glabra* (Shah *et al.*, 1976).

The interaction between planting materials and stage of harvest was found to be significant only with respect to root volume, fresh and dry root yield per plant and fresh root yield per hectare. The most significant interaction was observed for seedlings harvested at 18 months after planting.

Planting materials and stage of harvest exhibited significant influence on driage and harvest index. Among the planting materials seedlings showed the best performance with respect to these characters (35.99% and 0.52 respectively). On the contrary Hegde (1988) found that harvest index was not influenced by the method of propagation. Driage tended to increase with the increase in the age of plant and maximum (39.30%) was realised at the highest harvest interval (18 months after planting). Harvest index declined (0.40) at S_2 (12 months after planting) and then increased (0.57) at S_3 (18 months after planting) which can be explained by the predominance of vegetative growth after rejuvenation with the onset of monsoon coinciding with S_2. In concurrence with the present finding propagation method and harvesting time was reported to influence the driage in ginger and kacholam (Nybe, 1978 and Rajagopalan, 1983).

5.4 Qualitative characters

Chemical analysis of the root sample resulted in the isolation of six amino acids which were identified as alanine, aspartic acid, glycine, valine, serine and threonine which is in line with the reports of Ramiah *et al.* (1954). No difference was observed between planting materials and stage of harvest for the number of free amino acids, suggesting that the amino acid pattern in a species may be genetically controlled. The total amino acid content was found to be unaffected by the type of planting materials, but it showed an increase with increase in age of the plants (Table 8). The content was maximum (0.014%) in 18 month old roots. Increase in protein content with enhancement in maturity was also reported in Holostemma (Samuel *et al.*, 1993).

The planting materials and stage of harvest exhibited a significant influence on the soluble carbohydrate content. Maximum content was recorded by seedlings (4.92%) followed by root stumps (4.86%), vine cuttings 2 nodes (4.81%), vine cuttings 3 nodes (3.97%) and root cuttings (3.82%) which was the lowest. Stage of harvest also exhibited a significant influence on soluble carbohydrate percentage. The maximum content was obtained from 18 months old roots (7.42%) and minimum from 9 months old roots. In conformity with the present finding increase in the alkaloid content with advance in age of plants was observed in *Rauvolfia serpentina* (Sobti *et al.*, 1975), in ipecac (Banerjee, 1974) and ginsengosides in *Panax ginseng* (Soldati and Tanaka, 1984). The treatments were not interacting with respect to amino acid and soluble carbohydrate content. Since the carbohydrate accumulation is related with amino acid accumulation in an increasing order the pathway of general metabolism in this plant may be an exception to the already established pathways. This is in agreement with the total yield as well as the special medicinal properties of Holostemma where amino sugar or amino acids are the chemical constituents.

5.5 Correlation studies

Correlation between biometrical characters and dry root yield per plant was worked out (Table 9). The characters such as internodal length, diameter of the vine, number of branches and root volume showed significant positive correlation with root yield. This observation can be further substantiated by the fact that seedlings which recorded the highest root yield recorded significantly highest diameter of the vine, internodal length, root volume and highest number of branches. Even though different planting materials showed significant difference in leaf area and root length, the correlation was found to be insignificant. Therefore the difference in these characters may be genetically controlled which is naturally unaffected by soil, nutritional and external conditions.

5.6 Economics of cultivation

The economics of cultivation was worked out for one hectare of adakodien crop raised from seedlings and harvested 18 months after planting (Table 10). The net return realised was Rs.2,48,830/. With a cultivation cost of Rs.93,170/. The benefit cost ratio worked out indicates the profitable nature of the crop.

The present investigation highlights the superiority of seedlings as the economical planting material for realising maximum root yield with quality components. The cross pollinated nature of Holostemma a member of Asclepiads, reported elsewhere points to the variability in the seed propagated progenies. Barring the propagation of elite planting material for which vegetative propagules are suggested, seedlings can be recommended as the economic planting material. The progressive increase in yield as well as the chemical components with advance in age suggest that stage of harvest above 18 months needs to be tried for fixing the optimum stage. The benefit cost ratio worked out for the crop indicates the high profitability of its cultivation and the possibility for grooming this as a commercial crop under Kerala conditions.

Summary

SUMMARY

An investigation was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1992-'94 to standardise the most ideal and economic method of propagation and optimum stage of harvest in adakodien *Holostemma annulare* K. Schum.). The salient results are summarised below.

The plant bears large number of flowers but the fruit set (%) was found to be low. But this was compensated by the large number of seeds and high germination percentage.

The five propagules selected for field trials based on nursery screening were in the order of seedling < root stumps 10cm < 3 noded vine cuttings < 2 noded vine cuttings < main root cuttings 5 cm.

Fifteen treatments comprising combinations of the above five planting material and three stages of harvest (9, 12 and 18 months after planting) were tried in a factorial RBD with 3 replication.

Among the five propagules seedlings showed the highest field establishment followed by root stumps, root cuttings and vine cuttings.

Planting materials exhibited a significant influence on diameter of the vine, internodal length, number of leaves, total leaf area/plant, dry weight of stem, fresh and dry weight of leaf whereas the vine length, number of branches and fresh weight of stem were not significantly influenced by the type of planting material. Seedlings were significantly superior with respect to yield related characters such as

diameter of the vine and internodal length and recorded the highest vine length and number of branches.

Yield characters such as root length, root volume, fresh and dry root yield per plant and per hectare were significantly influenced by the planting material where as root diameter was unaffected by the type of planting material. Seedlings showed its superiority over vegetative propagules with respect to all the root characters studied and recorded maximum fresh and dry root yield (3.49 tonnes ha^{-1} and 1.19 tonnes ha^{-1}). Root stumps stands next to seedlings and recorded fresh and dry root yield of 2.96 tonnes ha^{-1} and 1.05 tonnes ha^{-1} respectively. This was followed by vine cuttings. Main root cuttings was inferior with respect to all the root characters and root yield and is the least acceptable commercial planting material. Driage and harvest index was also significantly influenced by the planting material and seedling was superior in this respect also. The rating of the propagules in the nursery stage stands true with regard to yield under field condition.

Age of the plant showed significant influence on all the biometric characters studied. The vine length, branches per vine, diameter of the vine and internodal length showed a progressive increase with increase in the duration of the crop. The number of leaves, total leaf area per plant, fresh and dry weight of stem and leaf first showed an increasing trend upto 12 months and thereafter declined.

Stage of harvest exhibited a significant influence on the yield characters, driage and harvest index. Increasing the crop duration brought out a progressive increase in all the root characters such as root length, diameter, volume and the fresh and dry root yield per hectare. Maximum root yield was realised by the last harvesting interval of 18 months after planting (4.14 tonnes ha^{-1} of fresh yield and

1.56 tonnes ha^{-1} of dry yield). Driage and harvest index was also maximum at harvesting interval of 18 months after planting.

Qualitative analysis of roots revealed the presence of six free amino acids identified as alanine, asparatic acid, glycine, valine, serine and threonine which were not altered by the type of planting material and different stages of harvest.

The total amino acid content was not influenced by the planting material but a progressive increase with advance in age of the plant was observed and maximum content was realised at 18 months after planting.

Soluble carbohydrate content was significantly influenced by planting material and stage of harvest. Maximum content was recorded by seedlings and at the last harvest interval of 18 months after planting.

Interaction effect between planting material and stage of harvest was found to be significant with respect to root volume, fresh and dry root yield per plant and fresh root yield per hectare. The most significant interaction was observed for seedlings harvested at 18 months after planting.

Correlation between biometric characters and dry root yield indicated that selection for yield could be based on internodal length, diameter of the stem, number of branches and root volume.

The economics of cultivation worked out for one hectare of adakodien crop revealed a high benefit cost ratio of 3.67 suggesting the suitability of grooming this as a commercial crop under Kerala conditions.

References

REFERENCES

Abrol, B.K. and Kapoor, L.D. 1962. Distribution of *Dioscorea deltoidea* in north-western Himalayan region. *Bull. Reg. Lab.*, Jammu. 1:30-31

Arumugam, R. and Kumar, N. 1980. Effect of leaves on rooting of stemcutting of bergamot mint *(Mentha citrata* Ehrh). *Indian Perfumer,* 24(3):166-167

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

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

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

* Badalov, M.M. 1973. The effect of cutting size on the development of liquorice plants. *Opyt. Kul'tury Novykh Syr'evykh Rastenii* - Tashkent, Uzbek, USSR, Fan., 1: 137-40

Balyan, S.S.; Sobti, S.N; Pushpangadan, P; Singh, A; and Atal, C.K. 1982. Cultivation and utilization of Cymbopogon In *Cultivation and Utilization of aromatic plants* by Atal, C.K. and Kapur, B.M. (Eds.); Regional Research Laboratory, Jammu, P:481-86

Banerjee, P.K. 1974. Phytochemical study of successive ages of ipecac. *Plant Sci.,* 6:38-40

Bavappa, K.V.A. and de A Gurusinghe, P. 1980. New technique for the rapid multiplication of pepper planting material. *Indian Cocoa Arecanut Spices J.*3(3) :53-55

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

Biswas, P.K. 1988. Root yield of *Rauvolfia serpentina* by different methods of propagation. *Environ. Ecol.,*6(2):486-487

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

Chinnamma, N.P. 1985. Influence of applied nutrients and stage of harvest on the yield and physico-chemical properties of essential oil of palmarosa (*Cymbopogon martini* Stapf var. motia). Ph.D. Thesis. Kerala Agricultural University, Vellanikkara, Thrissur.

Choudhury, S.N. and Bordoloi, D.N. 1984. Yield and eugenol content of *Ocimum gratissimum* Linn. under different plant densities. Pafai J.,6(4)24-28

Coffman, C.B. and Gentner, W.A 1979. Green house propagation of *Cannabis sativa* L. by vegetative cuttings. Eco. Bot.,32(2):124- 127

CSIR. 1959. Importance of *Holostemma annulare* along with uses and active ingredients. *The Wealth of India* Vol. V, H-K. 111

Dhar, A.K., Rekha, K. and Koul, S. 1986. Comparison of vegetative and seed propagation in *Cymbopogon jawaracusa* Jones Schulz. *Indian Perfumer.,* 30(1):247-250

Dubois, M. Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. *Anal. Chem.,* 26:350

Duraiswamy, P.I. and Arumugam, R. 1980. Preliminary studies on the rooting of geranium cuttings as affected by soil reaction. *Indian Perfumer,* 24(3):168-169

El-Keltawi, N.E and Croteau, R. 1985. Single node cuttings as a new method of mint propagation. *Scientia Hort.,*29(1/2):101-105

Flemer, W. 1961. Propagating woody plants by root cuttings. *Proc Plant Prop. Soc.,* 11:42-47

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

Ghosh, M.L. and Chatterjee, S.K. 1976. Pattern of essential oil formation in relation to Nitrogen content of 2 species of *Cympopogon. Indian perfumer,*20:71-72

Govind, S. 1987. Studies on optimum harvesting time of turmeric. *Haryana J. Hort. Sci.,* 6(3,4):257-263

Gulati, B.C., Garg, S.N. and Duhan, B.P.S. 1978. Effect of period of harvest on the yield and quality of oil of *Mentha piperita* Linn. *Indian Perfumer,*22:21

Gupta, R. 1972. Grow palmarosa oil grass for rose like perfume. *Indian Fmg.,*22:17-22

Gupta, B.K. and Jain, N. 1978. Cultivation and utilisation of the grass *Cymbopogon* in India. *Indian Perfumer,*22:79-87

Gupta, M.M., Farooqui, S.U. and Lal, R.N. 1981. Diosgenin content of *Costus speciosus* at different stages of growth. *Indian Drugs,*18(8):285-286

Hartmann, H.T and Kester, D.E. 1989. *Plant Propagation: Principles and Practices.* 4th ed. Prentice Hall International, INC. London. p. 29

Hegde, D.M., Randhawa, G.S., Selvaraj, Y. and Subashchander, M. 1981. Effect of planting materials and time of harvesting on tuber yield, diosgenin content and sprouting in medicinal yam. *Indian drugs,*18(4);145-148

Hegde, G.S. 1983. Effect of number of nodes on cuttings and growth regulators on rooting of Panniyur -1 pepper (*Piper nigrum* L.) vine. Thesis Abstracts. Haryana Agriculture University. 9(1):64

Hegde, D.M. 1988. Growth and productivity of periwinkle [*Catharanthus roseus* (L) G. Don] in relation to plant type and method of propagation. *Agric. Res. J. Kerala*, **26**(1):50-58

*Helmi, S., Wijandi, S. and Ketaren, S.1975. Influence of age at harvest and place and duration of storage on the yield and quality of ginger oil. *Bulletin Penelitian Hasil Pertanian*, No.1 4.

Huges, J. 1966. Vegetative propagation of pepper under mist spray. *S.Pacif. Bull.*, **16**(3):41-2

*Ivanova, Z.Y.Z. and Gladun, M.I. 1986. Propagation of essential oil rose by softwood cuttings. *Byulletin' Gosudarstvenogo Nikitskogo Botanicheskogo* Sada, 60:72-76

Jogi, B.S., Singh, I.P., Due, H.S and Sukhija. 1972. Changes in crude fibre, fat and protein content in ginger (*Zingiber officinale* R.) at different stages of ripening. *Indian J. agric. Sci.*,**42**(11):1011-1015

Joseph, E.J. 1983. Effect of spacing, rhizome weight and time of harvest in the yield and quality constituents in *Costus speciosus* M.Sc. (Hort) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

K.A.U. 1982. Palmarosa. Package of practices recommendations. Kerala Agricultural University. p. 104-105

Kolammal, M. 1979. *Holostemma annulare* K. Schum. Pharmacognosy of Ayurvedic Drugs (Kerala), Series 10, p. 21-24

*Kodash, A.G. Monina, O.I., Masavena, V.I. and Zakharov, A.N. 1977. Changes due to age and season in the weight and diosgenin content of rhizomes of *Dios Caucasia* in the northern Cancasus. *Restilelnye Res. Rursy*,13(1): 72-74

*Krishevetskaya, 1940. Essential oils of *Ocimum species. Doklady Vsesoyuz. Akad. Sel'skkhoz Naum im. Lenina.*,**6**:15

Kumar, T.V. and Hegde, D.M. 1983. Comparative performance of Patchouli strain in relation to cutting interval. 9th Internat. Congress of Essent. Oil 13th to 17th March, 1983, Singapore. p.16

Kurian, A., Nair, E.V.G. and Rajan K.C. 1984. Clocimum - A new source of eugenol. *Indian Perfumer* 28(1):33-34

Lal, G. 1935. Some observation on the essen*tial oil content of rosha grass Cymbopogon Martini* stapf var. motia. *Indian J Agric. Sci.* 5:415-421

Mathai, C.K. 1976. Seasonal variation in the chemical constituents of Spice Cultivars. *Ann. Prog. Rep.* CPCRI. Kasargod p. 166

Maheshwari, S.K. Chouhan, G.S., Trivedi, C.K. and Gangrade, S.K. 1992. Effect of irrigation and stage of crop harvest on oil yield and quality of palmarosa (*Cymbopogon martini*) oil grass. *Indian J. Agron.* 37(3):514-517

Malik, C.P. and Singh, M.B. 1980. *Plant Enzymology and Histo enzymology - A Text Manual.* Kalyan Publishers, New Delhi. p. 257- 60

Mehta, K.G., Raghava Rao, D.V. and Patel, S.H. 1980. Relative curcumin content during various growth stages in the leaves and rhizomes of three cultivars of *Curcuma longa* and *Curcuma amada. Proceedings of the National Seminar on Ginger* and *Turmeric,* Calicut p.76-78

Mitra, S. and Kushari, D.P. 1985. Rooting *Solanum khasianum* stem cutting in relation to season, light and cutting age. *Plant Propagator.* 31(3):3-6

*Mizrahi, A. 1988. *Biotechnology in Agriculture.* Alan R. Liss, Inc., New York. Vol. 9, p.2-3

Nair, E.V.G., Chinnamma, N.P. and Pushpa kumari, R. 1980. Influence of spacing on the grass and oil yield of palmarosa (*Cymbopogon martini* var. *motia*). *Indian Perfumer* 24(1):25-26

Nair, E.V.G., Nair, K.C., Chinnamma, N.P. and Mariam, K.A. 1983. Optimum time of harvest of *Eucalyptus citriodora* Hooker. grown in plains. *Indian Perfumer*,27(1):54-55

Nair, K.V., Yoganarasimhan, S.N. Keshavamurthy, K.R. and Mary, Z. 1982. Studies on some South Indian market sample of Ayurvedic drug. 1. *Ancient Sci. Life* 2(2):71-78

Nambiar, P.K.V., Pillai, V.S. and Sasikumar, S.1977. A note on rapid multiplication of Panniyur-1 pepper. *Arecanut Spices Bull.* 8(3):57-58

Nambiar, P.K.V., Pillai, V.S., Sasikumar, S. and Chandy, K.S. 1978. Pepper Research at Panniyur - A Resume. *J. Plant. Crops.* 6(1):4-11

Nybe, E.V. 1978. Morphological studies and quality evaluation of ginger (*Zingiber officinale* Rosc.) types. M.Sc. (Hort) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Pal, M., Mishra and Bhandari, H.C.S. 1993. Promotion of rooting of shoot cuttings of *Datura stramonium* by IBA. *Indian J. Forestry*, 80(11):36

Panse, V.G. and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers.* (3rd ed.) Indian Council of Agricultural Research, New Delhi. p. 157-164

Pareek, S.K., Saxena, D.B., Maheswari, M.L. and Gupta, R. 1981. Study of palmarosa oil grass for higher yield, oil and its quality under cultivation. *Indian Perfumer*,25(3&4):64-70

Pareek, S.K., Srivastava, V.K., Sarbjit Singh, Mandal, S., Maheswari, M.L. and Gupta, R. 1981. Advances in Periwinkle cultivation. *Indian Fmg.* 31(6):18-21

Philip, J., Nair, G.S., Premalatha and Sudhadevi, P.K. 1991. Standardisation of vegetative propagation techniques in some of the medicinal plants grown in Kerala. *Indian Cocoa Arecanut Spices J.* 15(1):12-14

Pillai, R.R. 1990. Quality of oil of clocimum (*Ocimum gratissimum* Linn.) as influenced by stages of harvest and shade. M.Sc.(Ag) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Rajukannu, K., Dhakshinamoorthy, M., Arumugam, R. and Durasiwamy, P. 1981. Seasonal influence on the total glycoside content of foxglove (*Digitalis lanata*). *J. agric. Sci. Camb.*, 96(4):255

Ramiah, N., Nair, G.A. and Prasad, N.B.R. 1981. Chemical components of *Holostemma annulare* K. Schum. *J. Sci. Res. Plants Med.*, 2(3):76-78

Rajagopalan, A. 1983. Standardisation of propagation method,time of planting, time of harvest and phytochemical analysis of *Kaempferia galanga* L. M.Sc.(Hort) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Rybacek, V. 1984. Biological and economic properties of vegetatively and generatively propagated hop sets. *Hort. Abstr.*, 55(3):2105

Sadasivam, S. and Manikam, A. 1992. *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Limited, New Delhi. p.10-11

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

Sahu, B.N. 1979. *Rauvolfia's: Botany and Agronomy* Vol.I. Today and Tomarrow Printers and Publishers, New Delhi. p.595

Samuel, S., Nair, E.V.G., Kumar, K.V.D. and John, R. 1993. Domestication of high value medical plants for Ayurvedic formulations - needs of the day. *Traditional Medical Systems*, 3 (3): 17: 21

Sarin, Y.K., Singh, A., Bedi, K.L., Kapur, S.K., Kapathi, B.K. and Atal, C.K. 1977. Observations on *Costus speciosus* as a source of diosgenin: In *Cultivation and Utilization of Medicinal and Aromatic Plants*. Atal, C.K. and Kapur, B.M. (Eds.), Regional Research Laboratory, Jammu, p.33-38

Selvarajan, M. and Madhava Rao, V.N. 1982. Studies on rooting of patchouli cuttings under different environments. *South Indian Hort.*, 30(2): 121

Shah, A.R., Amin, D.R., Patel, R.B. and Dalal, K.C. 1979. Yield performance and sennoside content of senna leaflets in relation to days to stripping. *Indian J. Pharm. Sci.* 41(4):157-160

Shah, C.S., Bhasvar, G.C. and Kapadia, N.S. 1976. Ontogenic variation of diosgenin in the leaves of *Balanites roxburghii. Proceedings of the second All India Workshop on Medicinal and Aromatic Plants,* Gujarat Agricultural University, Anand. 170-171

Shanthamalliah, M.R., Sulladmath, U.V. and Krishnamurthy, K. 1974. Influence of maturity and time of planting on the rooting of stem cuttings of pepper (*Piper nigrum* L.). *Indian J. Hort.* 31(3):250-254

*Shi, D.S., Zheng, L., Wang, J.L., Cheng, C.H. and Luzy. 1990. Trials on propagating Artemisia by cuttings. *Pratacult. Sci.* 7(4):6-8

Singh, D.V. and Anwar, M. 1985. Effect of soil salinity on herb and oil yield and quality of some Cymobopogn species, *J. Medic. Soc. Soil Sci.* 33:362-365

Singh, V.P., Duban, S.P.S. and Bhist, H.S. 1979. Response of Japanese mint (*Mentha arvensis* L.) to N, P and K under tarai conditions of Nainital. *Pantnagar J. Res.* 4:24-26

Snedecor, G.W. and Cochran, W.G. 1967. *Statistical methods.* Oxford and IBH Publishing Co., New Delhi, 6th Ed. p.173, 381-403

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

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

Sobti, S.N., Khosla, M.K. and Pushpangadan, D. 1978. Inheritance pattern of certain phenolic and sesquiterpene constituents of essential oils in the interspecific hybrids of *Ocimum species. Indian Perfumer,* 22(4):256-258

Soldati, F. and Tanaka, O. 1984. *Panax ginseng:* Relation between age of plant and content of ginsengosides. *Planta Med.* 50(4):351- 352

Sreedevi, P. 1989. A study on pollination, fertilization and seed yield using Asclepiads. *Proceedings of the Kerala Science Congress,* February 1989, Cochin. p.23-29

Staikova, V; Bypdiieva, B.C., Irinchev, 1. and Bozhkov, G. 1980. Lavender products manufactured in Bulgaria. *Pharmacheim,* Bulgaria. p:1-69

Stoutmeyer, V.T. 1968. Root cuttings. *The Plant Propagator,* 144:4-5

Subba Rao, T.V., George, A.A., Rao, R.L. and Desikacher, M. 1948. Palmarosa oil. Part I. *Indian Group J.* 14(6):159

Subha, S. 1990. Effect of spacing and planting material on the growth, yield and active principle in *Plumbago rosea* L. M.Sc. (Hort) Thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Sudhadevi, P.K. 1992. Ethnobotany of Medicinal plants used by Tribes of Kerala. Ph. D. Thesis. Kerala Agricultural University, Vellanikkara, Thrissur.

Swamy, A.Y. and Kalyansundaram, S. 1960. Propagation of *Pelargonium graveolens* Ait. by root suckers, single node stem cuttings and root cuttings. *South Indian Hort.* 11(3&4):34-5

Whitaker, R.J. 1972. *Principles of enzymology for the food sciences.* Marcel Decker, INC, New York. p.37

Wilson, M.F., Bertin, R.L. and Price, P.W. 1979. Nector production flower and visitors of *Asclepias verticillata. The American Midland Naturalist,* 102(1):23-35

*Originals not seen

Appendices

APPENDIX-I

Meteorological data (monthly average) for the crop period (May 1992 to January 1994)

Month	Air temperature mean (°C)	Relative humidity (%)	Total rainfall (mm)	Mean sunshine hours
1992				
May	29.3	73	90.6	7.4
June	26.9	84	979.8	3.3
July	25.7	87	874.5	2.1
August	26.1	88	562.9	2.7
September	26.6	82	302.9	4.1
October	26.8	82	386.7	4.6
November	27.1	77	2.0	5.5
December	26.1	61	0	8.9
1993				
January	26.7	53	0	8.1
February	28.1	62	6.6	9.4
March	29.6	63	0	9.0
April	29.8	69	32.1	9.1
May	29.6	74	131.1	6.5
June	27.0	86	700.3	3.3
July	25.7	87	661.6	2.4
August	26.5	87	276.7	4.8
September	26.8	81	85.3	6.4
October	27.0	83	74.6	4.8
November	27.6	73	18.0	5.8
December	27.4	66	0	7.5
1994				
January	27.8	58	19.4	9.1

Appendix - II
Abstract of ANOVA

Influence of planting materials on biometric characters at nursery stage.

Source	Degrees of freedom	Mean square					
		Sprouting percentage	Shoot length (cm)	Shoot girth (cm)	Number of leaves	Root length (cm)	Root girth (cm)
Treatment	8	149.988	8.226*	0.099	6.761*	62.71**	0.024
Error	36	64480.032	3.034	0.077	3.054	13.88	0.013
Total	44						

* Significant at 5% level

** Significant at 1% level

Appendix - III

Abstract of ANOVA

Effect of planting materials and age of the plant on morphological characters

Source	Degrees of freedom	Mean square					
		Length of vine	Number of branches	Number of leaves	Total leaf area/plant	diameter of the vine	internodal length (cm)
Factor A	4	1734.946	3.062	59.040	64415.602*	0.013**	0.014**
Factor B	5	26635.961	235.635	911.861**	4171755.677**	0.254**	0.571**
AB	20	77.005	0.509	8.911**	3522.591**	0.001	0.001
Error	58	1275.245	1.626		19000.402	0.001	0.001
Total	87						

* Significant at 5% level

** Significant at 1% level

Appendix - IV

Abstract of ANOVA

Effect of planting materials and stage of harvest on fresh and dry weight of aerial parts

Source	Degrees of freedom	Fresh weight of stem (g)	Dry weight of stem (g)	Fresh weight of leaf (g)	Dry weight of leaf (g)
Factor A	2	7706.222**	535.685**	1247.034**	5.603**
Factor B	4	58.251	23.628**	15.483**	96.582**
AB	8	8.936			
Error	28	30.007			
Total	42				

** Significant at 1% level

Appendix - V

Abstract of ANOVA

Effect of planting materials and stage of harvest on root characters.

Source	Degrees of freedom	Length of root (cm)	Diameter of root (cm)	Volume of root (ml)
Factor A	2	2681.101**	17.926**	57.290**
Factor B	4	59.963**	0.123	7711.44**
AB	8	5.380	0.028	32.534**
Error	28	13.797	0.105	3.508
Total	42			

** Significant at 1% level

Appendix - VI

Abstract of ANOVA

Effect of planting materials and stage of harvest on root yield.

Source	Degrees of freedom	Fresh weight of roots per plant (g)	Dry weight of roots per plant (g)	Fresh root yield (tha^{-1})	Dry root yield (tha^{-1})	Driage (%)	Harvest index
Factor A	2	8656.243**	1859.046**	22.736**	4.298**	419.826**	0.106**
Factor B	4	529.522**	93.17**	1.386**	1.132*	9.586**	0.009**
AB	8	79.162**	9.957**	0.210**	0.007	4.707	0.0001
Error	28	6.872	0.665	0.018	0.025	3.086	0.0002
Total	42						

* Significant at 5% level

** Significant at 1% level

STANDARDISATION OF PROPAGATION AND STAGE OF HARVEST IN ADAKODIEN

(*Holostemma annulare* K. Schum.)

By

MEERA, N.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
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ABSTRACT

Investigations on standardisation of propagation and stage of harvest in adakodien (*Holostemma annulare* K. Schum.) were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1992-94.

The trial was laid out in a factorial RBD with 3 replications. The treatments comprised of fifteen combinations of five planting materials (seedlings, 2 noded vine cuttings, 3 noded vine cuttings, root stumps 10 cm and main root cuttings 5 cm) and three stage of harvest (9, 12 and 18 months after planting).

Seedlings were rated as the best planting material realising maximum yield potential of fresh and dried roots (3.49 t ha^{-1} and 1.19 t ha^{-1} respectively).

Root characters contributing to yield, driage and harvest index and biometric characters related with yield such as internodal length, diameter of the vine and number of branches were highest for seedlings.

The soluble carbohydrate content was also maximum for seedlings.

Rating of the planting material under field condition followed the same trend as in the nursery. The preference for the five planting material in the decreasing order were seedling, root stump 10 cm, 3 noded vine cutting, 2 noded vine cutting and main root cuttings 5 cm.

The presence of large number of seeds in a fruit coupled with high germination per cent and field establishment and the high yielding nature of seedlings make this a commercially acceptable planting material.

Harvesting the roots 18 months after planting was found to be most beneficial for achieving maximum yield (4.14 t ha^{-1} and 1.56 t ha^{-1} of fresh and dry roots respectively).

Biometric characters related to yield such as internodal length, diameter of the vine and number of branches and all the root characters, driage and harvest index were maximum at this stage.

Total aminoacid and soluble carbohydrate content showed a progressive increase with advance in age and highest content was recorded at 18 months after planting.

Six free aminoacids were isolated from the roots and they were identified as alanine, aspartic acid, glycine, valine, serine and threonine.

Seedlings harvested after 18 months was the most desirable treatment combination for realising maximum yield of roots and chemical components.

Characters such as internodal length, diameter of vine, number of branches and root volume could be taken as selection indices for high root yield.

The benefit cost analysis suggest that adakodien is a profitable crop which can be groomed as a commercial crop for Kerala.