VARIABILITY IN MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERS IN KALMEGH

(*Andrographis paniculata* Nees.)

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Variability in morphological, physiological and biochemical characters in kalmegh *(Andrographis paniculata* Nees.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled **"Variability in morphological, physiological and biochemical characters in kalmegh *(Andrographis paniculata* Nees.)"** is a record of research work done independently by **Mr.K. Laju Paul**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him

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We, the undersigned members of the Advisory Committee of **Mr. K.Laju Paul,** a candidate for the degree of Master of Science in Agriculture with major in Plant Breeding and Genetics, agree that the thesis entitled '**Variability in morphological, physiological and biochemical characters in kalmegh *(Andrographis paniculata* Nees.)**' may be submitted by Mr. K.Laju Paul, in partial fulfillment of the requirement for the degree.

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EXTERNAL EXAMINER

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K. Laju Paul

Dedicated To My
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ABBREVIATIONS

ANOVA	-	Analysis of variance
CD	-	Critical difference
CGR	-	Crop growth rate
DAP	-	Days after planting
DAS	-	Days after sowing
DAT	-	Days after transplanting
df	-	Degrees of freedom
Fig.	-	Figure
GCV	-	Genotypic coefficient of variation
LAR	-	Leaf area ratio
MAP	-	Months after planting
MAT	-	Months after transplanting
NAR	-	Net assimilation rate
OD	-	Optical density
PCV	-	Phenotypic coefficient of variation
r	-	Regression coefficient
Rf	-	Relative front
RGR	-	Relative growth rate
TLC	-	Thin layer chromatography
UV	-	Ultra violet

Introduction

INTRODUCTION

India is one of the twelve mega biodiversity centres having over 45,000 plant species. It is also one of the richest countries for 'Medicinal and Aromatic Plant' genetic resources in the world. India apportions 11 per cent of total known world flora, though its total land mass is only 2.0 per cent of the whole world (Sharma, 1999). Around 3000 plant species are known for their medicinal value in India. Over 430 of these have been used in Ayurveda; 67 plant species are mentioned in Rigveda, 81 in Yajurveda and 289 in Atharvaveda. However nearly 550 botanical species are used commercially (Prakash, 1998).

More than 60 per cent of Indian population still depend on Ayurveda for the treatment of common diseases (Nair *et al.*, 1992). Plant and plant products are the main sources for various Ayurvedic preparations. Herbal medicines occupy an important position in India and other countries of world. Herbal medicines are also in great demand in the developed World for primary health care because of their biotic origin, efficacy, safety and lesser side effects. The turnover of herbal medicines in India is about $ 1 billion with a meagre export of about $ 80 million (Kamboj, 2000). Shortage in the availability of crude drugs of good quality is projected as the major limitation for the manufacture of medicines to meet the increasing demands. So to cope with this, there is an urgent need to scale up production by undertaking commercial cultivation.

Kalmegh (*Andrographis paniculata* Nees.) belonging to the family Acanthaceae is one of the 21 species of the genus. It is indigenous to India and has been used in Indian systems of medicines since time immemorial. It is one among the 23 red listed plants documented more than 300 years ago in Hortus Malabaricus (Kareem, 1996). It is an annual herb chiefly found in the plains throughout India from Himachal Pradesh to Assam and Mizoram and all over South India. The plant is known as 'Rice bitters' in West Indies and 'King of bitters' or 'Chiretta' in England. It is known as 'Kirata' in Sanskrit, 'Kirayat' in

Hindi, 'Kalmegh' in Bengali, 'Nilaveppu' and 'Kiriyat' in Malayalam, 'Nilavembu' in Telugu and 'Nelabevinagida' in Kannada (Vijaya and Nanavati, 1978).

Medicinal uses of the plant are many. The herb is reported to possess an astringent, anodyne, tonic and alexipharmic properties and is useful in dysentery, cholera, diabetes, consumption, influenza, bronchitis, swellings and itches, piles and gonorrhoea. A decoction of the plant is a blood purifier. It is used as a cure for torpid liver and jaundice. Macerated leaves of kalmegh when mixed with certain spices are found to relieve from gripe and other ailments in infants. A decoction or infusion of the leaves is useful in general debility and dyspepsia. The leaves and roots are also used as febrifuge, tonic, stomachache, cholagogue and aperient. In addition, anti-fungal, anti-typhoid and antibiotic activities are also reported in kalmegh (Anon., 1985).

The whole herb of *Andrographis paniculata* is the source of several diterpenoids of which andrographolide is important and is distributed all over the plant body. The plant is also an important source of flavonoids, sesquiterpenes and phenylpropanoids.

In spite of its numerous medicinal values, the crop is not being grown on a commercial scale anywhere in India. At present it is being collected generally from the forest areas, in which lot of variation in morphology, growth and the active ingredients are seen. Moreover, the availability of the plant through natural sources has also diminished considerably due to unscrupulous exploitation (Rajesh, 1994).

Variation within a single species is common in medicinal plants as in most of the other group of plants. Some of the common examples of medicinal plants in which intraspecific variation is prominently seen are *Abrus precatorius, Gloriosa superba, Clitorea ternatea and Withania somnifera* (Seeni *et. al.*, 1998).

In such cases it is necessary to collect and assess the variability in uniform situations to find out the existence of genetic variability. Growing of genetically superior types that are identified may bridge the gap between demand and supply to some extent. Since the crop is amenable to vegetative propagation through stem cuttings, superior genotypes identified can be maintained.

It is important to identify the optimum stage of growth at which the plant should be harvested for maximum benefit in terms of biomass and chemical content. Such stages for harvest had been standardised for several medicinal plants, for example *Adhatoda vasica* (Pandita *et al.*, 1983), *Solanum wrightii* (Indrayanto *et al.*, 1985) *Catharanthus roseus* (Sen and Datta, 1986) *Azhadirachta indica* (Banerjee and Datta, 1991).

The present study was undertaken at the College of Horticulture, Vellanikkara during the period from 1998 to 1999 with the following objectives.

1. Descriptive study on various morphological characters and their variation among the accessions.
2. To analyse the growth pattern of the plant and to estimate the optimum stage of harvest to get the maximum herbage yield.
3. To analyse variations for different biochemical compounds among the accessions.

Review of Literature

REVIEW OF LITERATURE

2.1 Origin and distribution

Acanthaceae is one of the largest tropical families having about 240 genera and over 2200 species. It has four centres of distribution namely, Indo Malaya, Africa, Brazil and Central America (Lawrence, 1969).

Hooker (1892) mentioned the occurrence of nineteen species of Andrographis in India, which according to him are very closely connected and identical in respect of form, colour of flowers and of seeds. Gamble (1921) reported twenty-one distinct species of Andrographis to occur in India. The genus Andrographis is comprised of annual herbs and shrubs including about 40 species distributed in the tropical Asia (Anon., 1948). Among the 21 species of genus Andrographis reported in India, 18 species are endemic and 10 species possess medicinal values (Ahmedullah and Nayar, 1986).

Andrographis paniculata known as kalmegh in Ayurveda is one of the important ingredients in various Ayurvedic preparations. It is also reported from Sri Lanka, Java, Mauritius, China, Thailand and several of the West Indian Islands (Bentley and Trimen, 1880). Dymock (1890) assigned the Isle of France as the origin of kalmegh in South India. According to Aiyar and Kolammal (1962) the plant is found in a wild condition of occasionally in cultivation throughout tropical India from Uttar Pradesh to Assam, to the extreme south. It has been recorded from Bengal, N. Circars, Deccan, Carnatic, Kerala *etc.* and it is quite common in moist uncultivated ground and also as an undergrowth of scrubby or deciduous forests as well as in dry ground under shade of trees, bushes etc. It has been recorded as occurring in the plains throughout India from Himachal Pradesh to Assam and Mizoram, and all over South India (Anon., 1985).

2.2 Economic Importance of the crop

Medicinal properties of kalmegh are many. Even in the 17th century, Rheede (1678-1708) in his Hortus Malabaricus described the morphology of the crop and its medicinal values under the name Cara-Caniram (Appendix IV). It forms the major ingredient of a household medicine called 'Alui' extensively used in West Bengal for general debility and certain forms of dyspepsia amongst adults and infants. The expressed juice of leaves is prescribed with cardamom, cloves and cinnamon in the form of globules to infants for their relief of bowel Complaints, irregular stools and loss of appetite (Dymock, 1890; Kirtikar and Basu, 1935 and Chopra *et al.*, 1958). The drug is reported to be a specific remedy for all types of fever especially intermittent fevers. It is laxative, dry cooling, bitter, light and overcomes *sannipata* type of fever, difficulty in breathing, *kapha*, *pitta*, vitiation of blood, burning sensation, cough, oedema, thirst, skin diseases, fever, ulcer and worms (Aiyar and Kolammal, 1962). Nadkarni (1954) reported that the roots of kalmegh are used as antipyretic, alterative and cholagogue agents. In the Pharmacopoeia of India kalmegh has been made official and directions for making a compound infusion and compound tincture are given (Anon., 1966).

Anti-inflammatory activity of kalmegh extracts was reported by Shahid (1985) and Madav *et al.* (1996). Hepatoprotective property of kalmegh was studied by many (Handa and Sharma, 1990; Rana and Avadhoot, 1991 and Trivedi and Rawal, 1998) and reported to be the most active. The plant is considered to be highly efficacious against chronic malaria (Vijaya and Nanavati, 1978; Dua *et al.*, 1999 and Rahman *et al.*, 1999). Reddy (1988) studied ethnobotany of *A. paniculata* and reported its use as a herbal drug in the treatment of Jaundice. Antipyretic properties of kalmegh was reported by Kanniappan *et al.* (1991) and Balu *et al.* (1993).

In Bangladesh, the hot water extracts of leaf and stem are prescribed as a powerful tonic (Ahmed and Talukder, 1977). Two new compounds carvacrol and

eugenol extracted from kalmegh were reported to show promising antifungal properties (Ojha, 1983). The decoction of the plant is used in some cases of anaemia (John, 1984) and in high blood pressure (Panthong *et al.*, 1986). Fresh leaf juice of kalmegh along with the leaf juice of neem and *Tinospora cordifolia* is taken to cure cholera (Reddy *et al.*, 1988).

In India, the entire plant is used to treat snakebites. Yanadees tribe uses pills made of fresh leaves of kalmegh and pulp of ripe tamarind as antidote to cobra venom. Snake repellent properties of kalmegh are discussed by Govindamenon (1931) and Nizamudeen *et al.*, (1978).

The important Ayurvedic preparations using the drug are *Tiktakaghrtam*, *Gorocanadi gulika*, *Candanasavam* etc. (Sivarajan and Balachandran, 1994). Girach *et al.* (1994) have discussed the possibility of substituting the much expensive and imported drug *Swertia chirayita* with kalmegh and found that it was highly promising.

2.3 Plant morphology

Kalmegh is a small, erect and branched herbaceous to semi woody annual. Bentley and Trimen (1880) described *A. paniculata* as a plant with stem about 1½-2 feet high, erect, stiff, thickened at lower nodes, quadrangular with the angles winged and having numerous long divaricate branches. Leaves opposite, lanceolate or oval, entire, dark green above and paler beneath. Flowers numerous, distantly arranged in a much branched cymose panicle; bracts very small, subulate, usually 3 at the base of each pedicel. Calyx small divided into 5 equal linear to subulate segments covered with stalked glandular hairs. Corolla 2-lipped; upper lip arched and bifid; lower lip cut into 3 short acute lobes. Stamens 2, inserted in the throat of the corolla; filaments flattened, tapering, ciliate above with a large tuft of hairs beneath the anthers; anthers 2-celled, sub-basifixed. Ovary small, laterally compressed with a small annular disc, 2 celled, with a few ovules in each cell. Style long as the stamens; stigma slightly bifid. Fruits ¾-1 inch long, oblong or

linear, acute, compressed, 2 celled, dehiscing foculicidally. Seeds few (6-10), round to ovoid, slightly compressed with retinaculum. Testa thick; hairy embryo curved; cotyledons ovate, thick; endosperm absent.

According to Hooker (1892) base of stem is not pubescent in kalmegh. Leaves are never spathulate but ovate at base. Petiole 0-1/4 inch, Racemes 1-4 inch, pedicels 0-1/6 inch, bract 1/16 inch, leaves 2½ by 1/2 – 3/4 inch in size respectively. Bracteoles smaller or absent. Inflorescence mostly sympodal. Sepals 1/8 inch in size and corolla 1/2 inch in size. Filaments hairy upwards. Capsules 3/4 by 1/8 inch in size.

Gamble (1921) has described inflorescence of kalmegh as an elongate raceme and sometimes subpaniculate. Aiyar and Kolammal (1962) described the leaves of kalmegh as simple, short petioled, opposite, lanceolate, entire and glabrous, narrowed at both ends from 2.5 cm to 7.5 cm long and about 12 mm wide. According to them inflorescence of kalmegh is an axillary horizontal simple dichotomous raceme with small distantly spaced whitish and purplish dotted irregular bilabiate flowers, one at each node. Fruits are linear-oblong to elliptic compressed capsules, 18 mm to 22 mm long and 3 mm wide containing 6-12 ovoid rugose, glabrous and flattened seeds.

Kirtikar and Basu (1935) and Datta and Mukerji (1952) have described *Andrographis paniculata* as an erect branched annual 0.3-0.9 m high; branches sharply quadrangular, often narrowly winged in the upper part. Leaves 5-7.5 cm by 1.2-2.5 cm, lanceolate, acute, glabrous, slightly undulate, pale beneath, base tapering, main nerve 4-6 pairs, slender petioles 0-6 mm long. Flowers small, solitary, distant in lax spreading axillary and terminal racemes or panicles, the whole forming a large pyramidal paniculate inflorescence; bracts 2.5 cm long, lanceolate; bracteoles smaller or absent, pedicels 0.8-0.4 mm long glandular and pubescent. Calyx 3 mm long, sepals equal, linear, lanceolate, glandular and pubescent. Corolla rose coloured, 1 cm long hairy outside, 2 lipped; corolla tube

5 mm long, slightly enlarged below the limb, upper lip equal in length, deeply 3-lobbed, the lobes 2.5 mm long linear, oblong sub-obtuse. Filaments flattened, hairy in the upper part; anthers bearded at the base; ovary glabrous, style slightly pubescent. Capsules 20 by 3 mm, linear oblong, acute at both ends. Seeds numerous, sub-quadrate, pitted, glabrous and yellowish brown.

2.4 Morphological and physiological variability

Andrographis paniculata has been reported to start its life cycle with first shower, whereas the second flush starts from the root stock during the end of winter season and the plant continues as perennial (Wahi, 1978). The morphological and histological aspects of these species were studied and reported to be different in different stages of development (Chen and Jiang, 1980). Wahi (1980) studied the pattern of energy accumulation in kalmegh. Plants grown under deep shade were found to conserve maximum energy provided maximum dry matter. Perennial nature in kalmegh was reported by Das and Das (1989).

Morphometric observations of kalmegh taken from natural habitat in Salem, revealed the following variation in plant characters. Leaf length varied from 15.1 to 18.0 cm and breadth between 5.0 and 6.1 cm. Average leaf area ranged between 12.65 and 39.85 cm^2. The basal leaf area varied between 452.3 and 1320.2 cm^2. The dry biomass of the individuals varied from 4.8 to 10.1 g .Shoot system recorded a maximum length of 126 cm and root system with a maximum of 16.3 cm (Alagesaboopathi, 1993).

The effect of sowing dates and spacing in kalmegh was studied by Rajesh (1994), and he observed that the plants sown at first June and harvested at 100 per cent flowering stage recorded maximum plant height (29.35 cm), plant spread (343.18 cm^2), number of branches (23.69), number of leaves (32.86), leaf area (134.84 cm^2), fresh weight per plant (15.64 g), dry weight per plant (8.81 g) and maximum number of days for 50 per cent flowering (126 DAS). At 30 x 30 cm

spacing the plant recorded maximum fresh weight/plant (10.17 g) and dry weight/plant (5.26 g).

Variation for internodal length (2.0 cm to 11.5 cm) and maximum root length (3.4 cm to 6.1 cm), measured at 115 days after planting was observed in *A. lineata* (Balu and Alagesaboopathi, 1995).

Alagesaboopathi and Balu (1995) recorded variation in internodal length (2.4 cm to 8.5 cm) and maximum root length (8.4 cm to 47.5 cm) in *Andrographis alata* when observed 190 days after planting.

Balu and Alagesaboopathi (1996) studied the morphometrics of the cuttings of kalmegh and observed variation for initial internodal length (1.5 cm to 2.2 cm), final internodal length (3.5 cm to 6.0 cm) and maximum root length (1.4 cm to 2.7 cm) when observed 110 days after planting.

Significant variation in plant height (23 to 48.5 cm), number of leaves/plant (20.85), fresh weight/plant (9.2 g to 23 g), dry weight/plant (4.3 g to 11.5 g); pods/plant (25 to 145), pod length (1.2 cm to 2.2 cm) and number of seeds/pod (4-6 to 8-10) were observed in different kalmegh accessions. (Anon., 1996).

Jamwal *and* Kaul (1997) evaluated three population samples of kalmegh for eight morphological characters and observed that a wide range of variation existed for most of the characters within the population as well as among population samples. They noted variability in plant height, stem diameter, total number of branches, leaf length, leaf breadth, total number of pods/plant, fresh weight of plant and dry weight of plant.

Significant variation in herbage yield was noticed at different stages of growth in kalmegh ranging from 760 to 3740 kg ha^{-1}. Total yield varied from 0.345 g /plant at 15 DAT to 41.17 g/plant at 140 DAT. The linear increase in the

dry yield of root was identical with that of stem, whereas there was a quantum increase in foliage upto 90 DAT (Anon., 1998).

Studies carried out on 52 germplasm collections of *Andrographis paniculata* at TBGRI showed that morphological variation observed in many accessions persisted through generation and kalmegh was identified as a medicinal plant showing prominent intraspecific variation (Seeni *et al.*, 1998).

Abnormal ternate phyllotaxy for leaves in *Andrographis paniculata* was reported by Alagesaboopathi and Balu (1998) from Thanjavur.

Kapur (1997) studied dry matter allocation in kalmegh under varying light intensities and reported that total dry matter production of the full light grown plants were maximum.

Alagesaboopathi and Balu (1996a) studied germination pattern and growth of kalmegh seedlings. They noted that seeds started to germinate 8 days after sowing and the percentage of germination was 80.

Trofimova (1994) studied seed germination and seedling development in 52 species of Acanthaceae under green house condition and classified *Andrographis paniculata* under the category of plants taking 7-14 days for germination.

Seed germination studies in *A. echioides* conducted at Tamil Nadu revealed that germination started after 11 days. The first 2 leaves appeared after a month and the percentage of germination was 75 (Alagesaboopathi and Balu, 1996b).

Pareek *et al.* (1981) noted that in periwinkle harvesting at 200 DAP gave maximum yield of leaves, stem and root.

A study on growth parameters of 10 late maturing pigeonpea genotypes showed considerable variability for physiological parameters such as leaf area index and specific leaf weight (Mehra *et al.*, 1987).

Moreno *et al.* (1987) reported large variations in plant morphology particularly in shoot architecture and types of inflorescence, flower, fruit and leaf characters among 17 introductions of the genus Ocimum.

Phadnis (1994) studied growth cycle of *Phyllanthus niruri* for 12 weeks and observed that major share of dry matter was concentrated in leaves and stem (75%) and that dry weight/fresh weight ratio varied with plant parts.

Studies on 18 collections of *Swertia chirayita* revealed that plant height, number of branches/plant, number of leaves/plant, leaf area and root thickness were significantly correlated with herbage yield and bitter content in herb (Rastogi and Srivastava, 1995).

A collection of 182 Ocimum accessions when evaluated for growth characteristics and yield components showed a wide range of variation within the accessions (Hamer *et al.*, 1996).

Growth analysis on castor cultivars showed significant difference for plant height, number of nodes, LAI, CGR, NAR, dry matter production and yield contributing factors (Reddy *et al.*, 1997).

Field investigation of 41 genotypes of soybean showed significant variation for days to 50 per cent flowering and maturity, leaf area/plant, plant height, root dry matter in yield, total dry matter/plant and yield/plant (Mehetre *et al.*, 1998).

Genetic divergence among 37 accessions of ashwagandha (*Withania somnifera*) were quantified for 6 characters namely plant height, plant canopy, leaf

area, root length, root diameter and dry yield. Five accessions were identified as widely divergent from each other (Misra *et al.*, 1998).

Dwivedi *et al.* (1999) studied 15 morphological traits in 26 genotypes of periwinkle and observed variation in most of the characters including total herbage yield, total leaf area, LAI and plant height.

Fifteen accessions of *Centella asiatica* when studied for growth, herbage yield and active principle content showed significant variation (Singh *et al.*, 1999).

2.5 Biochemical studies

2.5.1 Andrographolide

The active principle of kalmegh was first mentioned by Dymock (1890). According to him the aqueous infusion of the herb exhibits a slight acid reaction and has an intensely bitter taste, which appears due to an indifferent, non basic principle, for the usual reagents do not indicate presence of an alkaloid. Tannic acid produces abundant precipitate with the bitter principle. The infusion contains considerable quantity of chloride of sodium. It was Boorsma in 1911 who first attempted to isolate the active principle and he obtained a colourless neutral substance which he called as andrographide. The first distinctive contribution to the chemistry of *Andrographis paniculata* was that of Gorter in 1911 isolated the bitter principle in a pure cystalline form. He changed its name from andrographide to andrographolide, which is still in use today. He studied its reactions and transformations. According to him andrographolide has the molecular formula $C_{20}H_{30}O_5$ (mp. 278°C) and though neutral in character, dissolves slowly in alkali on warming and yields andrographolic acid ($C_{20}H_{32}O_6$) on acidification. Gorter also concluded that andrographolide is a lactone with three hydroxyl groups (one of which is tertiary) and more than one double bond.

Andrographolide is extremely bitter, sparingly soluble in benzene, ether, acetone, freely soluble in chloroform, methyl alchohol, ethyl alchohol, and almost insoluble in water. Moktader and Sircar (1939) re-examined andrographolide and confirmed the melting point, molecular formula and lactone nature of the compound as given by Gorter. But they failed to get a triacetyl derivative, and they claimed the presence of a methylene dioxy group instead. Rangaswami and Rao (1951) reinvestigated andrographolide and confirmed most of Gorter's findings. The sturucture and stereochemistry of andrographolide was determined by Arya (1962). Later andrographolide and itsderivatives were extracted from various plant parts of *Andrographis paniculata* and analyzed for their structure by many workers (Govindachary *et al.*, 1969; Xianglin *et al.*, 1981; Hu and Zhou, 1982; Gupta *et al.*, 1983; Ojha, 1983; Fujitha *et al.*, 1984 and Kuroyanagi *et al.*, 1987).

Maiti (1964) critically reviewed the chemistry of andrographolide the active principle of kalmegh. Analysis of the whole plant of kalmegh gave the following lactones (dry basis) andrographolide, 0.6 per cent; 14-deoxy-11-oxo-andrographolide ($C_{20}H_{28}O_5$, mp-98 to 100°), 0.121 per cent; 14-deoxy-11, 12 didehydroandrographolide ($C_{20}H_{30}O_4$ mp-203-204°), 0.06 per cent; 14-deoxyandrographolide ($C_{20}H_{30}O_4$ - mp 175°), 0.02 per cent and non bitter constituent neoandrographolide ($C_{26}H_{40}O_8$, mp 167-168°), 0.005 per cent. From the petroleum ether extract of the leaves α-, ß unsaturated lactone and homoandrographolide ($C_{22}H_{32}O_3$, mp 115°) were also identified (Anon., 1985).

Though several methods were suggested for the estimation of andrographolide, none of them were found to be satisfactory. The assay method described in the Pharmacopoeia of India is lacking in details and is unsatisfactory. The colorimetric method proposed by Maiti *et al.* (1959) was also found to be unsatisfactory as the colour was unstable and could not be measured satisfactorily in any photoelectric absorptiometer. Modified method of Srivastava *et al.* (1959) was tedious and not accurate. Subba Rao (1962) suggested a chemical method

involving a lactone titration but the method was reported to be not suitable for detecting quantities less than 100 mg. Talukdar *et al.* (1968) developed an improved titrimetric method for assay for andrographolide. Gaind *et al.* (1963) described a spectrophotometric method of assay of andrographolide by measuring its absorption at 226 nm. This method has been claimed to be more rapid and more accurate than the previously mentioned methods. Chauhan *et al.* (1999) developed a precise and sensitive high performance thin layer chromatography (HPTLC) method for the estimation of andrographolide. They found that the sensitivity was 0.10 µg and linearity in the range 0.1 to 1.0 µg.

The whole herb of kalmegh is an important source of andrographolide and is distributed all over the plant body. In Vietnam it is obtained from leaves alone (Tuu *et al.*, 1984). In India the entire plant is used (Randa and Sharma, 1990). Andrographolide content varied among plant parts and among different accessions of kalmegh. Moktader and Sircar (1939) reported varying yields (1.5 to 2.5%) in andrographolide from the leaves of fully mature plants grown under field conditions. But the samples purchased locally gave as low as 0.8 to 1 per cent. According to Chakravarti and Chakravarti (1952) leaves of kalmegh contain the maximum (2.5%) andrographolide content, while the stem contains lesser amount (2.0%). Datta and Mukerji (1952) noticed andrographolide content in leaves of kalmegh varying from 0.5 to 6.08 per cent. Maiti *et al.* (1959) pointed out that andrographolide content of kalmegh varied considerably with seasons, maximum of 2 per cent in October-November and minimum 0.5 per cent after January.

According to Pharmacopoeia of India, kalmegh yielding less than 1% andrographolide may not be considered as standard raw drug.The cytotypes of kalmegh collected from different localities in India and Bangladesh were found to contain varied quantities of andrographolide in leaves. The cytotype collected from Jessore in Bangladesh recorded the maximum (3.84%) and that from Trivandrum showed minimum (0.98%) (Roy and Datta, 1987).

Studies conducted by Alagesaboopathi (1993) on three different species of Andrographis recorded wide variation in the andrographolide content. Petroleum ether extracts of plant samples yielded 1.5 per cent andrographolide in kalmegh and 0.45 and 1.3 per cent in *Andrographis alata* and *Andrographis lineata* respectively. But ethanol extracts of plant samples yielded 11.8, 6.88 and 8.75 per cent in *Andrographis paniculata, Andrographis alata* and *Andrographis lineata* respectively. Variation in andrographolide to the range 1.2 to 3.55 per cent has also been reported in *Andrographis paniculata* (Anon., 1996). Jamwal and Kaul (1997) studied three population samples of kalmegh and observed andrographolide varying from 1.87 to 2.21 per cent. Andrographolide content was variable in each plant part at different stages with maximum recorded at leaves and stem (Anon., 1998).

Farooqi *et al.* (1999) reported a maximum of 2.5 per cent andrographolide in leaves and a minimum of 2 per cent in stem. Analysis of 52 germplasm collections of kalmegh maintained under uniform condition at TBGRI showed variation in andrographolide content at a range from 0.5 to 1.5 per cent dry weight (Padmesh *et al.*, 1999). Investigation carried out on collections of chirata revealed a great deal of variation ranging from 0.75 to 1.14 per cent with respect to bitter content in the crop (Dutt *et al.*, 1999).

2.5.2 Phenolic compounds

The term phenolic compound embraces a wide range of plant substances, which possess in common an aromatic ring bearing one or more hydroxyl substituents. Among the natural phenolic compounds of which over a thousand structures are known the flavonoids form the largest group, but simple monocyclic phenols, phenyl propanoids and phenolic quinones all exist in considerable numbers (Harborne, 1973).

Since total phenols and flavonoids come under phenolic compounds they are reviewed here together. In *Andrographis paniculata* chemical investigations on phenolic compounds have been confined to flavonoids only, hence here stress is given to the literature related with flavonoid compounds only.

Bhardwaj *et al.* (1981) isolated two flavones from kalmegh namely 5 hydroxy-7,8,2' trimethoxy flavone and 5,2' dihydroxy-7,8 dimethoxy flavone.

Gupta *et al.* (1983) isolated and characterised two flavonoides 5 hydroxy-7,8-dimethoxy flavanone and 5 hydroxy-3,7,8,2' tetramethoxy flavone. In addition they reported 5-hydroxy-7,8 dimethoxy flavone (7-0-Methyl Wogonin) for the first time in *A. paniculata*. Phenyl propanoid and eugenol has been reported in the aerial parts of kalmegh showing considerable variation in content (Ojha, 1983).

Roots of kalmegh were reported to possess 0.006 per cent of the natural flavone, 5-hydroxy-7,8,2',3'-tetramethoxy flavone (Anon., 1985).

In Japan, kalmegh roots were extracted for several flavanoids namely Andrographidin A, B, C, D, E and F whose contents varied from 0.015 to 0.15 per cent (Kuroyanagi *et al.*, 1987).

Roots of kalmegh gave flavones apigenin-7,4'-di-O-methyl ether andrographis and panicolin (Hussain *et al.*, 1992).

Gupta *et al.* (1996) took ethanol extracts of the roots of kalmegh on column chromatography and furnished two flavonoid glycosides namely 2'-5'dihydroxy'7,8-dimethoxy flavone, 2'-O-ß(D)-glucoside and 3ß-hydroxy-5-stigmasta-9(11), 22(23)-diene.

HPLC analysis of leaf extracts of *Glycyrrhiza glabra* has shown considerable differences in the content of flavonoids (Hayashi *et al.*, 1995).

Alagesaboopathi and Balu (1996c) studied three different species of Andrographis and reported the presence of flavonoids in all the species.

Sarma (1998) investigated the chemotaxonomy of 42 taxa of acanthaceae family and observed positive reaction for flavonoids in all taxa including *Andrographis paniculata* and *Andrographis echioides.*

2.5.3 Other compounds

Nadkarni (1954) has reported occurrence of a bitter substance in an amorphous form from the kalmegh plant. Sesquiterpenes like paniculide A, B and C have been reported to be obtained from callus tissues of kalmegh (Allison *et al.*, 1968). Presence of α-sitosterol was reported in kalmegh roots by Govindachari *et al.*, 1969.

Ghose (1984) mentioned about the occurrence of traces of essential oil in *Andrographis paniculata.*

Petroleum ether extracts of kalmegh leaves collected from types in Bangladesh yielded andrographosterol, andrographane, andrographone, a wax and two esters containing hydroxyl groups (Anon., 1985).

Polyphenols, caffeic and chlorogenic acids, and a mixture of dicaffeolyquinic acid from leaves of kalmegh have been reported (Asolkar *et al.*, 1992).

Three diterpenoids namely andrographanin, andrographanoside and 14-deoxy-12-methoxy andrographolide were isolated and characterized from kalmegh (Rastogi and Mehrotra, 1993).

Dried and powdered leaf samples were tested for availability of phytochemicals and presence of steroids, alkaloids, tannins, and saponins were reported in *Andrographis paniculata, Andrographis lineata* and *Andrographis alata* (Alagesaboopathi and Balu, 1996c).

Materials and Methods

MATERIALS AND METHODS

The present investigation on evaluation of variability in morphological, physiological and biochemical characters of kalmegh (*Andrographis paniculata* Nees.) was conducted in the Department of Plant Breeding and Genetics and the Biochemistry Laboratory, College of Horticulture, Vellanikkara, Thrissur. The crop was raised during the period from June 1999 to December 1999. The location is situated at an altitude of 40.29 m above MSL at 10° 31' N latitude and 76° 13' E longitude.

3.1 Materials

Germplasm of kalmegh including different local collections and accessions maintained at various institutes formed the materials for the present study. The details of these genotypes are given in Table 1.

Table 1. Details of sources of accessions utilised in the study

Sl No	Accession	Source		
		Village/Institute	District	State
1	Ac-1	GKVK campus	Bangalore	Karnataka
2	Ac-2	Kanchikode	Palakkad	Kerala
3	Ac-3**	Vellanikkara	Thrissur	Kerala
4	Ac-4	Aluva	Ernakulam	Kerala
5	Ac-5*	Chevakkadu	Tuticorin	Tamil Nadu
6	Ac-6	Thrissur	Thrissur	Kerala
7	Ac-7**	Vellanikkara	Thrissur	Kerala
8	Ac-8*	Semmedu	Namakkal	Tamil Nadu
9	Ac-9	Kottackal	Malappuram	Kerala
10	Ac-10	Murukkumpuzha	Thiruvananthapuram	Kerala

* Accessions collected and maintained by NBPGR Regional Station Vellanikkara, as IC-210699 and IC-210635 respectively

** Accessions maintained by AICRP on medicinal and aromatic plants, College of Horticulture, Vellanikkara

3.2 Methods

3.2.1 Cultivation

3.2.1.1 Nursery

Nursery beds of 60 cm x 60 cm size and 25 cm height were prepared. Dried cow dung @ 1 kg per bed was incorporated. Seeds collected from mature capsules of various accessions were mixed with sand and sown in separate rows at spacing of 10 cm between rows. Sowing was done in the month of June.

3.2.1.2 Land preparation

The experimental field (main field) was thoroughly ploughed thrice to provide uniform soil conditions. Beds of 3 m x 1.8 m size and 25 cm height were prepared with 30 cm wide channels between them.

3.2.1.3 Planting

Seedlings 45 DAS were used for planting. At this stage seedlings were 4-5 leafed with a shoot length ranging from 5 to 12 cm and a root length ranging from 2 to 7 cm. Seedlings were planted at a spacing of 30 cm x 30 cm. Dried and powdered cow dung @ 20 t ha^{-1} was applied before planting. A population of 60 plants including border plants (six rows @ ten plants per row), per plot was given (one lakh eleven thousand one hundred and eleven plants per hectare). Planting was done in the first week of August 1999.

3.2.2 The experimental design

The experimental design was RBD with ten accessions as treatments and three replications (Appendix I).

3.2.3 Sampling technique

Random sampling technique was adopted to select the sample plants for destructive sampling. Three plants were selected randomly eliminating the border rows from each plot, labelled and used for growth analysis. Data for morphological

characters were recorded from five plants, located centrally in the plot. For the biochemical analysis, these five sample plants were bulked together at the time of final harvest to get a representative sample.

3.2.4 Morphological studies

The description of morphological characters was based on the terms proposed by Radford (1986). Observations were recorded on the following morphological characters.

Character		Sample size
1.	General habit	
I	Appearance	
II	Nature of branching	
III	Plant height (cm)	: Mean of 20 plants
IV	Plant spread (cm)	: Mean of 20 plants (Spread of plant was measured as maximum plant circumference using a thread)
2. Stem		
I	Nature	
II	Diameter (cm)	: Mean of 20 plants (Measured at the base of stem)
III	Number of nodes	: Mean of 20 plants
IV	Internodal length (cm)	: Mean of 20 plants (Mean of four internodes at the middle portion of stem was taken from each plant)
3. Root		
I	Type	
II	Root length (cm)	: Mean of 20 plants

4.	Leaf	
I	Type	
II	Shape of leaf	
III	Leaf length (cm)	: Mean of 50 leaves
IV	Leaf width (cm)	: Mean of 50 leaves
V	Petiole length (cm)	: Mean of 20 leaves
VI	Leaf tip	
VII	Number of main nerves (in pairs)	
VIII	Leaf base	
IX	Leaf colour	
X	Leaf arrangement	
XI	Leaf margin	
XII	Leaf texture	

5. Inflorescence

I	Type	
II	Branching habit	
III	Peduncle length (cm)	: Mean of 15 inflorescence
IV	Inflorescence length (cm)	: Mean of 15 inflorescence (Three basal inflorescence from each plant)

6. Flower

I	General	
	a) Type	
	b) Pedicel length (cm)	: Mean of 15 flowers
II	Calyx	
	a) Sepal length (cm)	: Mean of 15 flowers
	b) Sepal apex	

III Corolla

a) Type

b) Corolla tube length (cm) : Mean of 15 flowers (From base to tip of lower lip of corolla)

IV Androecium

a) Type

b) Filament length (cm) : Mean of 15 flowers

c) Hairiness of filament

V Gynoecium

a) Type

b) Ovary size (cm) : Mean of 15 flowers

c) Style length (cm) : Mean of 15 flowers

d) Ovary shape

7. Fruit

I Type

II Fruit colour

III Fruit shape

IV Fruit length (cm) : Mean of 20 fruits

V Fruit breadth (cm) : Mean of 20 fruits

VI No. of seeds/fruit : Mean of 20 fruits

8. Seeds

I Type

II 100 seed weight (g) : Mean of 3 lots (100 seeds/lot) From each accession

3.2.2 Physiological studies

Destructive sampling was done at monthly intervals after transplanting. Three plants were uprooted randomly from each plot and used for observations on plant characters and physiological parameters and average worked out.

3.2.5.1 Biometrical observations

3.2.5.1.1 Total plant fresh weight (g)

Plants uprooted from each plot were washed thoroughly to remove soil particles adhering to roots. Plants were then kept under shade for fifteen minutes to dry. The whole plant was then weighed. Average weight was recorded.

3.2.5.1.2 Stem dry weight (g)

Plants after harvest were separated into stem, root and leaves. Each component was separately dried at 70-80°C in a hot air oven until the constant weight was reached. Average weight of stem was recorded.

3.2.5.1.3 Root dry weight (g)

Average weight of roots was recorded after drying at 70-80°C in a hot air oven till the constant weight was reached.

3.2.5.1.4 Leaf dry weight (g)

Average weight of leaves was recorded after drying at 70-80°C in hot air oven till the constant weight was reached.

3.2.5.1.5 Total plant dry weight (g)

Sum of dry weights of stem, root and leaf were recorded as total plant dry weight.

3.2.5.1.6 Shoot length (cm)

Length of stem from the base to the tip was measured for each plant and the average worked out.

3.2.5.1.7 Root length (cm)

Length of root from the base to the tip was measured for each plant and the average worked out.

3.2.5.1.8 Dry weight/fresh weight

Ratio of total plant dry weight to total plant fresh weight was worked out.

3.2.5.1.9 Days to 50 per cent flowering

Approximate number of days taken for 50 per cent flowering in each accession were recorded.

3.2.5.1.10 Moisture content (%)

Moisture content in plant sample was worked out as the difference in total plant dry weight from total plant fresh weight expressed as percentage.

$$MC = \frac{(FW-DW)}{FW} \times 100$$

Where

MC = Moisture content

FW = Fresh weight (g)

DW = Dry weight (g)

3.2.5.2 Physiological parameters

3.2.5.2.1 Leaf area (cm^2)

Leaf area per plant was estimated following linear regression equation based on dry weight of leaves. Equation obtained was

$$y = a + bx$$

Where y = Leaf area (cm^2)
a = 0.6734
b = 132.98
x = Leaf dry weight (g)
Regression coefficient (r) = 0.89

3.2.5.2.2 Leaf area index (LAI)

Leaf area index was estimated as the ratio of total leaf area of plant to the ground area. LAI was worked out as suggested by Watson (1952) for all the accessions.

$$LAI = \frac{\text{Leaf area per plant}}{\text{Land area occupied per plant}}$$

3.2.5.2.3 Leaf Area Ratio (LAR)

LAR is the ratio of leaf area to dry weight of plant expressed as cm^2g^{-1} (Whitehead and Mycersough, 1962).

$$LAR = \frac{LA}{W}$$

Where

LA = Total leaf area per plant

W = Total plant dry weight

3.2.5.2.4 Relative Growth Rate (RGR)

RGR expresses the dry weight increase in a time interval in relation to the initial weight and is expressed as g g^{-1} day^{-1}. It was calculated following the formula given by Blackman (1919).

$$RGR = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where

In = logarithm to the base 'e' (Naperian constant)

W_2 and W_1 = Total plant dry weight at time T_2 and T_1 respectively

3.2.5.2.5 Net Assimilation Rate (NAR)

NAR is the net gain of assimilates (net photosynthesis) per unit leaf area time (Redford, 1967).

$$NAR = \frac{(W_2 - W_1)(\ln LA_2 - \ln LA_1)}{(T_2 - T_1)(LA_2 - LA_1)}$$

where

In = logarithm to the base 'e' (Naperian constant)

LA_2 and w_2 = Leaf area and total plant dry weight at time T_2

LA_1 and W_1 = Leaf area total plant dry weight at time T_1

NAR is expressed as g cm^{-2} day^{-1}

3.2.5.2.6 Crop Growth Rate (CGR)

The gain in weight of a community of plants on a unit land per unit time is called CGR, expressed as g cm^{-2} day^{-1} (Watson, 1952).

$$CGR = \frac{W_2 - W_1}{P\,(T_2 - T_1)}$$

where

P = Land area occupied by the plant

W_2 and W_1 = Total plant dry weight at T_2 and T_1 time respectively

3.2.5.2.7 Vigour Index (VI)

Vigour Index was estimated using the method suggested by Abdul-Baki and Anderson (1973). Ten seeds from each accession were taken and placed in the middle of a blotting paper (20 x 20 cm) at equal distance. Blotting paper was then rolled in the form of a cylinder. These rolls are dipped in a beaker filled with water. Plumule length and radicle lengths were measured after 25 days. Germination percentage was also noted.

$$VI = [RL + SL] \times \text{germination } \%$$

where

RL = Root length (cm)

SL = Shoot length (cm)

3.2.6 Biochemical studies

3.2.6.1 Total phenol content

Total phenol content was estimated using Folin-Ciocalteau method (Sadasivam and Manickam, 1966). 0.5 gram of finely powdered plant sample was ground in a pestle and mortar with ten times volume of water and made up to 50 ml. Made up solution was further diluted ten times. 1 ml of diluted solution was taken in a testube, and made up to 3 ml with water, followed by the addition of 2 ml 20 per cent Na_2CO_3 and 0.5 ml Folin Ciocalteau reagent and kept in a boiling

water bath for one minute cooled down to room temperature. The intensity of blue colour developed after cooling was read at 650 nm in a spectrophotometer. Total phenol content was calculated from a standard curve of catechol and was expressed as mg of phenol per g of plant sample.

$$\text{Total phenol content} = \text{Factor} \times \frac{\text{Absorbance}}{\text{Volume made}} \times \frac{\text{Dilution (ml)}}{\text{Weight of sample (mg)}} \times \frac{1000}{1}$$

3.2.6.2 Andrographolide content

Andrographolide content was estimated using spectrophotometric method (Gaind *et al.*, 1963). 0.5 g finely ground plant sample (whole plant) was refluxed with benzene (50 ml) thrice, to remove the green colouring matter of kalmegh. Pigment free residue was mixed with 5g Kiesselguhr and extracted with chloroform in a soxhlet apparatus for 5 hours. Chloroform extract was evaporated and the solvent free extract was dissolved in methanol and made up to 50 ml. One ml from this solution was taken and further diluted to 50 ml and the absorbance was read at 226 nm. Concentration of andrographolide was expressed in per cent of plant dry weight.

3.2.6.3 Flavonoid content

To determine presence of flavonoids from solvent extract, the method suggested by Harborne (1973) was followed. 5 g of finely powdered dry plant sample was extracted with methanol in a soxhlet apparatus for 5 hours. It was then concentrated to 5 ml and used for spotting on TLC plates. TLC plates were prepared by coating 300 µm thick layer of silica-gel-G on glass plates of 20 cm x 20 cm size. Spotting on TLC plates was done with the help of capillary tubes. The capillary tubes were calibrated to 5, 10, 15 and 20 µl marks. 20 µl volume was spotted per sample. Spotting was done 2 cm above the lower edge of plate in a straight line maintaining a distance of 1.5 cm between two consecutive spots.

Running solvent system was prepared by mixing hexane and ethyl acetate at various proportions. Spotted plates were placed in a CAMAG TLC developing glass tank for elution. After the solvent front had reached 2/3rd length of the plate, the plates were taken out for air drying and observed under UV light for the presence of fluorescent spots. Rf values for these spots were recorded.

3.2.7 Statistical analysis

The data collected were subjected to statistical analysis using MSTATC package. Genotypic and phenotypic variances (σ^2g and σ^2p respectively) were calculated as follows

$$\sigma^2_g = (MST-MSE)/r$$
$$\sigma^2_p = \sigma^2_g + MSE$$

Where MST, MSE and r are treatment (accession) mean square, error mean square and replication respectively.

GCV and PCV were worked out as (σ_g x 100)/mean and (σ_p x 100)/mean respectively. Where σ_g and σ_p are genotypic and phenotypic standard deviation respectively. Heritability (broad sense) was estimated as σ^2_g / σ^2_p.

Results

RESULTS

4.1 Morphological observations

The various morphological characters for different accessions (Plates I to IV) of kalmegh are presented in Table 2 and Appendix III.

Table 2. Morphological features of different accessions of kalmegh

Accession	Habit				Stem			
	Appearance	Nature of branching	Plant height (cm)	Plant spread (cm)	Nature	Diameter (cm)	Nodes/ plant	Internodal length (cm)
Ac-1	Annual erect herb	A few branches	59.16	57.29	Dark green quadrangular & woody	2.46	15.8	4.25
Ac-2	Annual erect herb	A few branches	57.56	55.30	Dark green quadrangular & woody	3.16	19.3	2.85
Ac-3	Annual erect subherb	Profuse	55.96	60.62	Dark green quadrangular & woody	3.38	20.9	2.95
Ac-4	Annual erect herb	Profuse	65.95	53.37	Dark green quadrangular & woody	2.90	21.6	2.70
Ac-5	Annual erect subherb	Profuse	63.98	69.72	Dark green quadrangular & woody	3.50	22.3	2.97
Ac-6	Annual erect subherb	Profuse	66.00	61.62	Dark green quadrangular & woody	3.30	22.1	2.88
Ac-7	Annual erect herb	A few branches	59.66	56.70	Green quadrangular & woody	2.82	18.1	2.81
Ac-8	Annual erect sub herb	A few branches	58.17	78.11	Dark green quadrangular, woody	3.87	17.5	2.87
Ac-9	Annual erect herb	A few branches	49.33	53.16	Green quadrangular herbaceous	2.98	19.9	3.38
Ac-10	Annual erect herb	A few branches	52.43	55.16	Dark green quadrangular herbaceous	2.83	19.9	2.93

Table 2. (continued)

Accession	Root		Leaf				
	Type	Root length (cm)	Type	Shape	Leaf length (cm)		
					Basal	Middle	Top
Ac-1	Primary	21.24	Simple	Lanceolate to ovate	7.2	6.1	3.7
Ac-2	Primary	18.96	Simple	Lanceolate to ovate	10.1	9.1	4.4
Ac-3	Primary	19.78	Simple	Lanceolate to ovate	8.8	8.0	3.5
Ac-4	Primary	21.13	Simple	Lanceolate to ovate	7.6	6.5	4.7
Ac-5	Primary	19.47	Simple	Lanceolate to ovate	7.7	6.7	3.8
Ac-6	Primary	22.04	Simple	Lanceolate to ovate	6.6	6.4	3.1
Ac-7	Primary	16.13	Simple	Lanceolate to Narrowly elliptic	6.5	5.6	3.7
Ac-8	Primary	22.83	Simple	Lanceolate to ovate	9.2	6.2	3.9
Ac-9	Primary	28.17	Simple	Narrowly elliptic	8.7	5.9	3.4
Ac-10	Primary	19.00	Simple	Lanceolate to ovate	5.3	6.1	3.2

Table 2. (continued)

Accession	Leaf					
	Leaf breadth (cm)			Petiole length (cm)	Leaf tip	Leaf margin
	Basal	Middle	Top			
Ac-1	2.5	1.7	0.6	0.23	Acuminate	Undulate to entire
Ac-2	3.3	2.3	1.1	0.32	Acuminate	Entire
Ac-3	2.8	1.9	0.7	0.31	Acuminate	Undulate to entire
Ac-4	2.7	2.0	1.2	0.21	Acuminate	Undulate to entire
Ac-5	2.5	1.7	0.7	0.13	Acuminate	Entire
Ac-6	2.7	2.1	1.1	0.18	Acuminate	Entire
Ac-7	1.8	1.5	0.8	0.24	Acuminate	Entire
Ac-8	2.4	1.4	0.8	0.32	Acuminate	Entire
Ac-9	2.5	1.4	0.5	0.33	Acuminate	Undulate to entire
Ac-10	1.8	1.6	0.6	0.16	Acuminate	Undulate to entire

Table 2. (continued)

Accession	Leaf								
	Main nerves (in pairs)	Base	Colour				Arrangement	Attachment	Texture
			Young leaves		Old leaves				
			Upper side	Lower side	Upper side	Lower side			
Ac-1	3-4	Acute	Dark green	Pale green	Reddish brown	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-2	3-6	Acute	Dark green	Pale green	Dark green	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-3	3-4	Acute	Dark green	Pale green	Dark green	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-4	3-4	Acute	Green	Pale green	Green with red patches	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-5	3-5	Acute	Green	Pale green	Yellowish green	Pale green	Opposite decussate	Sub sessile	Chartaceous
Ac-6	3-4	Acute	Green	Pale green	Green with red patches	Pale green	Opposite decussate	Sub sessile	Chartaceous
Ac-7	3-4	Acute	Green	Pale green	Yellowish green with red patches	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-8	3-5	Acute	Dark green	Pale green	Dark green	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-9	3-4	Acute	Dark green	Pale green	Dark green	Greyish green	Opposite decussate	Petiolate	Chartaceous
Ac-10	3-5	Acute	Green	Pale green	green	Pale green	Opposite decussate	Sub sessile	Chartaceous

Table 2. (continued)

Accession	Inflorescence				Flower	
	Type/Arrangement	Branching habit	Peduncle length (cm)	Inflorescence length (cm)	Type	Pedicel length (cm)
Ac-1	Panicle Terminal & axillary	Low	2.69	9.09	Pedicellate	0.43
Ac-2	Panicle Terminal & axillary	Medium	2.70	7.24	Pedicellate	0.56
Ac-3	Panicle Terminal & axillary	Medium	2.64	7.51	Pedicellate	0.47
Ac-4	Panicle Terminal & axillary	High	2.06	6.60	Pedicellate	0.38
Ac-5	Panicle Terminal & axillary	High	2.45	7.92	Pedicellate	0.42
Ac-6	Panicle Terminal & axillary	High	2.12	9.29	Pedicellate	0.51
Ac-7	Panicle Terminal & axillary	High	2.32	8.78	Pedicellate	0.37
Ac-8	Panicle Terminal & axillary	Medium	2.67	10.93	Pedicellate	0.44
Ac-9	Panicle Terminal & axillary	Low	3.10	7.27	Pedicellate	0.59
Ac-10	Panicle Terminal & axillary	Medium	2.43	9.24	Pedicellate	0.52

Table 2. (continued)

Accession	Calyx		Corolla		Androecium			Gynoecium			
	Sepal Length (cm)	Apex	Type	Corolla tube length (cm)	Type	Filament length (cm)	Hairiness of filament	Type	Ovary shape	Ovary Size (cm)	Style Length (cm)
Ac-1	0.34	Linear	Bilabiate	1.32	Stamens - 2, Sub basifixed	0.97	Hairy upward	Laterally compressed, bilocular	Oblong	1.56	1.35
Ac-2	0.32	Linear	Bilabiate	1.27	Stamens - 2, Sub basifixed	1.01	Hairy upward	Laterally compressed, bilocular	Oblong	1.78	1.24
Ac-3	0.34	Linear	Bilabiate	1.24	Stamens - 2 , sub basifixed	1.01	Hairy upward	Laterally compressed, bilocular	Oblong	1.43	1.34
Ac-4	0.36	Linear	Bilabiate	1.34	Stamens - 2 , sub basifixed	1.00	Hairy upward and at base	Laterally compressed, bilocular	Oblong	1.52	1.41
Ac-5	0.29	Linear	Bilabiate	1.07	Stamens - 2 , sub basifixed	0.98	Hairy upward	Laterally compressed, bilocular	Oblong	1.52	1.31
Ac-6	0.33	Linear	Bilabiate	1.15	Stamens - 2 , sub basifixed	1.11	Hairy upward	Laterally compressed, bilocular	Oblong	1.54	1.33
Ac-7	0.36	Linear	Bilabiate	0.97	Stamens - 2 , sub basifixed	1.08	Less hairy	Laterally compressed, bilocular	Oblong	1.56	1.18
Ac-8	0.32	Linear	Bilabiate	1.13	Stamens - 2 , sub basifixed	1.19	Hairy upward	Laterally compressed, bilocular	Oblong	1.41	1.30
Ac-9	0.39	Linear	Bilabiate	1.21	Stamens - 2 , sub basifixed	1.21	Hairy upward	Laterally compressed, bilocular	Oblong	1.53	1.32
Ac-10	0.37	Linear	Bilabiate	0.98	Stamens - 2 , sub basifixed	1.05	Hairy upward	Laterally compressed, bilocular	Oblong	1.32	1.27

Table 2. (continued)

Accession	Fruit							Seed	
	Type	Fruit colour		Fruit shape	Fruit length (cm)	Fruit breadth (cm)	No. Of seeds/fruit	Type	100 seed weight (g)
		Initiation	Mature						
Ac-1	Capsule	Pale green	Brownish yellow	Oblong	1.60	0.34	8-10	Subquadrate, osseous rugose with retinaculum	0.1663
Ac-2	Capsule	Pale green	Brownish yellow	Oblong	1.80	0.40	6-9	Subquadrate, osseous rugose with retinaculum	0.1717
Ac-3	Capsule	Pale green	Reddish brown	Oblong	1.87	0.32	6-10	Subquadrate, osseous rugose with retinaculum	0.1400
Ac-4	Capsule	Pale green	Reddish brown	Oblong	1.76	0.31	8-10	Subquadrate, osseous rugose with retinaculum	0.1367
Ac-5	Capsule	Pale green	Brown	Oblong	1.72	0.37	5-7	Subquadrate, osseous rugose with retinaculum	0.1692
Ac-6	Capsule	Pale green	Brown with reddish tinge	Oblong	1.80	0.40	6-8	Subquadrate, osseous rugose with retinaculum	0.1777
Ac-7	Capsule	Pale green	Brown	Oblong	1.83	0.37	6-9	Subquadrate, osseous rugose with retinaculum	0.1413
Ac-8	Capsule	Pale green	Brown	Oblong	1.72	0.31	8-10	Subquadrate, osseous rugose with retinaculum	0.1458
Ac-9	Capsule	Pale green	Reddish brown	Oblong	1.74	0.37	5-8	Subquadrate, osseous rugose with retinaculum	0.1674
Ac-10	Capsule	Pale green	Brownish yellow	Oblong	1.83	0.39	6-8	Subquadrate, osseous rugose with retinaculum	0.1734

Plate II General habit of different accessions of kalmegh

IIa Ac-1 and Ac-7
IIb Ac-2 and Ac-3

Plate I. General view of crop in the field

Ia. Front view

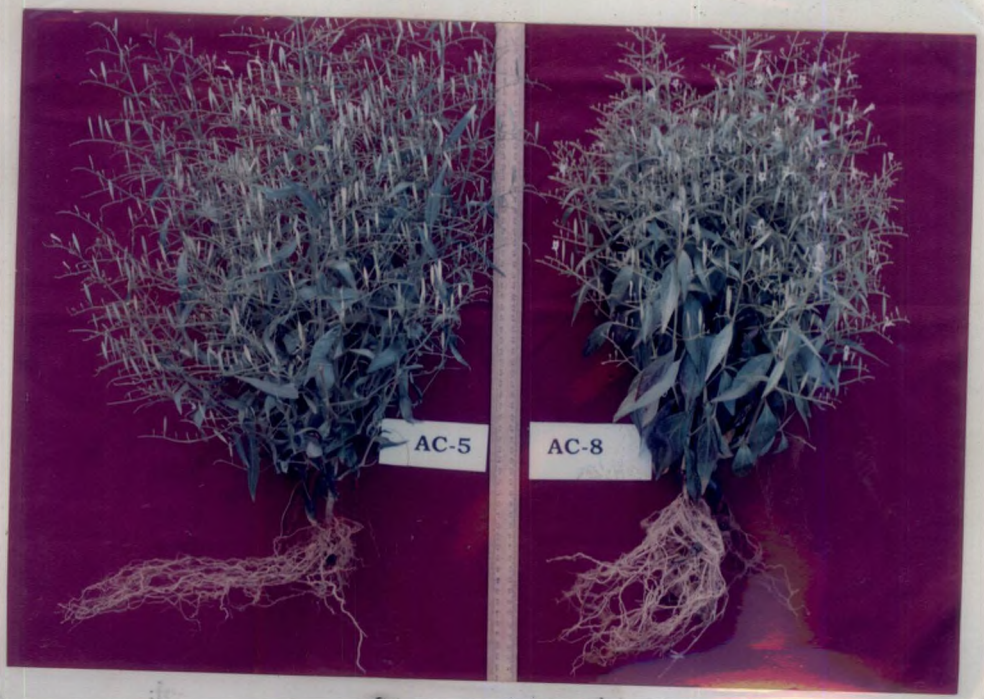
Ib. Side view

Plate II General habit of different accessions of kalmegh

IIc Ac-5 and Ac-8

IId Ac-6 and Ac-4

Plate – II



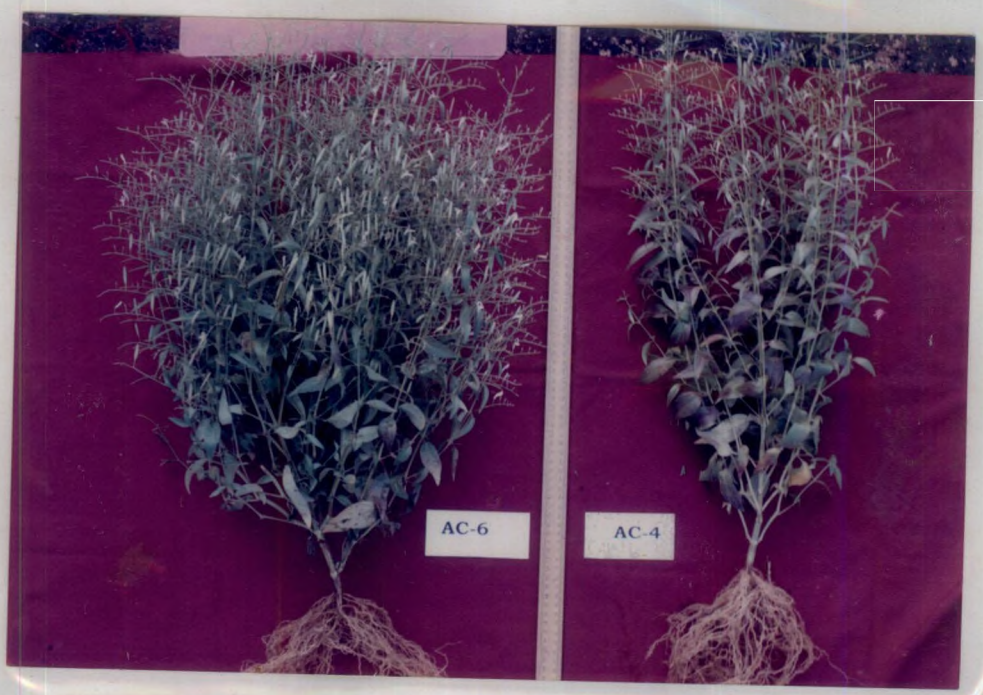


Plate II General habit of different accessions of kalmegh

IIe Ac-9 and Ac-10

Plate – II

Plate III Twig with inflorescence in different accessions of kalmegh

IIIa Ac-4, Ac-2 and Ac-7
IIIb Ac-6, Ac-1 and Ac-3

Plate – III

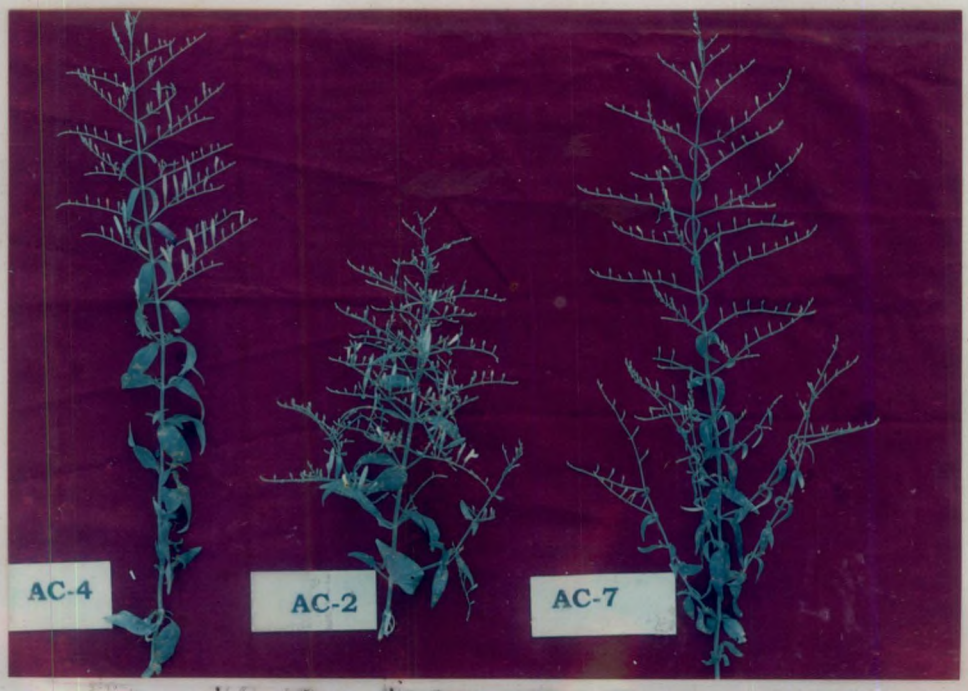


Plate – II

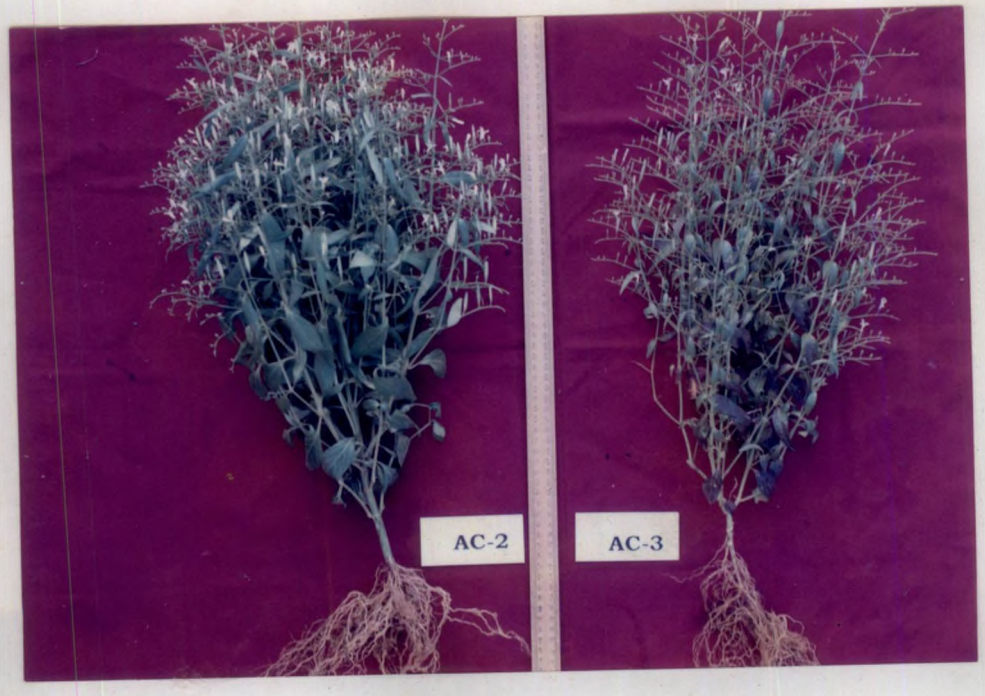


Plate III Twig with inflorescence in different accessions of kalmegh

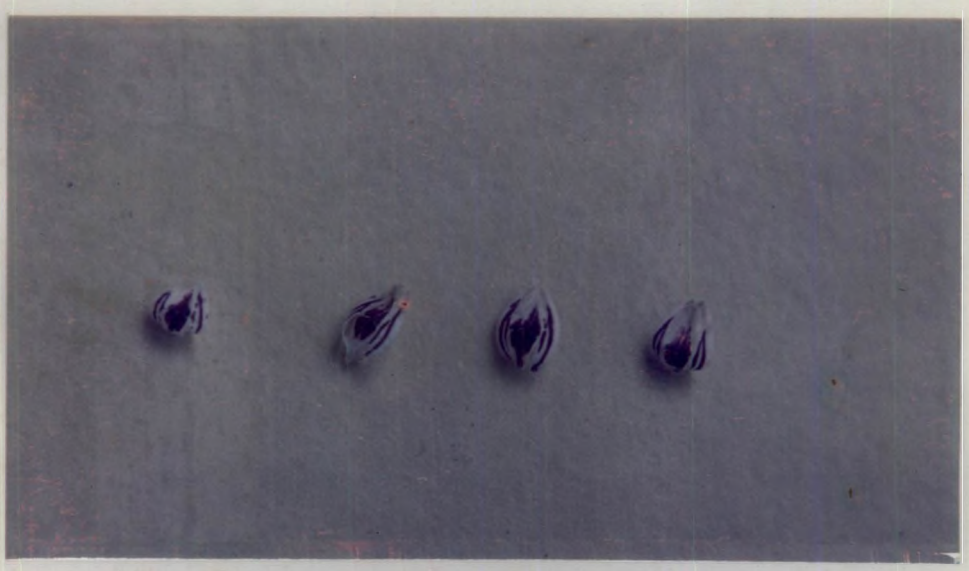
IIIc Ac-5 and Ac-1
IIId Ac-9 and Ac-10

Plate – III

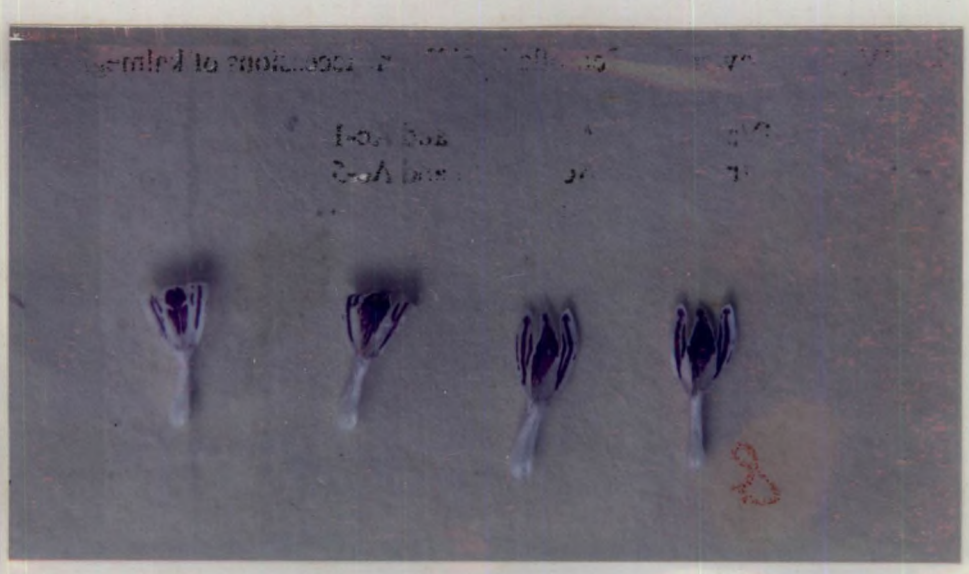
Plate IV Lower lip of corolla in different accessions of kalmegh

IVa Ac-4, Ac-3, Ac-2 and Ac-1
IVb Ac-8, Ac-7, Ac-6 and Ac-5
IVc Ac-9 and Ac-10

Plate – IV



Ac-4 Ac-3 Ac-2 Ac-1



Ac-8 Ac-7 Ac-6 Ac-5

4.2 Physiological observations

The analysis of variance showed significant differences among the accessions for most of the fourteen growth characters studied at various MAT (Appendix V and VI). Pooled ANOVA showed significant difference for months after transplanting (MAT) and accession x MAT interaction for all characters except NAR (Table 3 and 4). Therefore results are projected here at each MAT separately and accessions are not compared for their overall performance across the MAT.

4.2.1 Comparison of accessions and dates of harvest

4.2.1.1 Plant characters

4.2.1.1.1 Total plant fresh weight

Total plant fresh weight varied significantly among the accessions at all MAT except 2 MAT. Total plant fresh weight varied from 1.25 g (Ac-8) to 2.87 g (Ac-4) at 1 MAT (Table 5). At 2 MAT, Ac-9 showed the lowest value (17.40g) and the highest was recorded from Ac-6 (39.03 g). Ac-1 produced a lowest value of 63.10g and Ac-5 the highest (118.77g) at 3 MAT. At 4 MAT also, Ac-1 recorded the lowest (64.24g). The highest weight was observed for accession Ac-5 (127.58g).

All the accessions maintained consistent ranking at 3 MAT and 4 MAT. Ac-2, Ac-5 and Ac-9 maintained almost the same ranking positions irrespective of the dates of harvest. Ac-5 was in the top two positions irrespective of the date of harvest.

In general, plants harvested at 3 and 4 MAT yielded maximum fresh weight than when harvested at earlier dates. (Table 5, Fig.1 and 2). There was no significant difference in total plant fresh weight for any of the accessions harvested at 3 and 4 MAT.

Table 3. ANOVA (Mean squares) for seasonal effects on plant characters in kalmegh accessions

Source	df	Mean sum of squares										
		TPFW	SDW	RDW	LDW	TPDW	SL	RL	Dw/Fw	Leaf area	LAI	LAR
Replication	2	274.12	6.692	0.357	2.985*	17.153	122.71**	2.91	0.004	52793.4*	0.147*	18.84
Accession (A)	9	1339.95	23.500	0.746*	23.965**	79.688**	242.87**	13.64	0.007*	423789.1**	1.177**	405.39**
Month (M)	3	59995.69	3277.352**	32.459**	764.646**	8106.45**	14477.56**	1006.06**	0.212**	13521757.3**	37.560**	21547.78**
AxM	27	313.11	21.190*	0.278*	6.393**	40.699**	53.66**	15.26*	0.005*	113050.3**	0.314**	138.34**
Error	78	184.36	13.034	0.165	0.905	17.673	19.21	8.13	0.003	16008.8	0.044	46.31

TPFW-Total plant fresh weight SDW-Stem dry weight RDW-Root dry weight TPDW- Total plant dry weight SL-Shoot length RL-Root length

Dw/Fw-Dry weight/Fresh weight

* Significant at 5% level
** Significant at 1% level

Table 4 ANOVA (Mean squares) for seasonal effects on physiological parameters in kalmegh accessions

Source	df	Mean sum of squares		
		RGR	NAR	CGR
Replication	2	0.01	1.68	0.02
Accession (A)	9	162.85**	4.24**	0.10
Stage (S)	2	56193.93**	0.04	9.87**
AxS	18	205.33**	2.21	0.25**
Error	58	45.41	1.28	0.07

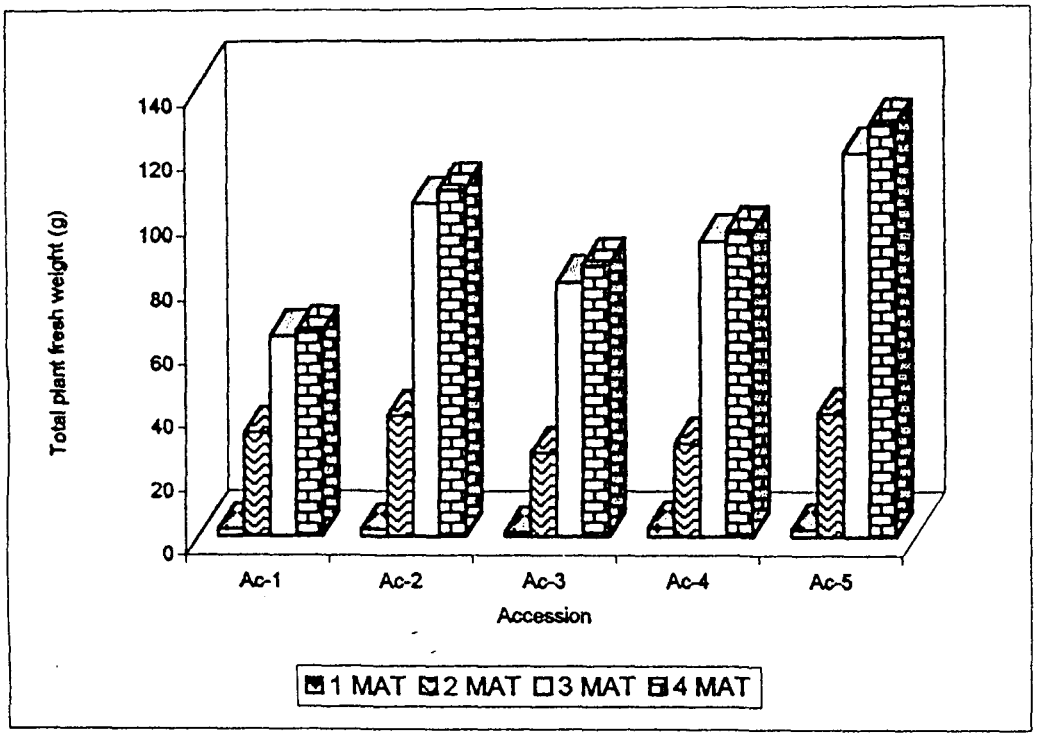
* Significant at 5% level
** Significant at 1% level

Table 5.Total plant fresh weight of kalmegh accessions on various dates of harvest (g)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	2.27^{abc}	5	32.47^{abc}	6	63.10^{f}	10	64.24^{d}	10	40.57^{d}
Ac-2	2.35^{ab}	4	37.93^{ab}	3	103.94^{ab}	2	108.11^{abc}	3	63.08^{ab}
Ac-3	1.36^{cd}	9	26.30^{abc}	8	79.87^{cdef}	6	85.01^{bcd}	7	48.14^{cd}
Ac-4	2.87^{a}	1	29.20^{abc}	7	92.00^{cde}	5	94.73^{abcd}	5	54.70^{bc}
Ac-5	2.37^{ab}	2	38.60^{a}	2	118.77^{a}	1	127.58^{a}	1	71.83^{a}
Ac-6	1.84^{bcd}	7	39.03^{a}	1	94.97^{bcd}	4	103.48^{abc}	4	59.83^{b}
Ac-7	2.26^{abc}	6	33.67^{abc}	4	73.83^{def}	8	78.76^{cd}	8	47.13^{cd}
Ac-8	1.25^{d}	10	21.33^{bc}	9	77.67^{cdef}	7	88.39^{bcd}	6	47.16^{cd}
Ac-9	1.45^{bcd}	8	17.40^{c}	10	70.60^{ef}	9	75.09^{cd}	9	47.14^{d}
Ac-10	2.36^{ab}	3	32.77^{abc}	5	101.18^{abc}	3	118.95^{ab}	2	63.82^{ab}
Overall mean	2.04		30.87		87.59		94.45		53.74
$CD_{5\%}$	0.92		17.27		23.76		35.50		11.09

$CD_{5\%}$ for comparing months within accession: 22.17
$CD_{5\%}$ for comparing months over the accession: 7.016

Values having any common superscript are not significantly different from one another



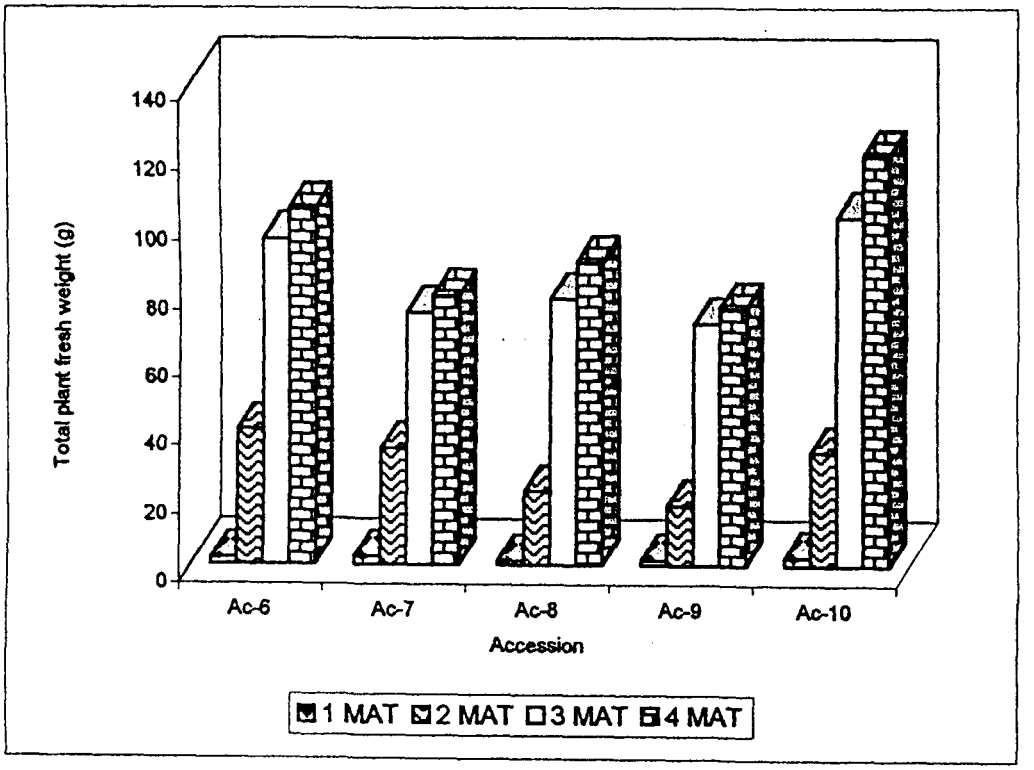


Fig. 1. Total plant fresh weight (g)

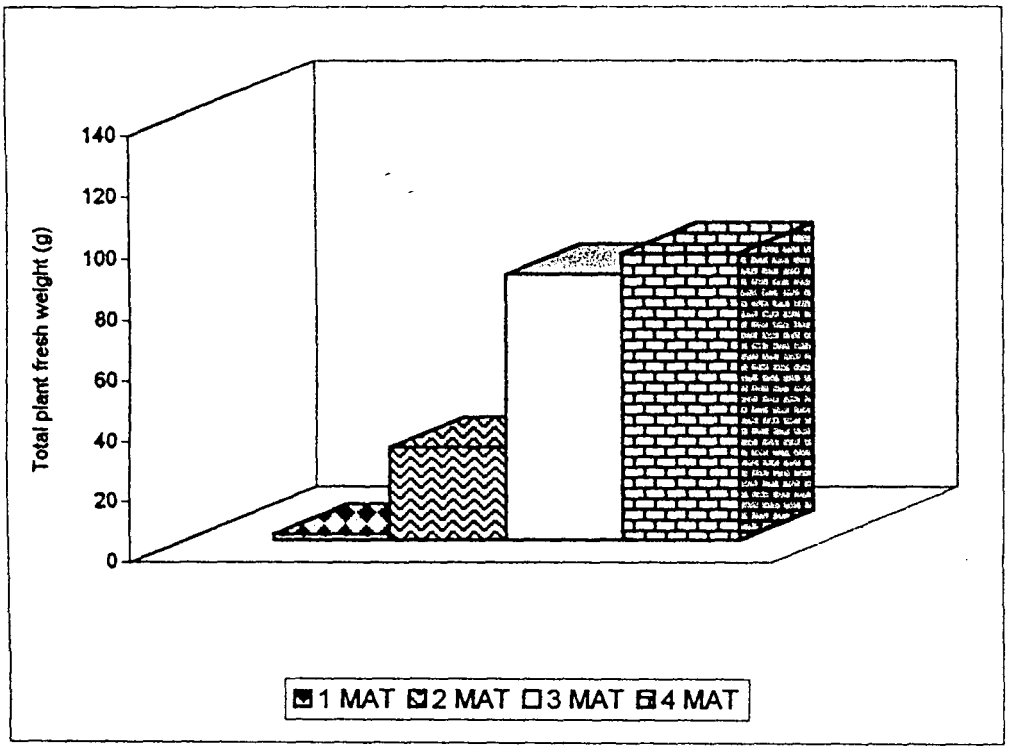


Fig. 2. Overall total plant fresh weight (g)

4.2.1.1.2 Stem dry weight

Stem dry weight showed significant differences among the accessions at all MAT except 4 MAT. Stem dry weight at 1 MAT varied from 0.034 g (Ac-9) to 0.070 g (Ac-1) (Table 6). At 2 MAT, Ac-8 produced the lowest stem dry weight (1.390 g) and Ac-6 produced the highest (3.630 g). Ac-8 produced the lowest (10.886 g) and Ac-2 produced the highest (22.615 g) at 3 MAT. At 4 MAT, Ac-1 produced the lowest stem dry weight (14.341 g) and the highest was recorded for Ac-5 (25.817 g).

Ranking of the accessions were not consistent in general. However, Ac-5 and Ac-9 maintained almost consistent ranking. In general plants harvested 4 MAT yielded higher stem dry weight than earlier dates (Table 6, Fig. 3 and 4). Ac-3, Ac-5 and Ac-8 produced significantly higher weight at 4 MAT than on all other dates. There was no significant difference in stem dry weight for other accessions between 3 and 4 MAT.

4.2.1.1.3 Root dry weight

Root dry weight showed significant differences among the accessions at all MAT except 4 MAT. Root dry weight at 1 MAT varied from 0.029 g (Ac-8) to 0.058 g (Ac-1) (Table 7). At 2 MAT, the lowest value was shown by Ac-9 (0.180 g) and the highest by Ac-7 (0.947 g). Ac-3 produced the lowest weight (1.369 g) and Ac-5 produced the highest (2.731 g) at 3 MAT. At 4 MAT, Ac-1 produced the lowest (1.758 g). The highest was recorded for accession Ac-4 (3.492 g).

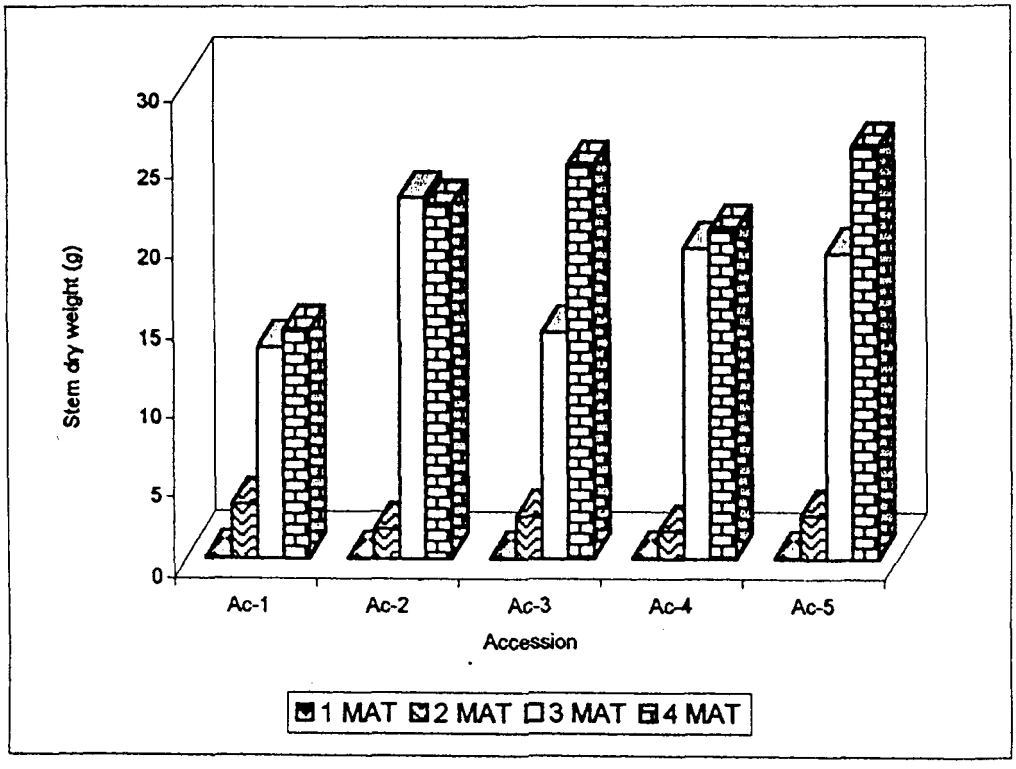
Ranking of some of the accessions varied under various MAT for example Ac-5 showed a moderate ranking at 1 and 2 MAT, but at 3 and 4 MAT it showed 1st and 2nd rank respectively. However accessions such as Ac-4 showed high ranking at all MAT.

Table 6. Stem dry weight of kalmegh accessions on various dates of harvest (g)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.070^{a}	1	3.333^{ab}	2	13.325^{c}	9	14.341^{a}	10	7.767^{d}
Ac-2	0.045^{cde}	7	1.823^{de}	7	22.615^{a}	1	22.111^{a}	5	11.648^{ab}
Ac-3	0.037^{de}	9	2.591^{bcd}	5	14.287^{c}	7	24.609^{a}	3	10.381^{ab}
Ac-4	0.067^{ab}	2	1.673^{e}	9	19.446^{a}	3	20.534^{a}	6	10.430^{abcd}
Ac-5	0.060^{abc}	4	2.656^{bc}	4	19.093^{a}	4	25.817^{a}	1	11.907^{abcd}
Ac-6	0.057^{abc}	5	3.630^{a}	1	18.710^{ab}	5	19.984^{a}	7	10.595^{a}
Ac-7	0.066^{ab}	3	2.873^{ab}	3	14.811^{bc}	6	16.491^{a}	9	8.560^{abcd}
Ac-8	0.037^{de}	8	1.390^{e}	10	10.886^{c}	10	25.477^{a}	2	9.448^{cd}
Ac-9	0.034^{e}	10	1.676^{e}	8	13.945^{c}	8	19.480^{a}	8	8.784^{abcd}
Ac-10	0.051^{bcd}	6	2.084^{cde}	6	20.087^{a}	2	23.172^{a}	4	11.348^{bcd}
Overall mean	0.052		2.373		16.720		21.202		10.086
$CD_{5\%}$	0.017		0.783		4.225		11.870		2.947

$CD_{5\%}$ for comparing months within accession: 5.895
$CD_{5\%}$ for comparing months over the accession:1.864

Values having any common superscript are not significantly different from one another



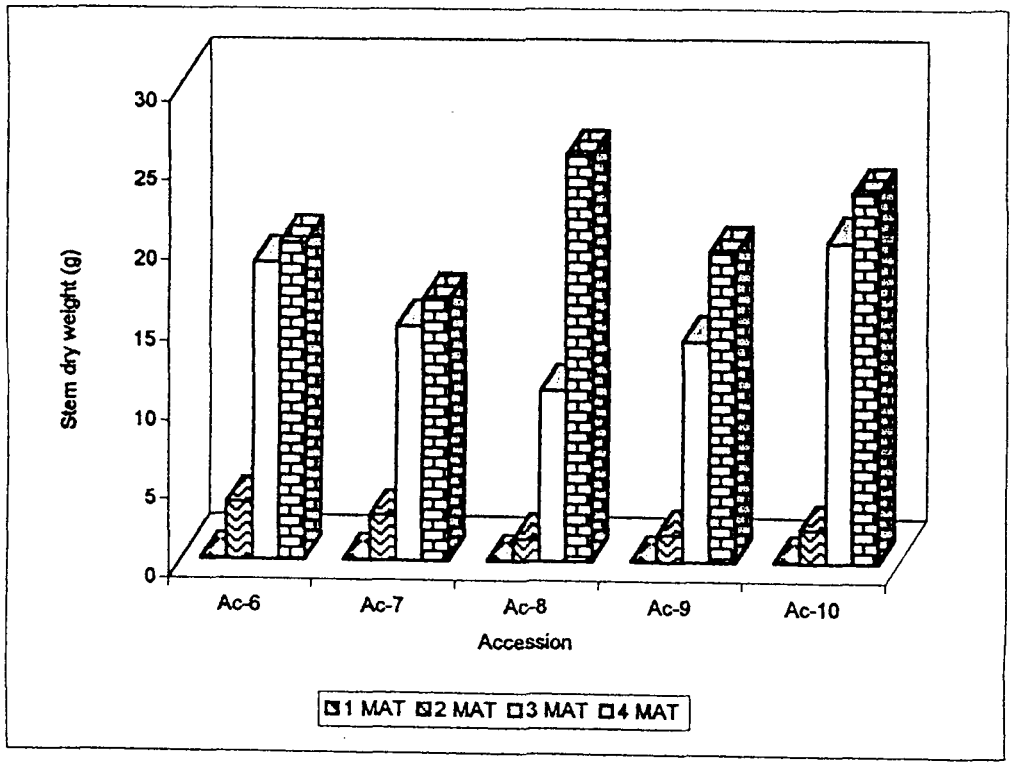


Fig. 3. Stem dry weight (g)

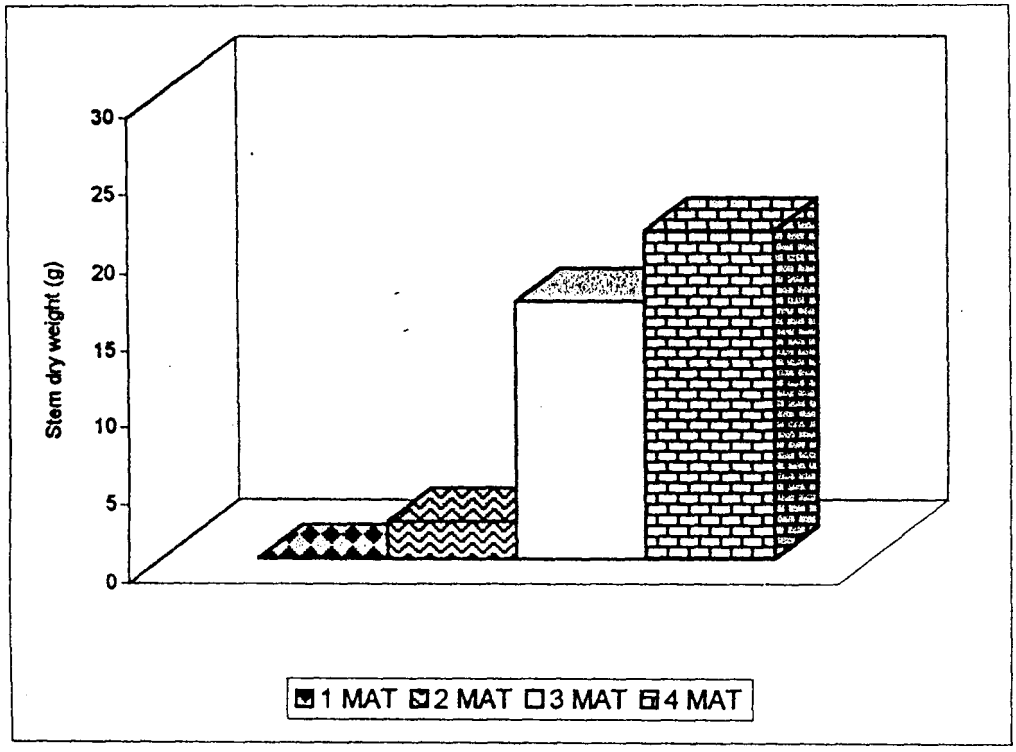


Fig. 4. Overall stem dry weight (g)

In general, plant harvested at 4 MAT yielded maximum root dry weight (Table 7, Fig. 5 and 6). Ac-3 and Ac-4 produced highest root dry weight at 4 MAT. There was no significant difference in weight for the other accessions between 3 and 4 MAT.

4.2.1.1.4 Leaf dry weight

Leaf dry weight showed significant differences among the accessions at all MAT. Leaf dry weight at 1 MAT varied from 0.161 g (Ac-8) to 0.430 g (Ac-5) (Table 8). At 2 MAT the lowest value was shown by Ac-1 (1.775 g) and the highest by Ac-6 (5.570 g). Ac-3 produced the lowest weight (7.175 g) and Ac-4 produced the highest (14.740 g) at 3 MAT. At 4 MAT also, Ac-4 produced the highest weight (14.390 g). The lowest was recorded for accession Ac-1 (6.274 g).

Ranking of the accessions for leaf dry weight was not consistent under various MAT, as suggested by a significant accession x MAT interaction. For instance Ac-10 which had a moderate ranking at 1, 2 and 3 MAT was ranked 2nd when harvested at 4 MAT. However, certain accessions showed appreciable consistency with respect to ranking. For example, Ac-5 was in the top three positions irrespective of the date of harvest.

Plants when harvested at 3 and 4 MAT produced higher weight than when harvested at earlier dates (Table 8, Fig. 7 and 8). Ac-8 and Ac-10 produced significantly higher yield at 4 MAT than on all other dates, whereas the highest yield was at 3 MAT for Ac-6.

4.2.1.1.5 Total plant dry weight

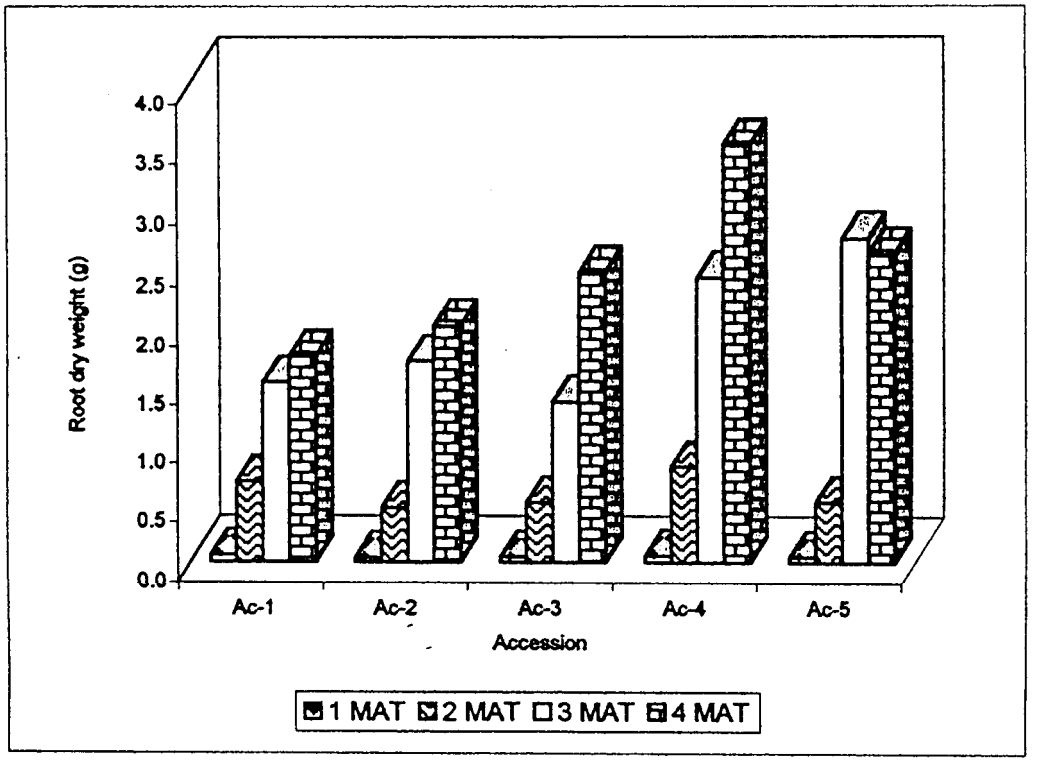
There was significant difference for total plant dry weight among the accessions at 1, 2 and 3 MAT. It varied from 0.228 g (Ac-9) to 0.550 g (Ac-4) at 1 MAT (Table 9). Ac-8 produced the lowest dry weight (3.456 g) and Ac-6 the highest (10.086 g) at 2 MAT. At 3 MAT, dry weight ranged from 21.390 g (Ac-8)

Table 7. Root dry weight of kalmegh accessions on various dates of harvest (g)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.058^{a}	1	0.682^{abc}	4	1.530^{cde}	7	1.758^{b}	10	1.007^{cd}
Ac-2	0.037^{cd}	8	0.461^{cd}	8	1.714^{cde}	5	2.014^{b}	7	1.057^{cd}
Ac-3	0.044^{bc}	5	0.507^{cd}	7	1.369^{e}	10	2.454^{ab}	3	1.093^{cd}
Ac-4	0.055^{ab}	2	0.815^{abc}	3	2.411^{ab}	2	3.492^{a}	1	1.693^{a}
Ac-5	0.044^{bc}	6	0.515^{cd}	6	2.731^{a}	1	2.605^{a}	2	1.474^{ab}
Ac-6	0.051^{ab}	4	0.887^{ab}	2	1.976^{bc}	3	2.310^{ab}	4	1.306^{bc}
Ac-7	0.052^{ab}	3	0.947^{a}	1	1.630^{cde}	6	1.843^{b}	9	1.118^{cd}
Ac-8	0.029^{d}	10	0.222^{d}	9	1.441^{de}	9	2.058^{b}	6	0.938^{d}
Ac-9	0.031^{d}	9	0.180^{d}	10	1.475^{de}	8	1.910^{b}	8	0.899^{d}
Ac-10	0.044^{bc}	7	0.519^{bcd}	5	1.867^{cd}	4	2.247^{ab}	5	1.169^{bcd}
Overall mean	0.045		0.573		1.814		2.269		1.175
$CD_{5\%}$	0.012		0.374		0.479		1.258		0.332

$CD_{5\%}$ for comparing months within accession: 0.663
$CD_{5\%}$ for comparing months over the accession: 0.210

Values having any common superscript are not significantly different from one another



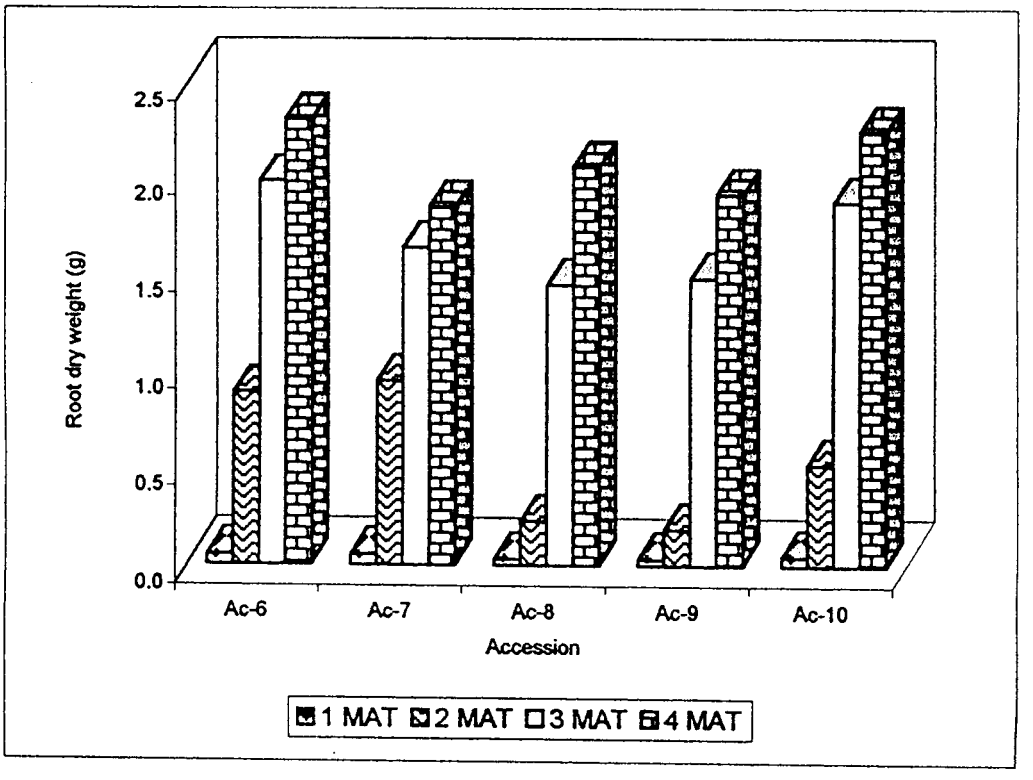


Fig. 5. Root dry weight (g)

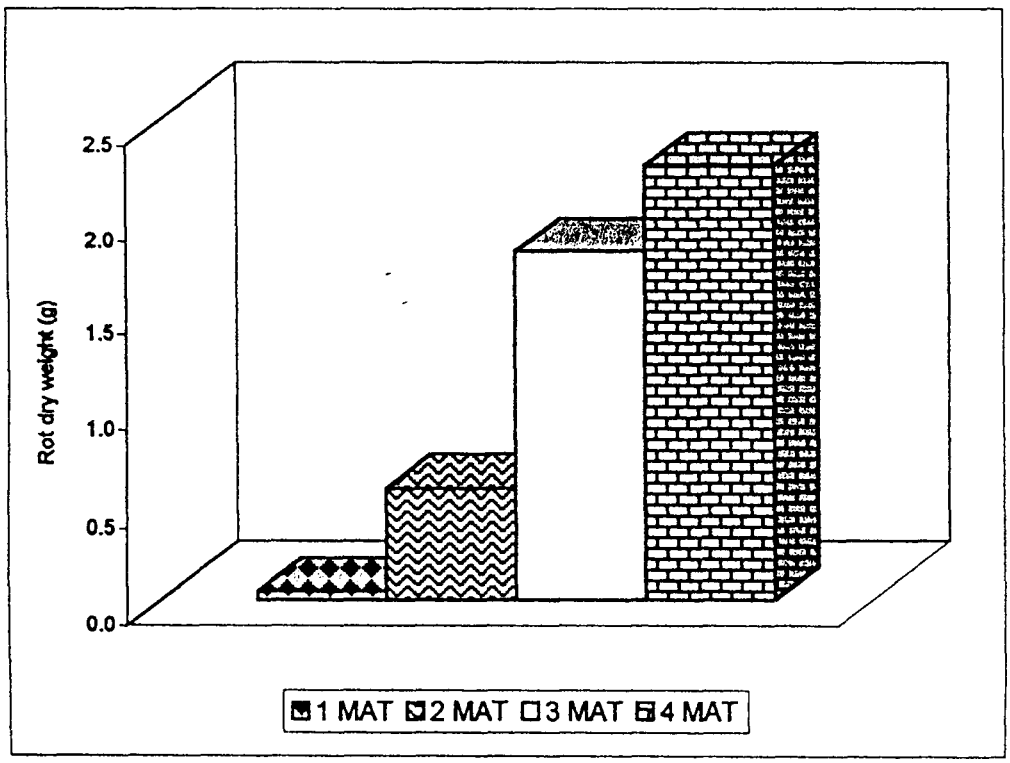


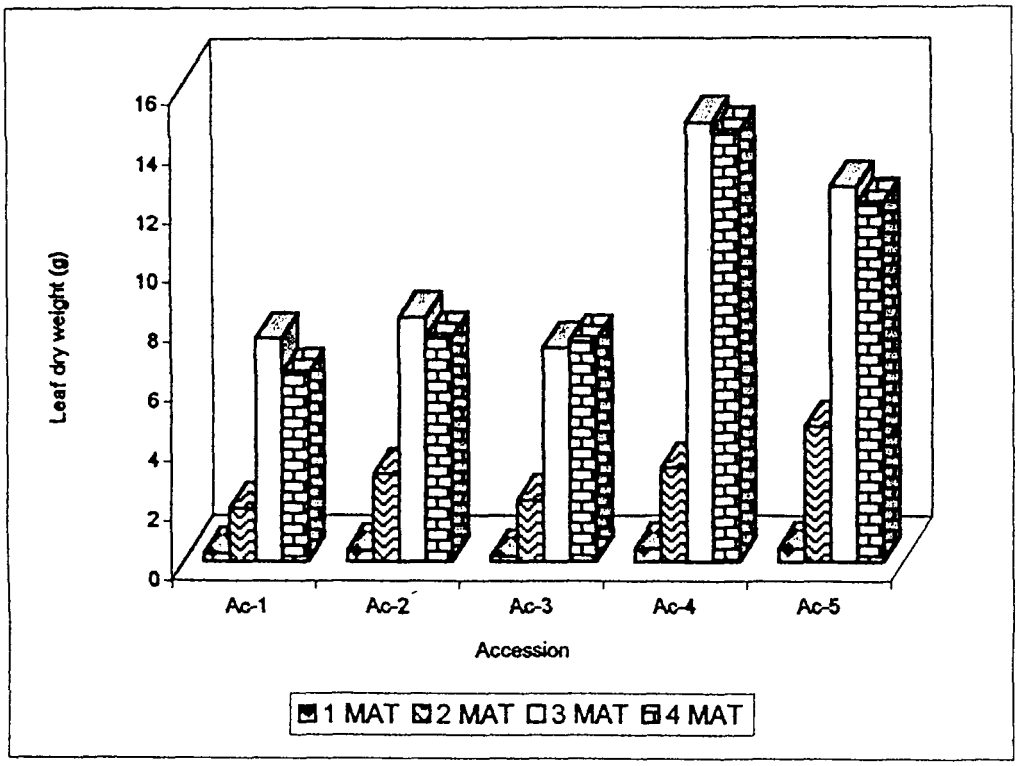
Fig. 6. Overall root dry weight (g)

Table 8. Leaf dry weight of kalmegh accessions on various dates of harvest (g)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.261^{cd}	7	1.775^{f}	10	7.471^{ef}	9	6.274^{e}	10	3.945^{g}
Ac-2	0.365^{ab}	9	2.918^{cd}	6	8.193^{ef}	8	7.512^{de}	8	4.747^{ef}
Ac-3	0.178^{de}	8	2.039^{ef}	8	7.175^{f}	10	7.396^{de}	9	4.197^{fg}
Ac-4	0.428^{a}	2	3.133^{cd}	4	14.740^{a}	1	14.390^{a}	1	8.173^{a}
Ac-5	0.430^{a}	1	4.543^{b}	2	12.600^{bc}	3	12.017^{abc}	3	7.398^{b}
Ac-6	0.316^{bc}	5	5.570^{a}	1	13.140^{ab}	2	9.754^{cd}	6	7.195^{b}
Ac-7	0.371^{ab}	3	3.450^{c}	3	9.170^{de}	6	9.651^{cd}	7	5.660^{d}
Ac-8	0.161^{e}	10	1.844^{ef}	9	9.063^{de}	7	10.741^{bc}	4	5.452^{de}
Ac-9	0.166^{e}	9	2.579^{de}	7	11.052^{c}	4	10.507^{bc}	5	6.076^{cd}
Ac-10	0.290^{bc}	6	3.016^{cd}	5	10.833^{cd}	5	12.493^{ab}	2	6.658^{bc}
Overall mean	0.297		3.087		10.344		10.073		5.950
$CD_{5\%}$	0.091		0.801		1.812		2.549		0.777

$CD_{5\%}$ for comparing months within accession: 1.553
$CD_{5\%}$ for comparing months over the accession:0 .491

Values having any common superscript are not significantly different from one another



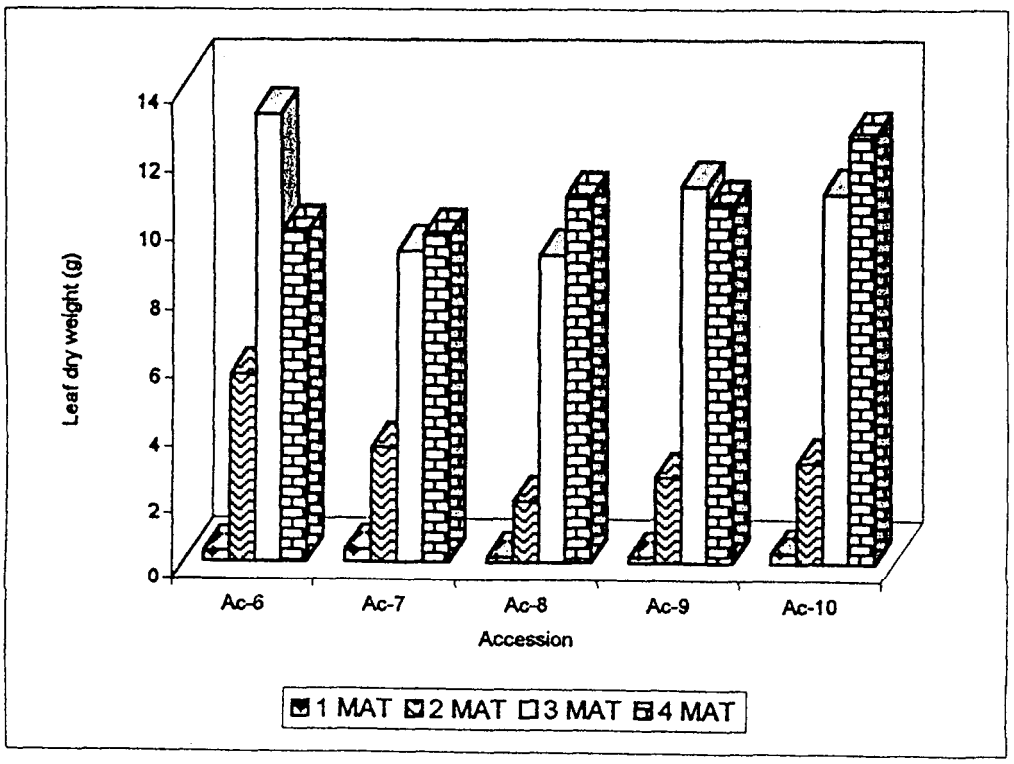


Fig. 7. Leaf dry weight (g)

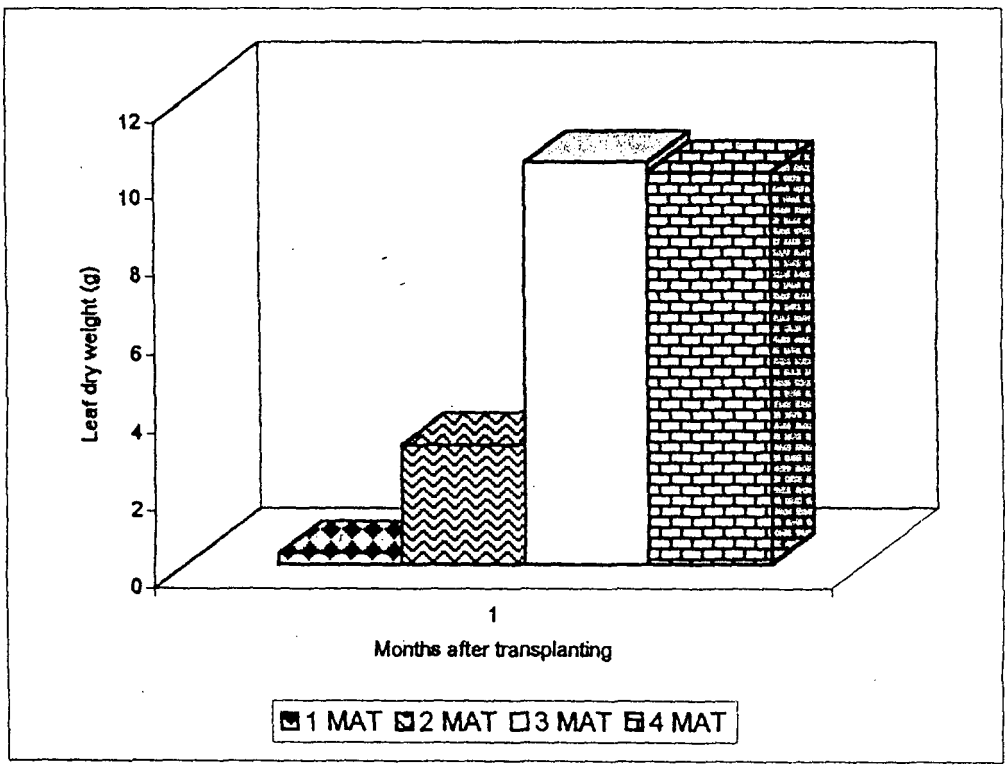


Fig. 8. Overall leaf dry weight (g)

to Ac-4 (36.602 g). However at 4 MAT Ac-1 produced the lowest value (22.373 g) and Ac-5 produced the highest (40.439 g).

Ranking was not consistent for most of the accessions. But Ac-5 was in the top two positions irrespective of the date of harvest. Similarly accessions such as Ac-3, Ac-8 and Ac-9 maintained almost the same ranks at various MAT. In general, plants harvested at 4 MAT yielded maximum weight (Table 9, Fig. 9 and 10). Ac-3 and Ac-8 produced highest yield at 4 MAT. There was no significant difference in total plant dry weight for other accessions between 3 and 4 MAT.

4.2.1.1.6 Shoot length

Shoot length showed significant differences among the accessions at all MAT. Shoot length at 1 MAT varied from 7.57 cm (Ac-9) to 12.80cm (Ac-1) (Table 10). At 2 MAT the lowest value was shown by Ac-9 (18.63 cm) and the highest by Ac-1 (42.33 cm). Ac-9 produced the lowest shoot length (35.57 cm) and Ac-6 the highest (60.83 cm). At 4 MAT, also Ac-9 produced the lowest (49.33 cm). The highest was recorded for accession Ac-6 (66.00 cm).

Ranking of the accessions for shoot length varied widely for most of the accessions at various MAT. However Ac-6 and Ac-7 maintained a high ranking at all the MAT. Ac-9 was consistently the lowest in rank. In general plants recorded maximum shoot length at 4 MAT (Table 10, Fig. 11 and 12). Ac-1, Ac-4, Ac-5, Ac-8 and Ac-9 recorded significantly higher shoot length at 4 MAT. There was no significant difference in shoot length for other accessions between 3 and 4 MAT.

4.2.1.1.7 Root length

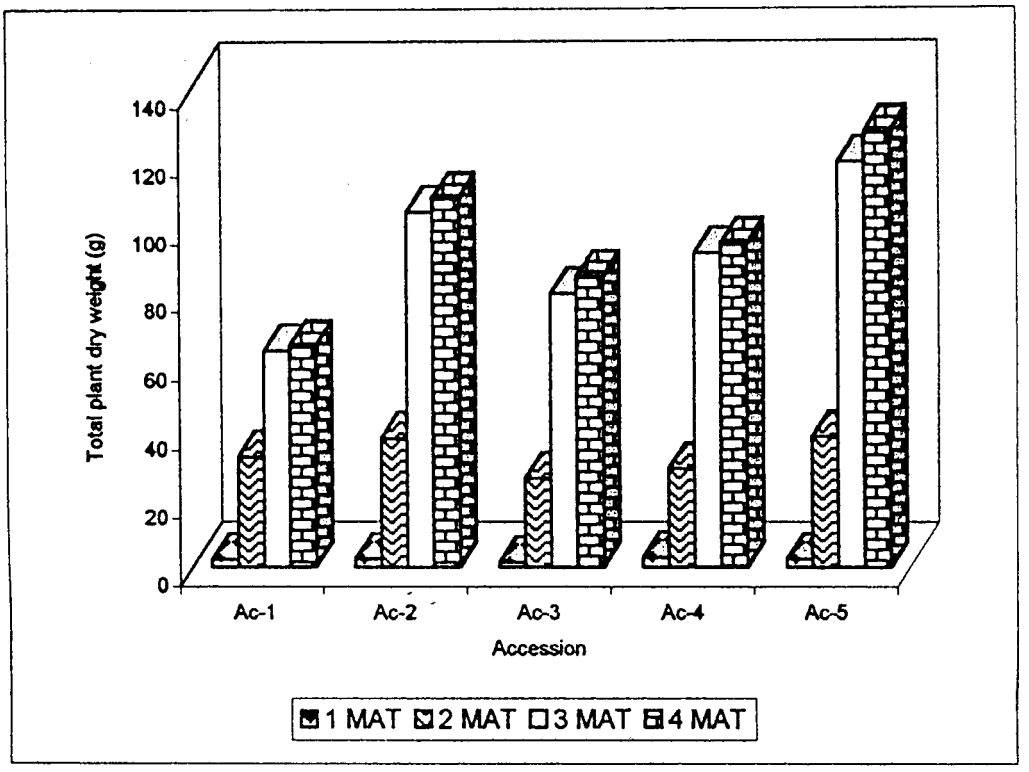
Root length showed significant differences among the accessions at 1 and 4 MAT. It varied from 4.73 cm (Ac-9) to 10.03 cm (Ac-4) at 1 MAT (Table 11). At 2 MAT, Ac-8 recorded the lowest value (8.07 cm) and the highest Ac-6 (13.73 cm). Ac-7 recorded lowest root length (11.73 cm) and Ac-9 the

Table 9 . Total plant dry weight of kalmegh accessions on various dates of harvest (g)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.389^{d}	6	5.790^{c}	4	22.325^{b}	9	22.373^{b}	10	12.719^{d}
Ac-2	0.447^{bcd}	4	5.202^{c}	7	32.521^{a}	5	31.638^{ab}	8	17.452^{abc}
Ac-3	0.259^{e}	8	5.137^{c}	8	22.831^{b}	8	34.457^{ab}	5	15.671^{cd}
Ac-4	0.550^{a}	1	5.622^{c}	5	36.602^{a}	1	38.414^{a}	2	20.297^{a}
Ac-5	0.535^{ab}	2	7.714^{b}	2	34.422^{a}	2	40.439^{a}	1	20.778^{a}
Ac-6	0.424^{cd}	5	10.086^{a}	1	33.825^{a}	3	32.047^{ab}	6	19.096^{ab}
Ac-7	0.488^{abc}	3	7.269^{b}	3	25.605^{b}	7	27.984^{ab}	9	15.337^{cd}
Ac-8	0.228^{e}	10	3.456^{d}	10	21.390^{b}	10	38.277^{a}	3	15.837^{bcd}
Ac-9	0.231^{e}	9	4.436^{cd}	9	26.473^{b}	6	31.897^{ab}	7	15.759^{bcd}
Ac-10	0.385^{d}	7	5.618^{c}	6	32.787^{a}	4	37.911^{a}	4	19.175^{ab}
Overall mean	0.394		6.033		28.878		33.911		17.212
$CD_{5\%}$	0.092		1.401		5.461		13.506		3.432

$CD_{5\%}$ for comparing months within accession: 6.864
$CD_{5\%}$ for comparing months over the accession: 2.170

Values having any common superscript are not significantly different from one another



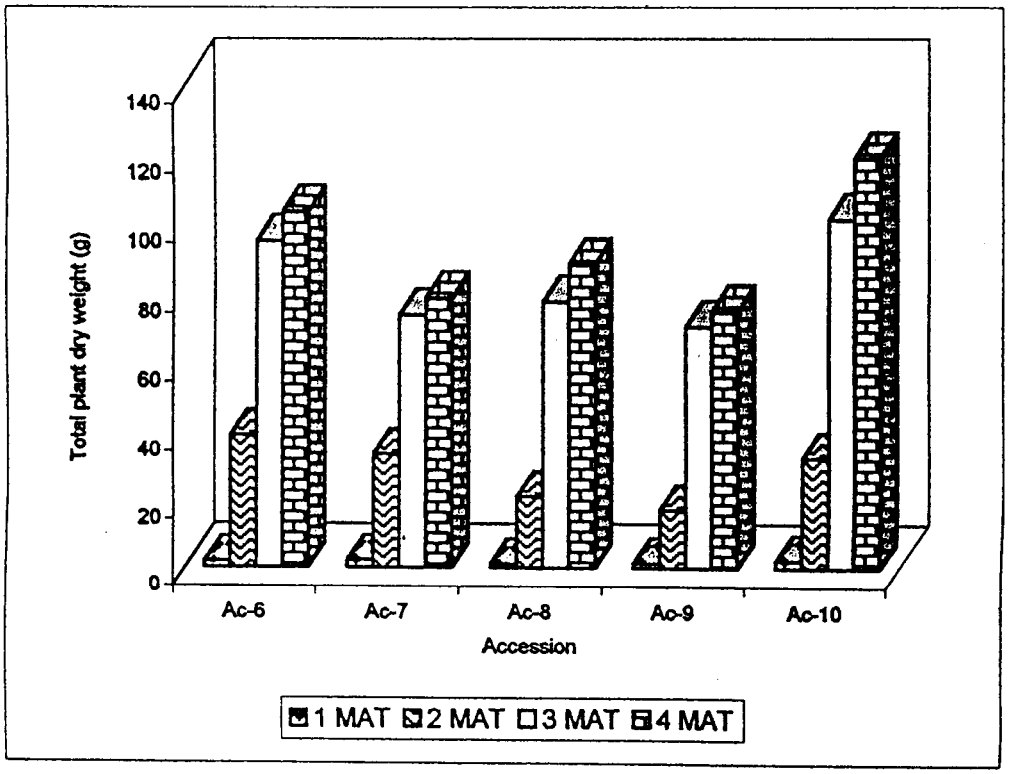


Fig. 9. Total plant dry weight (g)

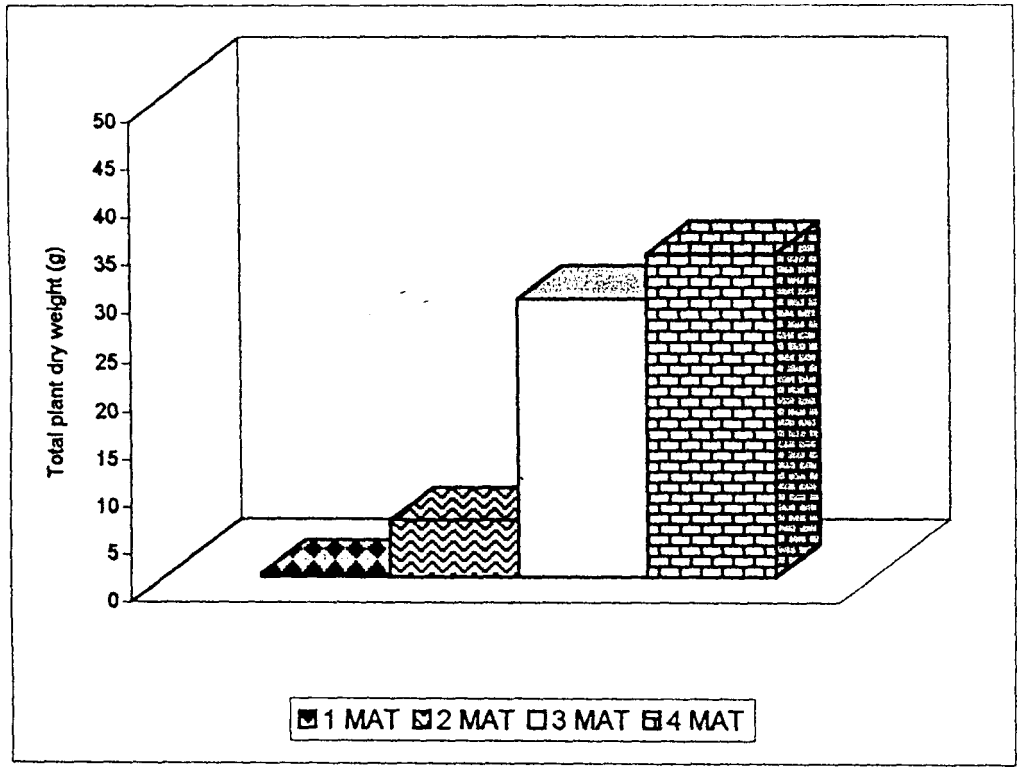


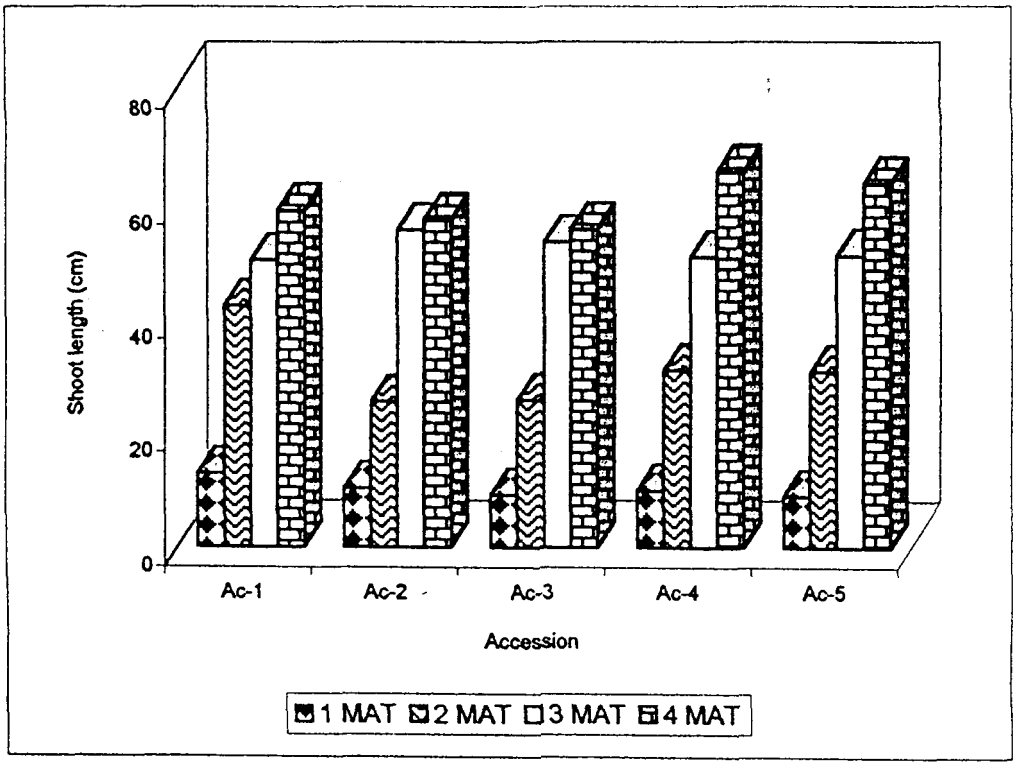
Fig. 10. Overall total plant dry weight (g)

Table 10. Shoot length of kalmegh accessions on various dates of harvest (cm)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	12.80^{a}	1	42.33^{a}	1	50.33^{b}	8	59.17^{abcd}	5	41.16^{ab}
Ac-2	10.30^{bc}	5	25.76^{def}	8	52.77^{ab}	2	57.56^{bcde}	7	37.35^{cde}
Ac-3	9.13^{cd}	8	26.13^{cde}	7	53.63^{ab}	4	55.97^{cde}	8	36.22^{de}
Ac-4	9.93^{c}	6	31.33^{bcd}	4	51.00^{b}	7	65.96^{ab}	2	39.56^{abcd}
Ac-5	8.97^{cd}	9	31.13^{bcd}	5	51.13^{b}	6	63.99^{abc}	7	38.86^{bcde}
Ac-6	10.77^{abc}	3	33.97^{b}	2	60.83^{a}	1	66.00^{a}	1	42.89^{a}
Ac-7	12.33^{ab}	2	33.33^{bc}	3	54.83^{ab}	3	59.67^{abcd}	4	40.04^{abc}
Ac-8	9.20^{cd}	7	19.97^{ef}	9	41.17^{c}	9	58.17^{abcd}	6	32.13^{f}
Ac-9	7.57^{d}	10	18.63^{f}	10	35.57^{c}	10	49.33^{e}	10	27.78^{g}
Ac-10	10.53^{bc}	4	27.63^{bcd}	6	52.33^{b}	5	52.43^{de}	9	35.73^{e}
Overall mean	10.15		29.02		50.68		58.83		37.17
$CD_{5\%}$	2.18		7.23		8.39		8.40		3.58

$CD_{5\%}$ for comparing months within accession: 7.162
$CD_{5\%}$ for comparing months over the accession: 2.265

Values having any common superscript are not significantly different from one another



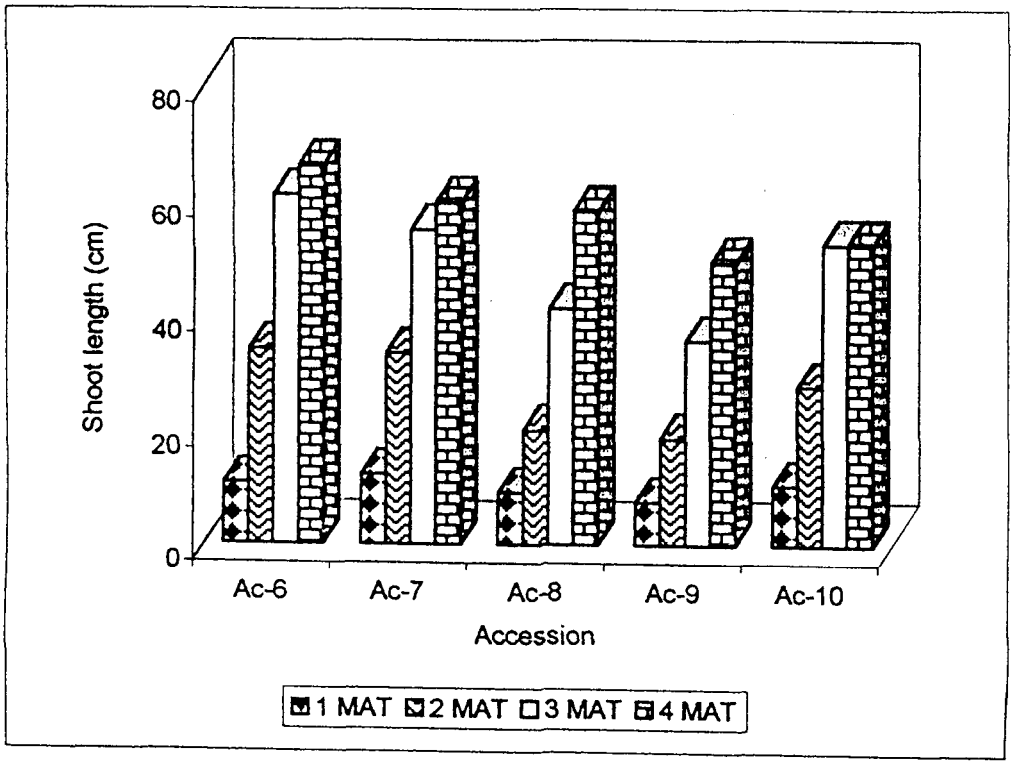


Fig.11. Shoot length (cm)

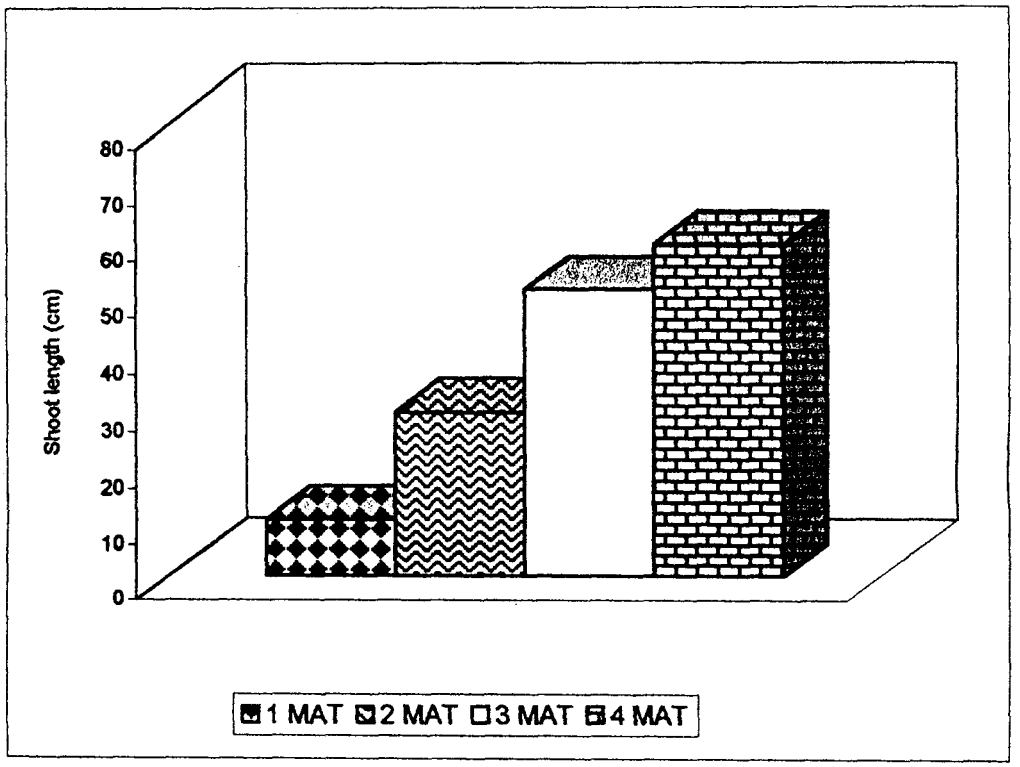


Fig. 12. Overall shoot length (cm)

highest (18.27 cm) at 3 MAT. At 4 MAT also Ac-7 (16.13 cm) and Ac-9 (28.17 cm) maintained the same position as in 3 MAT.

Ranking showed wide fluctuations for the accessions at various MAT. But Ac-1 maintained consistent ranking throughout its growth period.

In general, plants recorded maximum root length at 4 MAT (Table 11, Fig. 13 and 14). But Ac-1, Ac-2, Ac-3, Ac-5 and Ac-10 showed no significant differences when harvested at 3 and 4 MAT.

4.2.1.1.8 Dry weight/fresh weight

It showed significant differences among the accessions only at 4 MAT. Dry weight/Fresh weight at 1 MAT varied from 0.160 (Ac-9) to 0.247 (Ac-7) (Table 12). At 2 MAT, the lowest value was recorded for Ac-2 (0.141) and the highest by Ac-6 (0.290). Ac-4 produced the highest ratio (0.398) and Ac-8 recorded the lowest (0.280) at 3 MAT. At 4 MAT, Ac-8 recorded the maximum (0.438) and Ac-2 the minimum (0.293).

Ranking of the accessions for dry weight/fresh weight ratio was not consistent under various MAT. For instance Ac-8, which had a moderate ranking of 8 and 10 at 1 and 3 MAT, was ranked 5 at 2 MAT and 1 at 4 MAT.

Plants harvested at 3 and 4 MAT recorded the highest ratio (Table 12). Ac-3 and Ac-8 showed significantly higher ratio at 4 MAT than at all other dates.

4.2.1.1.9 Days to 50 per cent flowering

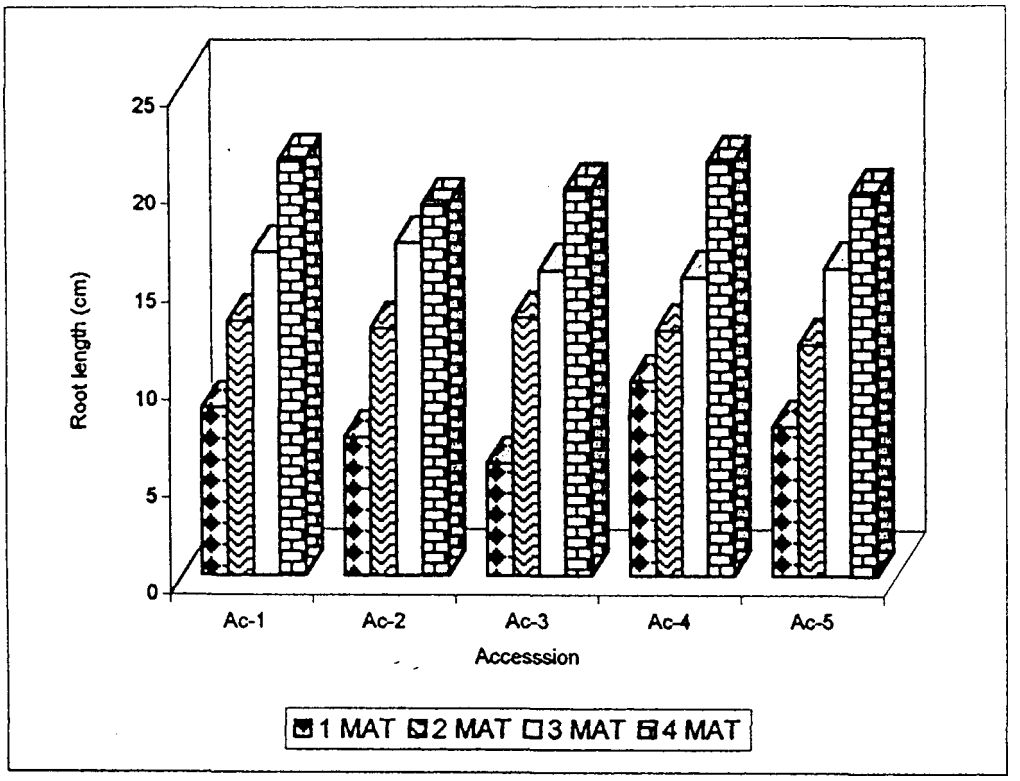
Among the ten accessions, Ac-1 reached 50 per cent flowering in the shortest period (95 DAS) followed by Ac-7 (105 DAS), (Table 20). Accession Ac-8 took maximum time to attain 50 per cent flowering (165 DAS). Other accessions reached 50 per cent flowering within 140 DAS to 150 DAS.

Table 11. Root length of kalmegh accessions on various dates of harvest (cm)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	8.67^{ab}	2	13.07^{a}	3	16.53^{a}	4	21.27^{b}	4	14.88^{a}
Ac-2	7.17^{bc}	7	12.67^{ab}	4	17.00^{a}	3	18.93^{bc}	9	13.94^{abc}
Ac-3	5.83^{cd}	8	13.23^{a}	2	15.63^{a}	6	19.80^{bc}	6	13.63^{abc}
Ac-4	10.03^{a}	1	12.60^{ab}	5	15.27^{a}	7	21.17^{b}	5	14.77^{ab}
Ac-5	7.73^{bc}	4	11.87^{ab}	6	15.70^{a}	5	19.47^{bc}	7	13.69^{abc}
Ac-6	7.73^{bc}	5	13.73^{a}	1	15.23^{a}	8	22.03^{b}	3	14.68^{ab}
Ac-7	8.53^{ab}	3	11.10^{ab}	7	11.73^{a}	10	16.13^{c}	10	11.88^{c}
Ac-8	4.97^{d}	9	8.07^{b}	10	14.13^{a}	9	22.83^{b}	2	12.50^{bc}
Ac-9	4.73^{d}	10	9.63^{ab}	9	18.27^{a}	1	28.17^{a}	1	15.20^{a}
Ac-10	7.67^{bc}	6	10.50^{ab}	8	17.77^{a}	2	19.00^{bc}	8	13.73^{abc}
Overall mean	7.31		11.65		15.73		20.88		13.89
$CD_{5\%}$	2.09		4.66		7.34		3.91		2.33

$CD_{5\%}$ for comparing months within accessions: 4.65
$CD_{5\%}$ for comparing months over the accessions: 1.47

Values having any common superscript are not significantly different from one another



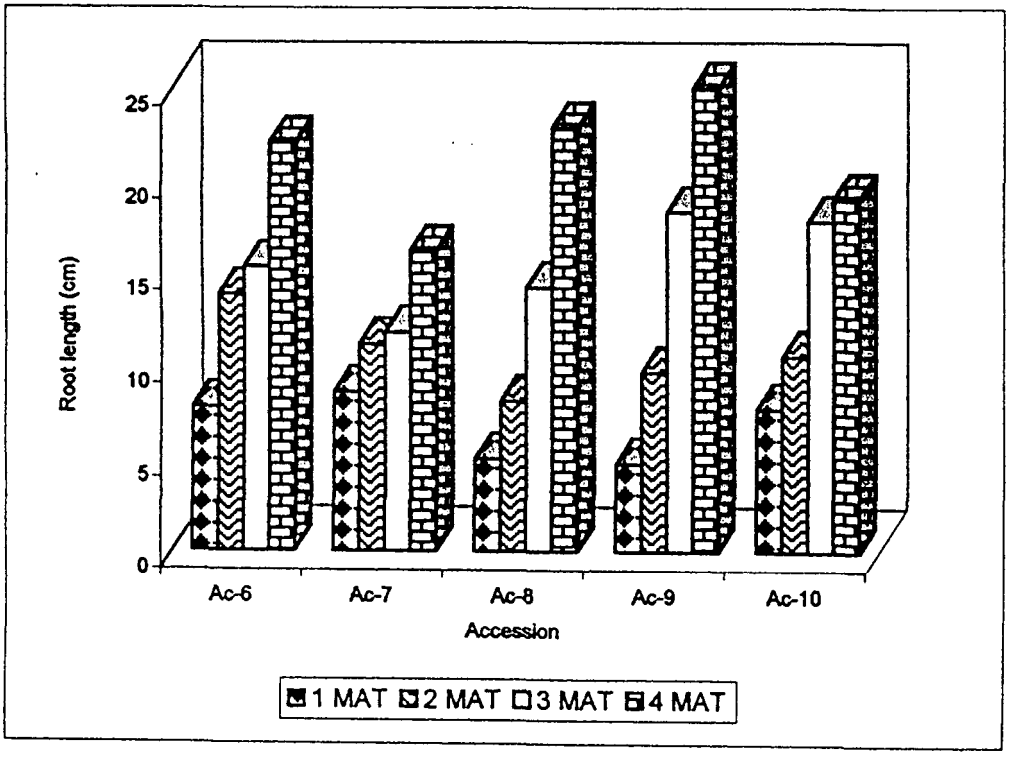


Fig. 13. Root length (cm)

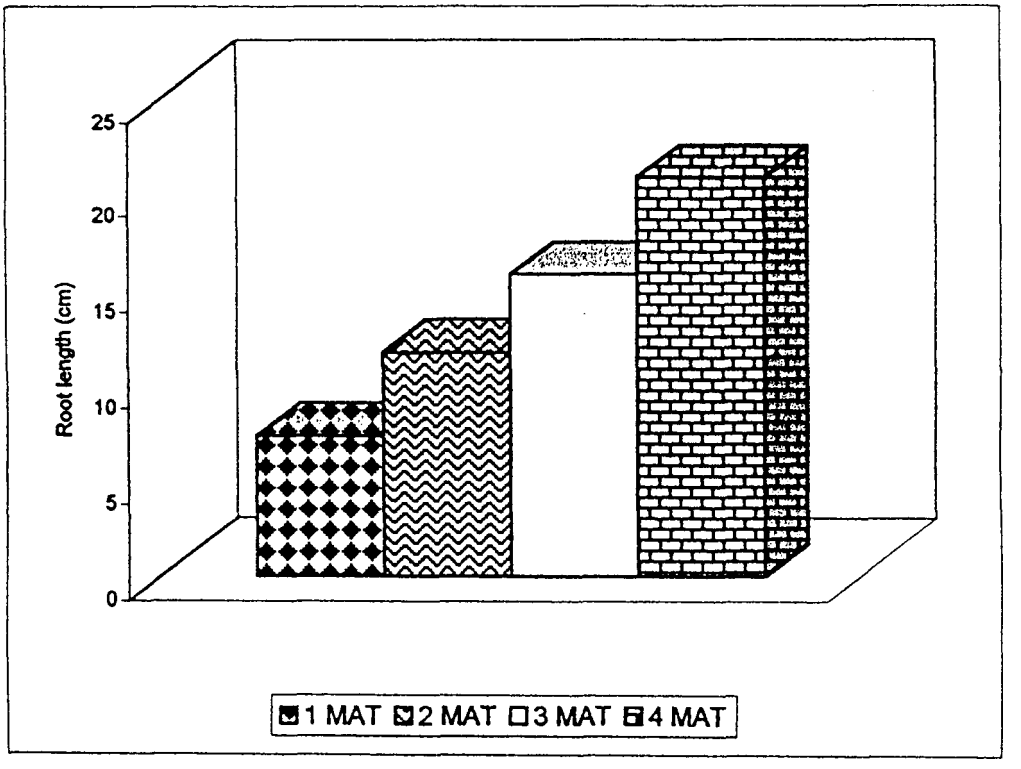


Fig. 14. Overall root length (cm)

Table 12. Dry weight/Fresh weight of kalmegh accessions on various dates of harvest

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.190^{a}	7	0.180^{bc}	9	0.354^{abc}	4	0.349^{bc}	6	0.268^{abc}
Ac-2	0.190^{a}	5	0.141^{c}	10	0.314^{bc}	7	0.293^{c}	10	0.235^{c}
Ac-3	0.190^{a}	6	0.197^{abc}	6	0.286^{c}	9	0.399^{ab}	4	0.268^{abc}
Ac-4	0.193^{a}	4	0.192^{abc}	7	0.398^{a}	1	0.408^{ab}	3	0.298^{a}
Ac-5	0.241^{a}	3	0.201^{abc}	4	0.309^{bc}	8	0.318^{c}	8	0.267^{abc}
Ac-6	0.245^{a}	2	0.290^{a}	1	0.354^{abc}	4	0.309^{c}	9	0.299^{a}
Ac-7	0.247^{a}	1	0.222^{abc}	3	0.350^{abc}	5	0.355^{bc}	5	0.293^{ab}
Ac-8	0.183^{a}	8	0.201^{abc}	5	0.280^{c}	10	0.438^{a}	1	0.275^{abc}
Ac-9	0.160^{a}	10	0.260^{ab}	2	0.380^{ab}	2	0.426^{ab}	2	0.306^{a}
Ac-10	0.166^{a}	9	0.189^{abc}	8	0.323^{abc}	6	0.318^{c}	7	0.249^{bc}
Overall mean	0.200		0.207		0.335		0.361		0.275
$CD_{5\%}$	0.094		0.108		0.075		0.077		0.043

$CD_{5\%}$ for comparing months within accessions: 0.085
$CD_{5\%}$ for comparing months over the accessions:0.027

Values having any common superscript are not significantly different from one another

4.2.1.1.10 Moisture content

Moisture content of plant samples varied from 56.7% (Ac-8) to 70.7% (Ac-2) (Table 20).

4.2.1.2 Physiological parameters

4.2.1.2.1 Leaf area

Leaf area showed significant differences among the accessions at all MAT. Leaf area at 1 MAT varied from 22.1 cm^2 (Ac-8) to 57.9 cm^2 (Ac-5) (Table 13). At 2 MAT, leaf area varied from 236.7 cm^2 (Ac-1) to 741.3 cm^2 (Ac-6). Ac-3 recorded the lowest leaf area (954.9 cm^2) and Ac-4 the highest (1960.8 cm^2) at 3 MAT. At 4 MAT, Ac-1 recorded a minimum of 834.9 cm^2 and Ac-4 the maximum (1914.3 cm^2).

Ac-1, Ac-3, Ac-4 and Ac-5 maintained almost consistent ranking at all MAT. Ac-5 maintained the top three positions irrespective of the date of harvest.

In general, plants recorded higher leaf area when harvested at 3 and 4 MAT than when harvested on earlier dates (Table 13, Fig. 15 and 16). Ac-8 and Ac-10 produced significantly higher leaf area at 4 MAT than all other dates. Ac-6 recorded a significantly higher yield at 3 MAT.

4.2.1.2.2 Leaf area index

Leaf area index showed significant differences among the accessions at all MAT. Leaf area index (LAI) at 1 MAT varied from 0.037 (Ac-8) to 0.096 (Ac-5) (Table 14). At 2 MAT, Ac-1 recorded the lowest LAI (0.395) and Ac-6 the highest (1.236). Ac-3 recorded the lowest LAI (1.592) and Ac-4 the highest LAI (3.268) when harvested 3 MAT. At 4 MAT, LAI varied from 1.392 (Ac-1) to 3.190 (Ac-4).

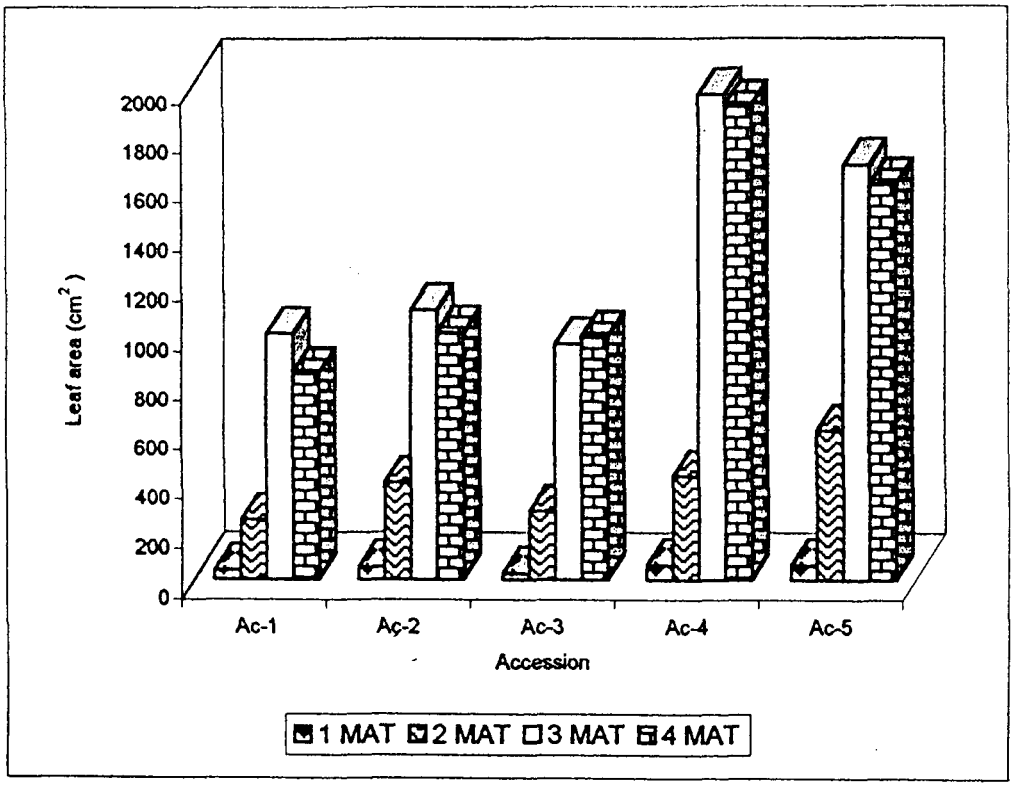
Ac-1, Ac-3, Ac-4 and Ac-5 maintained consistent ranking irrespective of the dates of harvest. Ac-5 was superior maintaining ranking in the top three

Table 13. Leaf area of kalmegh accessions on various dates of harvest (cm^2)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	35.4^{cd}	7	236.7^{f}	10	994.2^{ef}	9	834.9^{e}	10	525.3^{g}
Ac-2	49.2^{ab}	4	388.7^{cd}	6	1090.1^{ef}	8	99.7^{de}	8	631.9^{ef}
Ac-3	24.4^{de}	8	271.8^{ef}	8	954.9^{f}	10	984.2^{de}	9	558.8^{fg}
Ac-4	57.6^{a}	2	417.3^{cd}	4	1960.8^{a}	1	1914.3^{a}	1	1087.5^{a}
Ac-5	57.9^{a}	1	604.9^{b}	2	1676.2^{bc}	3	1598.7^{abc}	3	984.4^{b}
Ac-6	42.7^{bc}	5	741.3^{a}	1	1748.0^{ab}	2	1297.7^{cd}	6	957.5^{b}
Ac-7	50.0^{ab}	3	459.4^{c}	3	1220.1^{de}	6	1284.1^{cd}	7	753.4^{d}
Ac-8	22.1^{e}	10	245.8^{ef}	9	1205.9^{de}	7	1428.9^{bc}	4	725.7^{de}
Ac-9	22.8^{e}	9	343.7^{de}	7	1470.3^{c}	4	1397.9^{bc}	5	808.7^{cd}
Ac-10	39.2^{bc}	6	401.7^{cd}	5	1441.2^{cd}	5	1662.0^{ab}	2	886.1^{bc}
Overall mean	40.1		411.1		1376.2		1340.2		791.9
$CD_{5\%}$	12.1		106.5		241.0		338.9		103.3

$CD_{5\%}$ for comparing months within accessions: 206.6
$CD_{5\%}$ for comparing months over the accessions:65.3

Values having any common superscript are not significantly different from one another



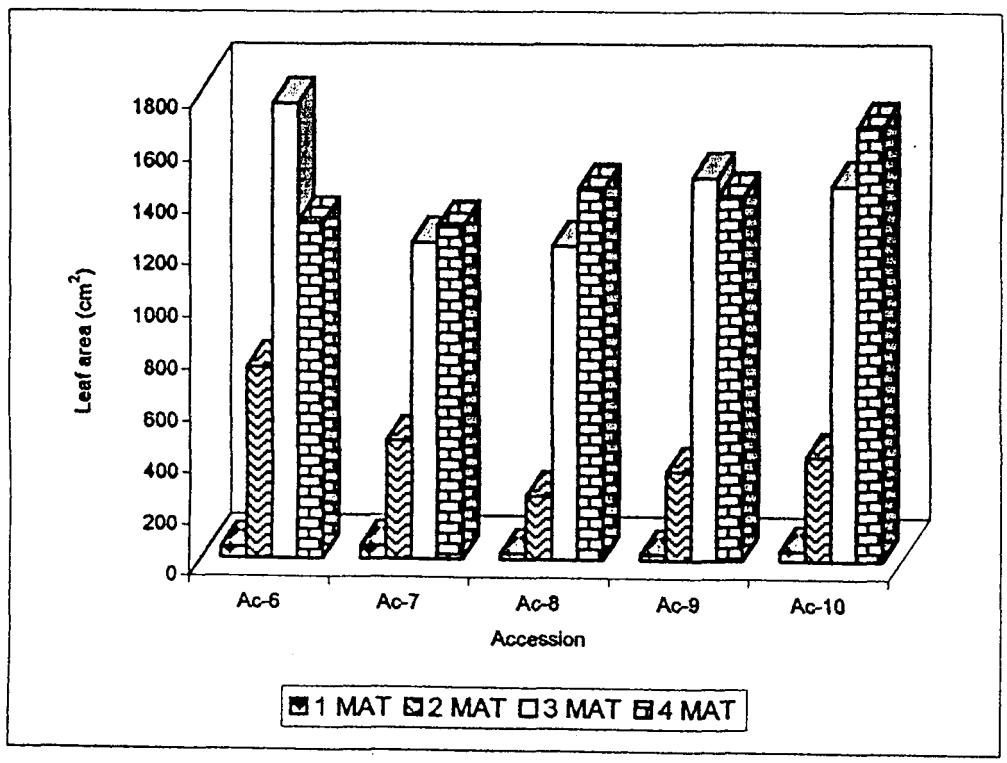


Fig. 15. Leaf area (cm^2)

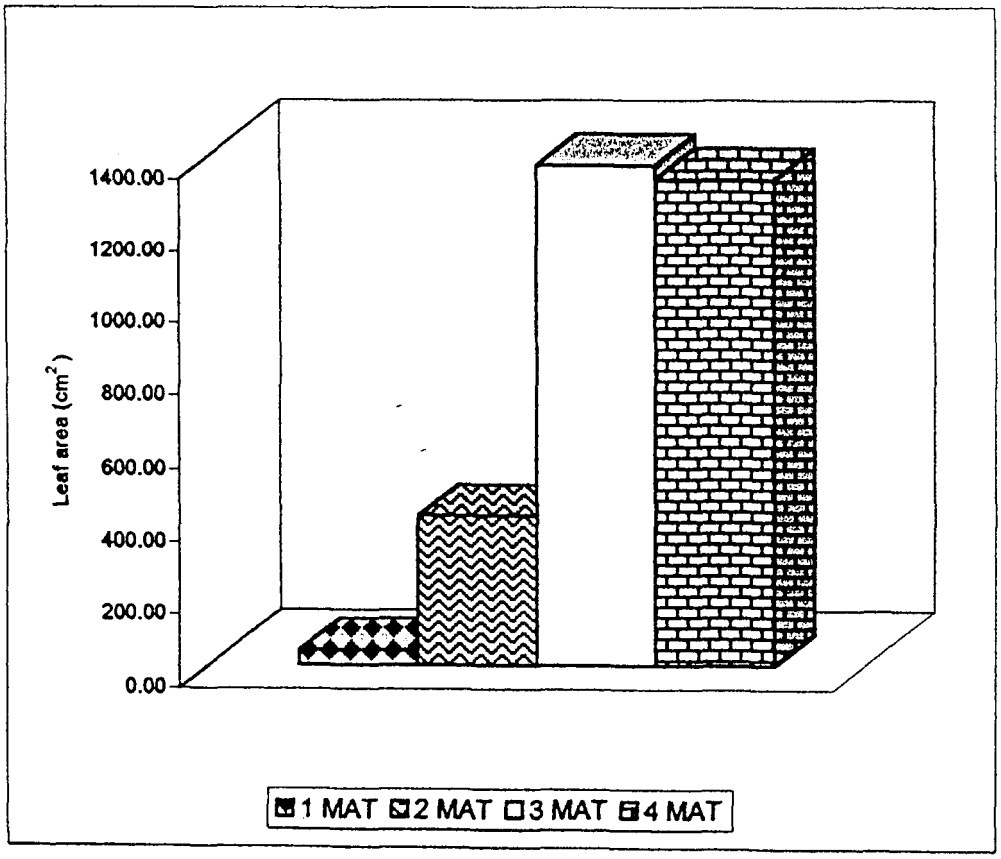


Fig. 16. Overall leaf area (cm^2)

positions at the entire MAT. Plants when harvested at 3 and 4 MAT produced higher LAI than when harvested at earlier dates (Table 14). Ac-8 and Ac-10 showed significantly higher LAI at 4 MAT. Ac-6 showed significantly higher LAI at 3 MAT.

4.2.1.2.3 Leaf area ratio

Leaf area ratio varied significantly among the accessions at all MAT except 4 MAT. LAR at 1 MAT varied from 90.39 $cm^2 g^{-1}$ (Ac-1) to 109.95 $cm^2 g^{-1}$ (Ac-2) (Table 15). At 2 MAT, LAR varied from 40.91 $cm^2 g^{-1}$ (Ac-1) to 78.51 $cm^2 g^{-1}$ (Ac-5). Ac-8 showed maximum LAR value (56.29 $cm^2 g^{-1}$) and Ac-2 showed minimum (33.50 $cm^2 g^{-1}$) at 3 MAT. LAR at 4 MAT ranged from 31.44 $cm^2 g^{-1}$ (Ac-2) to 49.88 $cm^2 g^{-1}$ (Ac-4).

Ac-3 showed the same rank for LAR irrespective of the dates of harvest. Ac-7, Ac-4 and Ac-6 maintained consistent ranking at various MAT.

All the accessions showed significantly higher LAR values at 1 MAT (Table 15). LAR was minimum for plants harvested at 4 MAT. Ac-8 and Ac-9 showed significantly lower LAR values at 4 MAT. However there was no significant difference in LAR for other accessions between 3 and 4 MAT.

4.2.1.2.4 Relative growth rate

RGR showed significant differences among the accessions at all stages. RGR at stage 1 varied from 77.28 g g^{-1} day^{-1}(Ac-4) to 105.62 g g^{-1} day^{-1} (Ac-6). At stage 2, RGR varied from 39.88 g g^{-1} day^{-1} (Ac-6) to 62.66 g g^{-1} day^{-1} (Ac-4). Ac-8 showed the highest RGR value (19.39 g g^{-1} day^{-1}) and Ac-6 the lowest (-2.42 g $g^{-1}day^{-1}$) at stage 3 (Table 16).

Ranking of the accessions for RGR fluctuated at various stages. But Ac-9 and Ac-10 showed consistent ranking irrespective of the dates of harvest. In

Table 14. Leaf area index of kalmegh accessions on various dates of harvest

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.059^{cd}	7	0.395^{e}	10	1.657^{ef}	9	1.392^{e}	10	0.876^{g}
Ac-2	0.082^{ab}	4	0.648^{cd}	6	1.817^{ef}	8	1.666^{de}	8	1.053^{ef}
Ac-3	0.041^{de}	8	0.453^{e}	8	1.592^{f}	10	1.640^{de}	9	0.931^{fg}
Ac-4	0.096^{a}	2	0.696^{cd}	4	3.268^{a}	1	3.190^{a}	1	1.812^{a}
Ac-5	0.096^{a}	1	1.008^{b}	2	2.794^{bc}	3	2.664^{abc}	3	1.641^{b}
Ac-6	0.071^{bc}	5	1.236^{a}	1	2.913^{ab}	2	2.163^{cd}	6	1.596^{b}
Ac-7	0.083^{ab}	3	0.766^{c}	3	2.033^{de}	6	2.140^{cd}	7	1.256^{d}
Ac-8	0.037^{e}	10	0.410^{e}	9	2.010^{de}	7	2.382^{bc}	4	1.209^{de}
Ac-9	0.038^{e}	9	0.573^{de}	7	2.450^{c}	4	2.330^{bc}	5	1.348^{cd}
Ac-10	0.065^{bc}	6	0.670^{cd}	5	2.402^{cd}	5	2.770^{ab}	2	1.477^{bc}
Overall mean	0.067		0.685		2.294		2.234		1.320
$CD_{5\%}$	0.020		0.178		0.402		0.565		0.172

$CD_{5\%}$ for comparing months within accessions: 0.344
$CD_{5\%}$ for comparing months over the accessions:0.109

Values having any common superscript are not significantly different from one another

Table 15. Leaf area ratio of kalmegh accessions on various dates of harvest ($cm^2 g^{-1}$)

Accession	Months after transplanting								Overall mean
	1		2		3		4		
	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	90.39^{e}	10	40.91^{d}	10	44.62^{bc}	7	37.46^{ab}	7	53.34^{d}
Ac-2	109.95^{a}	1	76.19^{ab}	3	33.50^{d}	10	31.44^{b}	10	62.77^{c}
Ac-3	93.76^{de}	9	52.88^{cd}	9	41.82^{c}	9	31.48^{b}	9	54.99^{d}
Ac-4	104.54^{abc}	3	74.20^{ab}	4	53.57^{a}	3	49.88^{a}	1	70.55^{a}
Ac-5	108.21^{ab}	2	78.51^{a}	1	48.96^{abc}	5	40.81^{ab}	6	69.12^{ab}
Ac-6	100.92^{abcd}	6	73.64^{ab}	5	52.37^{ab}	4	43.23^{ab}	5	67.54^{abc}
Ac-7	101.79^{abcd}	4	63.71^{bc}	8	48.67^{abc}	6	47.21^{a}	2	65.34^{abc}
Ac-8	96.21^{cde}	8	70.22^{ab}	7	56.29^{a}	1	37.22^{ab}	8	64.99^{bc}
Ac-9	98.64^{bcde}	7	77.26^{a}	2	55.41^{a}	2	43.79^{ab}	4	68.77^{ab}
Ac-10	101.09^{abcd}	5	71.16^{ab}	6	44.15^{c}	8	43.81^{ab}	3	65.05^{abc}
Overall mean	100.55		67.87		47.94		40.63		64.25
$CD_{5\%}$	10.33		13.49		7.77		14.23		5.55

$CD_{5\%}$ for comparing months within accessions: 11.11
$CD_{5\%}$ for comparing months over the accessions:3.51

Values having any common superscript are not significantly different from one another

Table 16. Relative growth rate of kalmegh accessions on various dates of harvest ($g\ g^{-1}\ day^{-1}$)

Accession	Stages						Overall mean
	1		2		3		
	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	90.14^{bc}	4	44.96^{cd}	8	0.08^{c}	8	45.06^{c}
Ac-2	81.40^{cd}	9	61.53^{a}	2	-1.07^{c}	9	47.28^{c}
Ac-3	99.72^{ab}	2	49.73^{bcd}	7	11.97^{ab}	2	53.80^{ab}
Ac-4	77.28^{d}	10	62.66^{a}	1	1.64^{bc}	7	47.19^{c}
Ac-5	88.66^{bcd}	8	50.18^{bc}	6	4.27^{bc}	5	47.71^{bc}
Ac-6	105.62^{a}	1	39.88^{d}	10	-2.42^{c}	10	47.69^{bc}
Ac-7	90.10^{bc}	6	41.79^{cd}	9	2.98^{bc}	6	44.96^{c}
Ac-8	90.02^{bc}	7	61.49^{a}	3	19.39^{a}	1	56.97^{a}
Ac-9	98.71^{ab}	3	59.60^{ab}	4	6.31^{bc}	3	54.88^{a}
Ac-10	90.10^{bc}	5	58.53^{ab}	5	4.97^{bc}	4	51.20^{abc}
Overall mean	91.18		53.03		4.81		49.67
$CD_{5\%}$	12.19		9.89		10.98		6.35

$CD_{5\%}$ for comparing months within accessions:11.00
$CD_{5\%}$ for comparing months over the accessions:3.48

Values having any common superscript are not significantly different from one another

general, all the accession recorded maximum RGR values at stage 1 and minimum values at stage 3.

4.2.1.2.5 Net assimilation rate

NAR values varied significantly among the accessions at all stages except stage-3. NAR varied from 0.93 g cm^{-2} day^{-1} (Ac-4) to 1.71 g cm^{-2} day^{-1} (Ac-1) at stage-1 (Table 17). At stage-2 NAR varied from 0.62 g cm^{-2} day^{-1} (Ac-6) to 1.98 g cm^{-2} day^{-1} (Ac-8). Ac-10 showed the lowest NAR value (-0.43 g cm^{-2} day^{-1}) and Ac-8 recorded the highest (4.18 g cm^{-2} day^{-1}) at stage-3

Ac-3 maintained the same rank (2^{nd} rank) for NAR in all the three stages. Most of the accessions showed consistent ranks at stage-2 and stage-3. In general, there was no significant difference in NAR values at all the stages. Ac-3 and Ac-8 showed significantly higher NAR values at stage-3.

4.2.1.6 Crop growth rate

CGR showed significant differences among the accessions at stage 1 and stage 2. CGR varied from 0.18 g cm^{-2} day^{-1} (Ac-8) to 0.54 g cm^{-2} day^{-1} (Ac-6) at stage 1(Table 18). At stage 2, CGR varied from 0.92 g cm^{-2} day^{-1} (Ac-1) to 1.72 g cm^{-2} day^{-1} (Ac-4). Ac-6 showed the lowest CGR value (-0.09 g cm^{-2} day^{-1}) and Ac-8 the highest (0.94 g cm^{-2} day^{-1}) at stage 3.

Here also ranking was not consistent under various stages, for most of the accessions. But Ac-5 and Ac-10 showed consistent ranking at all the stages. All the accessions showed significantly higher CGR values at stage 2.

4.2.1.2.7 Vigour index

Vigour index of accessions varied from 792 (Ac-5) to 1023.5 (Ac-6) (Table 19). Germination percentage varied from 78 per cent (Ac-3) to 89 per cent (Ac-7). Ac-9 recorded the highest plumule length (6.2 cm) and Ac-5 the lowest (4.8 cm). Radicle length varied from 4.7 cm (Ac-10) to 5.9 cm (Ac-9).

Table 17. Net assimilation rate of kalmegh accessions on various dates of harvest ($g\ cm^{-2}\ day^{-1}$)

Accession	Stages						Overall mean
	1		2		3		
	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	1.71^{a}	1	1.05^{c}	8	0.01^{b}	8	0.92^{b}
Ac-2	0.97^{de}	9	1.31^{abc}	4	-0.43^{b}	10	0.62^{b}
Ac-3	1.59^{a}	2	1.82^{ab}	2	3.91^{a}	2	2.44^{a}
Ac-4	0.93^{e}	10	1.10^{c}	6	0.29^{b}	5	0.77^{b}
Ac-5	1.02^{cde}	8	1.06^{c}	7	1.00^{ab}	7	1.02^{b}
Ac-6	1.32^{b}	3	0.62^{c}	10	-0.33^{b}	9	0.54^{b}
Ac-7	1.24^{bc}	4	0.89^{c}	9	0.62^{b}	6	0.92^{b}
Ac-8	1.18^{bcd}	6	1.98^{a}	1	4.18^{a}	1	2.44^{a}
Ac-9	1.19^{bc}	5	1.20^{bc}	5	1.31^{ab}	3	1.23^{b}
Ac-10	1.13^{bcde}	7	1.33^{abc}	3	1.09^{ab}	4	1.18^{b}
Overall mean	1.23		1.23		1.17		1.21
$CD_{5\%}$	0.22		0.71		3.29		1.07

$CD_{5\%}$ for comparing months within accessions:1.85
$CD_{5\%}$ for comparing months over the accessions:0.58

Values having any common superscript are not significantly different from one another

Table 18. Crop growth rate of kalmegh accessions on various dates of harvest ($g\ cm^{-2}\ day^{-1}$)

Accession	Stages						Overall mean
	1		2		3		
	Mean	Rank	Mean	Rank	Mean	Rank	
Ac-1	0.30^{cd}	4	0.92^{d}	10	0.003^{bc}	8	0.41^{b}
Ac-2	0.26^{d}	8	1.52^{ab}	2	-0.05^{c}	9	0.59^{ab}
Ac-3	0.27^{d}	7	0.98^{d}	9	0.65^{ab}	2	0.63^{ab}
Ac-4	0.28^{d}	6	1.72^{a}	1	0.10^{bc}	7	0.70^{a}
Ac-5	0.40^{b}	2	1.48^{ab}	4	0.33^{abc}	3	0.74^{a}
Ac-6	0.54^{a}	1	1.32^{bc}	5	-0.10^{c}	10	0.59^{ab}
Ac-7	0.38^{bc}	3	1.02^{cd}	7	0.13^{bc}	6	0.51^{ab}
Ac-8	0.18^{e}	10	1.00^{d}	8	0.94^{a}	1	0.71^{a}
Ac-9	0.23^{de}	9	1.22^{bcd}	6	0.30^{abc}	4	0.59^{ab}
Ac-10	0.29^{d}	5	1.51^{ab}	3	0.29^{abc}	5	0.70^{a}
Overall mean	0.31		1.27		0.26		0.61
$CD_{5\%}$	0.08		0.31		0.69		0.25

$CD_{5\%}$ for comparing months within accessions:0.43
$CD_{5\%}$ for comparing months over the accessions:0.13

Values having any common superscript are not significantly different from one another

Table 19. Vigour Index (25 DAS) of kalmegh accessions

Accession	Shoot length	Root length	Germination %	Vigour Index
Ac-1	5.4	5.1	82	861.0
Ac-2	5.2	5.2	88	915.2
Ac-3	6.0	5.1	78	865.8
Ac-4	5.8	4.9	86	920.2
Ac-5	4.8	5.1	80	792.0
Ac-6	5.2	5.3	82	861.7
Ac-7	6.1	5.4	89	1023.5
Ac-8	5.3	4.9	86	877.2
Ac-9	6.2	5.9	81	980.1
Ac-10	5.6	4.7	84	865.2

Table 20. Days to 50% flowering and moisture content of kalmegh accesssions

Accession	Days to 50% Flowering (DAS)*	Moisture content (%)
Ac-1	95	65.2
Ac-2	140	70.7
Ac-3	148	59.5
Ac-4	150	59.5
Ac-5	152	68.3
Ac-6	145	69.1
Ac-7	105	64.5
Ac-8	165	56.7
Ac-9	144	57.5
Ac-10	145	68.0

* Days after sowing

4.2.2 Genotypic and phenotypic variability

4.2.2.1 Plant characters

Plant characters except dry weight/fresh weight, shoot length and root length showed relatively high GCV at various MAT (Table 21). GCV was absent for stem dry weight at 4 MAT and for root length at 3 MAT, GCV was relatively low for total plant dry weight at 4 MAT. But PCV was high for most of the plant characters at various MAT. Shoot length showed comparatively low PCV at various dates of harvest.

4.2.2.2 Physiological parameters

Leaf area and LAI showed high GCV at various MAT (Table 22). LAR showed relatively low GCV. RGR, CGR and NAR recorded moderate GCV at stage 1 and stage 2. But both recorded high GCV and PCV at stage 3. PCV values were high for leaf area and LAR at all stages. But LAR recorded low PCV at 1 MAT.

4.2.3 Heritability

4.2.3.1 Plant characters

Among plant characters leaf dry weight showed maximum heritability (0.72 to 0.86) at various MAT (Table 21). Heritability was high for stem dry weight, total plant dry weight and root dry weight when harvested at various MAT except 4 MAT. Shoot length recorded moderate heritability on various dates. But root length and dry weight/fresh weight showed variation for heritability on various MAT. Total fresh weight recorded moderate heritability at various dates except 2 MAT.

4.2.3.2 Physiological parameters

Among physiological parameters, leaf area and LAI showed high heritability (0.72 to 0.86) at various MAT (Table 22). LAR showed moderate heritability at all stages except 4 MAT. CGR and NAR showed variation in

Table 21. Measures of variability and heritability for plant characters in kalmegh accessions

	Total plant fresh weight				Stem dry weight				Root dry weight			
	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Mean	2.04	30.87	87.59	94.45	0.052	2.373	16.720	21.202	0.045	0.573	1.814	2.269
Range	1.25-2.87	17.40-39.03	63.10-118.77	64.42-127.58	0.034-0.070	1.390-3.630	10.886-22.615	14.341-25.817	0.029-0.058	0.180-0.947	1.369-2.731	1.758-3.492
Standard error	0.31	5.81	8.00	11.95	0.006	0.263	1.432	3.996	0.004	0.126	0.161	0.423
GCV (%)	21.46	14.59	17.68	17.03	23.82	30.06	20.67	*	19.86	39.80	23.02	12.39
PCV (%)	33.90	35.71	23.72	27.78	30.35	35.70	25.44	32.12	24.88	54.91	27.69	34.62
Heritability	0.40	0.17	0.56	0.38	0.62	0.71	0.66	0	0.64	0.53	0.69	0.13

Table 21. (Continued)

	Leaf dry weight				Total plant dry weight				Shoot length			
	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Mean	0.297	3.087	10.344	10.073	0.394	6.033	28.878	33.544	10.15	29.02	50.68	58.83
Range	0.161-0.430	1.775-5.570	7.175-14.740	6.274-14.390	0.228-0.550	3.456-10.086	21.390-36.602	22.373-40.439	7.57-12.80	18.63-42.33	35.57-60.83	49.33-66.00
Standard error	0.031	0.269	0.610	0.858	0.031	0.472	1.838	4.546	0.73	2.44	2.84	2.83
GCV (%)	33.10	38.05	24.02	23.57	29.31	30.20	18.79	9.52	13.76	22.61	13.24	7.99
PCV (%)	37.89	40.95	26.10	27.80	32.44	33.09	21.79	25.33	18.59	26.87	16.39	11.54
Heritability	0.76	0.86	0.85	0.72	0.82	0.83	0.74	0.14	0.55	0.71	0.65	0.48

Table 21. (Continued)

	Root length				Dry weight/ Fresh weight			
	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Mean	7.31	11.65	15.73	20.88	0.200	0.207	0.335	0.361
Range	4.73-10.03	8.07-13.73	11.73-18.27	16.13-28.17	0.160-0.247	0.141-0.290	0.280-0.398	0.293-0.438
Standard error	0.70	1.57	2.47	1.31	0.032	0.036	0.025	0.026
GCV (%)	20.96	7.69	*	13.91	2.37	8.82	9.44	12.38
PCV (%)	26.79	24.57	25.23	17.67	27.64	31.80	16.35	17.52
Heritability	0.61	0.10	0	0.62	0.01	0.07	0.33	0.50

*Calculated genotypic variance was negative, but treated as zero for calculation of coefficient of variation and heritability

Table 22. Measures of variability and heritability for physiological parameters in kalmegh accessions

	Leaf area				LAI				LAR			
	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Mean	40.13	411.14	1376.17	1340.24	0.07	0.69	2.29	2.23	100.55	67.87	47.94	40.63
Range	22.1-57.9	236.7-741.3	954.9-1960.8	834.9-1914.3	0.04-0.09	0.39-1.23	1.59-3.27	1.39-3.19	90.39-109.95	40.91-78.51	33.50-56.29	31.44-49.88
Standard error	4.08	35.85	81.13	114.08	0.01	0.06	0.14	0.19	3.48	4.54	2.61	4.79
GCV	32.73	38.00	24.01	23.56	32.68	37.98	24.01	23.56	5.00	16.61	13.70	9.68
PCV	37.17	40.89	26.09	27.79	37.11	40.95	26.10	27.78	7.81	20.25	16.64	22.59
Heritability	0.78	0.86	0.85	0.72	0.78	0.86	0.85	0.72	0.41	0.67	0.68	0.18

Table 22. (Continued)

	RGR			NAR			CGR		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
Mean	91.18	53.03	4.81	1.23	1.23	1.16	0.31	1.27	0.26
Range	77.28-105.62	39.88-62.66	-2.42-19.39	0.93-1.71	0.62-1.98	-0.43-4.18	0.18-0.54	0.92-1.72	-0.09-0.94
Standard error	4.10	3.33	3.69	0.07	0.24	1.11	0.03	0.10	0.213
GCV	8.06	15.31	112.99	19.84	26.59	102.75	30.87	20.70	87.48
PCV	11.22	18.78	174.57	22.35	42.63	194.21	34.01	25.04	178.33
Heritability	0.52	0.67	0.42	0.79	0.39	0.28	0.82	0.68	0.24

Stage 1, stage 2 and stage 3 refer to periods of growth between 1st and 2nd, 2nd and 3rd and 3rd and 4th MAT respectively

heritability at different stages. However, RGR maintained moderate heritability at all stages.

4.3 Biochemical observations

4.3.1 Total phenol content (mg g^{-1})

Phenol content was estimated using catechol as the standard, and standard curve showed a progressive increase in OD value up to 0.5 per cent (Fig. 17). Dry sample (0.5 g) was taken for the estimation of total phenol. It was estimated from the plant samples collected after 4 MAT. There was wide variation among the accessions in the phenol content. Accession Ac-10 recorded the maximum (83.4 mg g^{-1}) and Ac-9 the minimum (28.2 mg g^{-1}) (Table 23). The percentage of phenol content varied from 2.82 to 8.34.

4.3.2 Andrographolide content (%)

Dried finely powdered plant samples (0.5 g) were taken for the estimation of andrographolide. It was estimated from the samples collected after 4 MAT. There was variation in the andrographolide content among the accessions. Accession Ac-2 recorded the maximum (4.29%) and Ac-4 the minimum (0.77%) (Table 23 and Fig.18).

4.3.3 Flavonoid content

Running solvent systems were prepared by mixing hexane and ethyl acetate in desired ratios. Among the various ratios tried hexane: ethyl acetate at 1:2 and 1:3 gave better results. It gave yellow to pale green fluorescent spots under UV light. At 1:2, the solvent mixture separated the spots into two distinct spots for accessions Ac-1, Ac-3, Ac-4, Ac-5 and Ac-7. The mixture of hexane and ethyl acetate in the ratio of 1:3 separated the spots into 3 distinct spots for accessions Ac-3 and Ac-4. It separated accessions Ac-1, Ac-5 and Ac-7 into two distinct spots and Ac-9 to a single distinct spot 1:3 (Table 24 and Fig.19_a and 19_b).

Table 23. Total Phenol and andrographolide content in kalmegh accessions

Accession	Andrographolide content			Phenol content		
	Per plant weight (g)	% of plant dry weight	% of plant fresh weight	Per plant weight (g)	% of plant dry weight	% of plant fresh weight
Ac-1	0.36	1.60	0.56	1.15	5.16	1.80
Ac-2	1.35	4.29	1.26	1.93	6.11	1.80
Ac-3	0.59	1.72	0.70	1.51	4.38	1.80
Ac-4	0.30	0.77	0.31	3.07	7.98	3.24
Ac-5	1.65	4.07	1.29	1.76	4.34	1.38
Ac-6	0.26	0.81	0.25	1.57	4.91	1.52
Ac-7	0.66	2.36	0.84	1.11	3.96	1.41
Ac-8	0.32	0.83	0.36	1.47	3.84	1.66
Ac-9	1.05	3.29	1.40	0.91	2.82	1.21
Ac-10	0.70	1.85	0.59	3.16	8.34	2.67

Table 24.R_f values for Flavanoids in kalmegh accessions

Accession	Hexane: Ethyl acetate (1:2)		Hexane: Ethyl acetate (1:3)		
	Spot 1	Spot 2	Spot 1	Spot 2	Spot 3
Ac-1	0.59	0.69	0.31	0.54	Nil
Ac-2	Nil	Nil	Nil	Nil	Nil
Ac-3	0.51	0.69	0.56	0.72	0.45
Ac-4	0.55	0.69	0.57	0.78	0.34
Ac-5	0.54	0.69	0.51	0.59	Nil
Ac-6	Nil	Nil	Nil	Nil	Nil
Ac-7	0.61	0.74	0.44	0.56	Nil
Ac-8	Nil	Nil	Nil	Nil	Nil
Ac-9	Nil	Nil	Nil	0.51	Nil
Ac-10	Nil	Nil	Nil	Nil	Nil

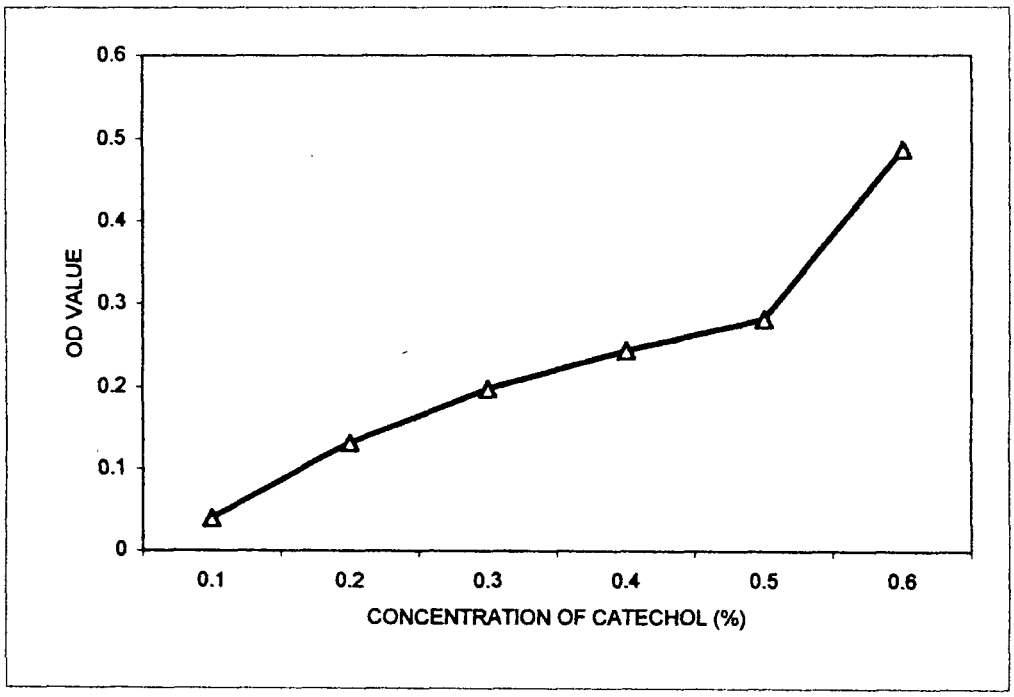
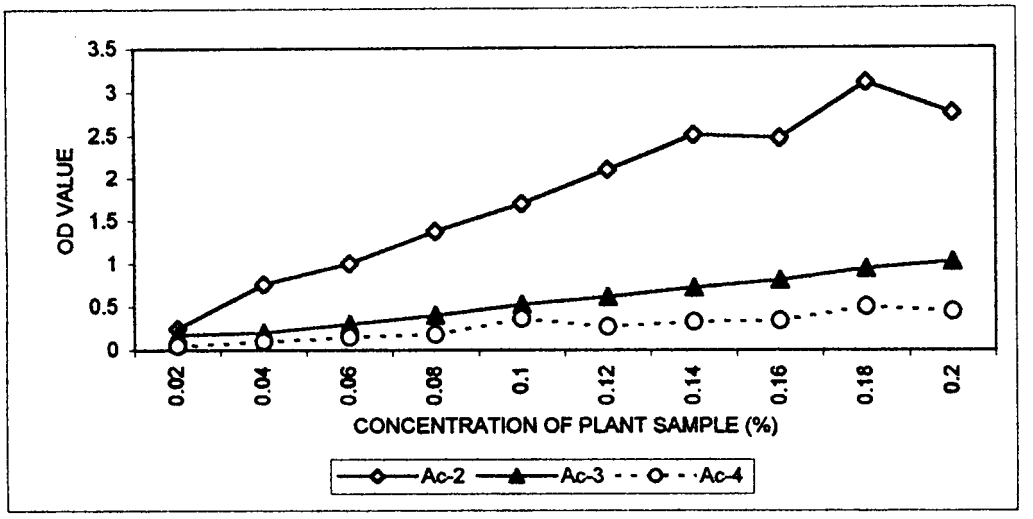
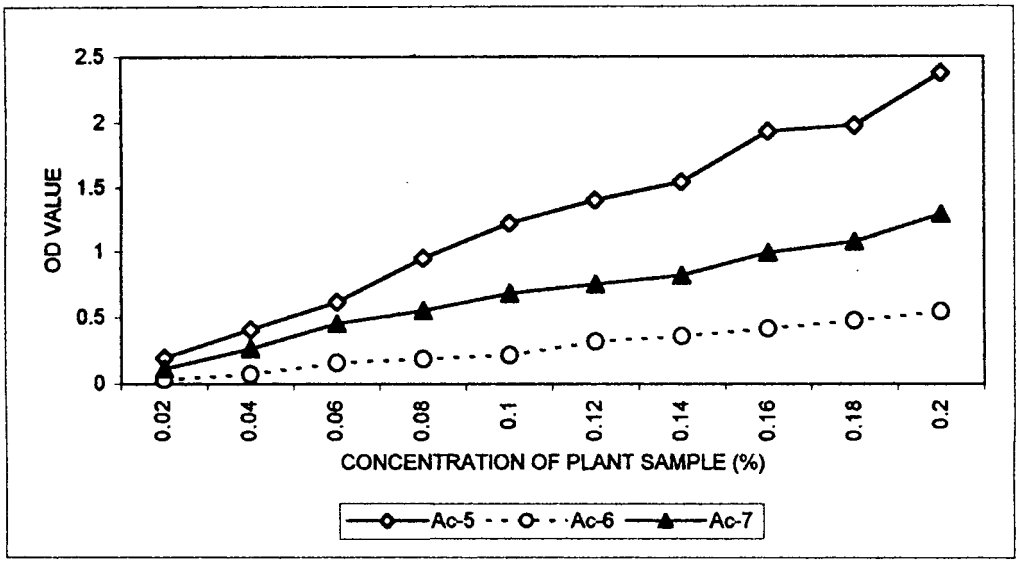


Fig.17. Standard curve for total phenol





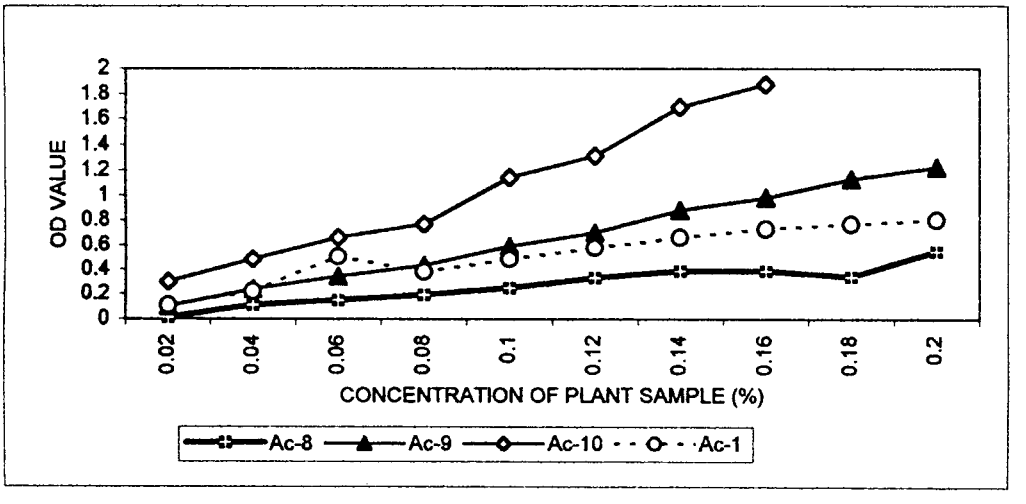


Fig.18. Absorbance of plant samples at 226 nm in various accessions of kalmegh

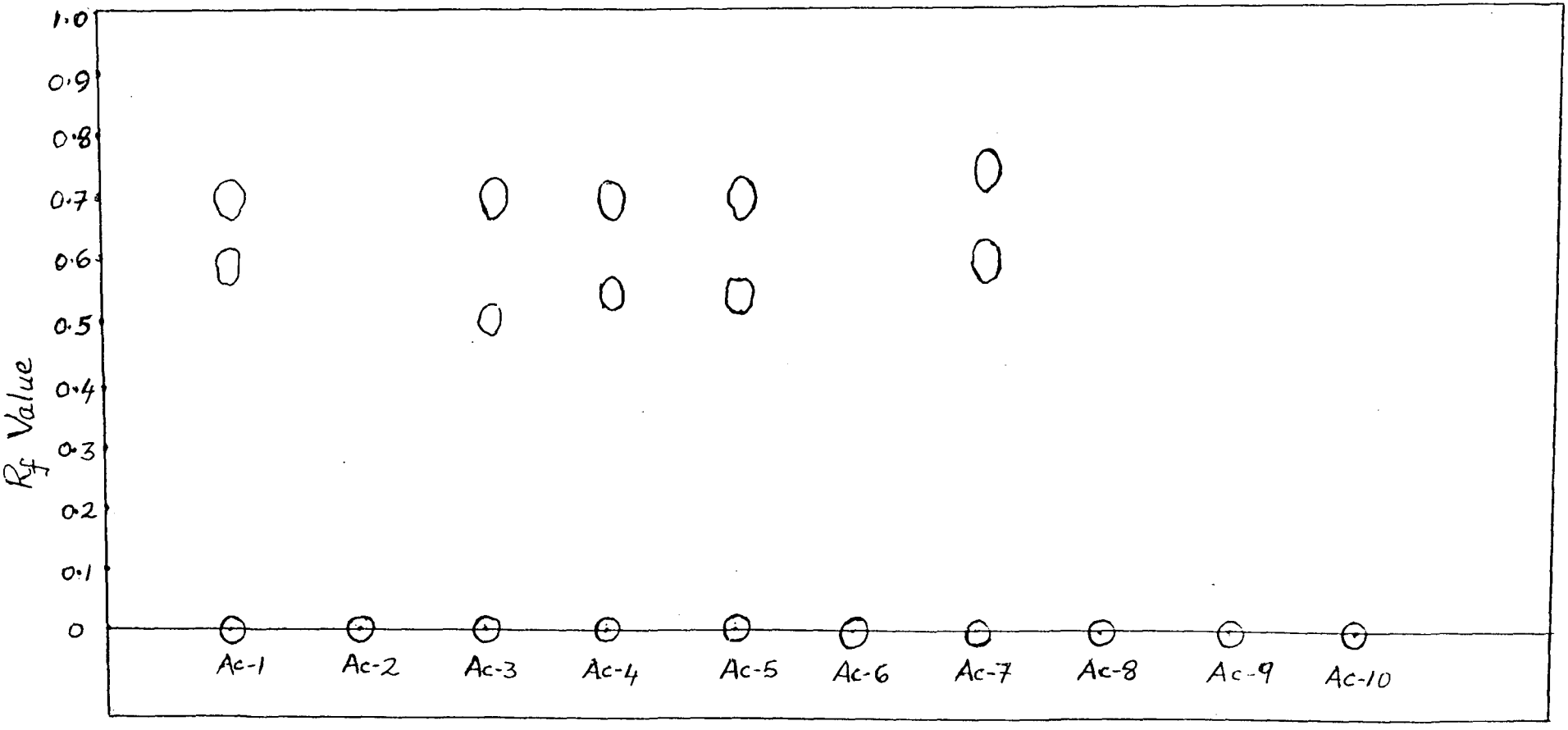


Figure 19a. Elution pattern of flavonoids using Hexane : Ethyl acetate [1: 2]

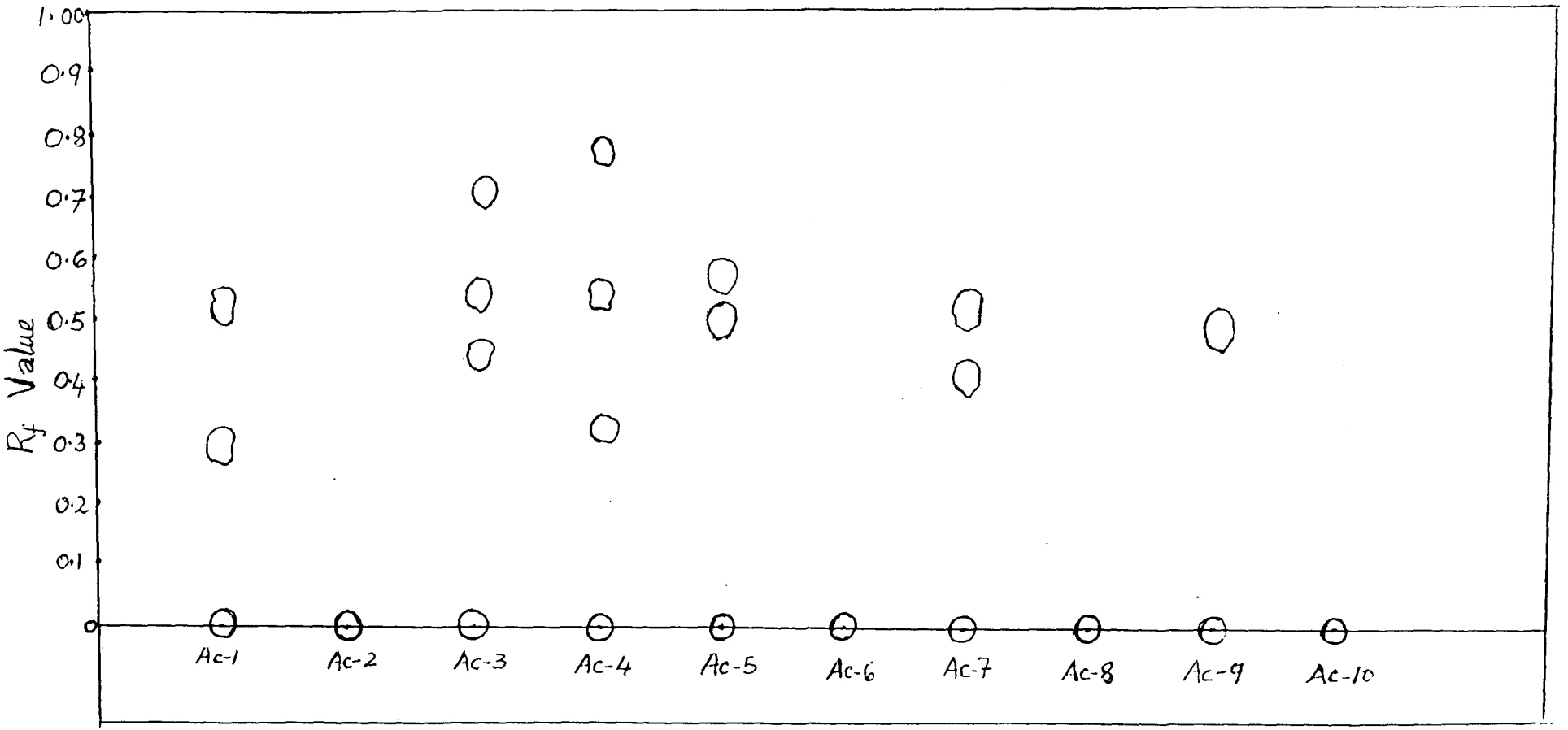


Figure 19b. Elution pattern of flavanoids using Hexane : Ethyl acetate [1 : 3]

Discussion

DISCUSSION

5.1 Morphology

Annual erect herbaceous nature of kalmegh had been described by many (Datta and Mukherji, 1952; Alagesaboopathi and Balu, 1999). In the present study consisting of ten accessions, they ranged from herbaceous to sub herbaceous (nearly shrubby) in nature. The genus Andrographis comprises species, which are herbaceous to shrubby in nature (Kirtikar and Basu, 1935 and Mathew, 1983).

Branching pattern varied among the accessions ranging from a few to many. Pareek *et al.* (1980) and Kurien *et al.* (1984) reported similar variation in branching pattern of Ocimum species. Accessions showed variability for stem thickness and number of nodes as previously reported by Rajesh (1994) and Jamwal and Kaul (1997). Further, number of nodes influenced the number of branches. Variability observed in the internodal length supports the earlier reports in this crop by Alagesaboopathi and Balu (1996).

Leaf characters such as type, leaf base, leaf tip, arrangement, texture and colour of younger leaves showed a little variation among the accessions (Figure 20), and were in conformity with the description by Gamble (1921) and Aiyar and Kolammal (1962). Similarly the observations that basal leaves are larger than others are in agreement with that of Alagesaboopathi (1993) and Jamwal and Kaul (1997). Gamble (1921) and Datta and Mukerji (1952) reported that the leaves were sessile or petiolate with a maximum length of 0.6 cm. In the present study, petiole length ranged from 0.13 to 0.33 cm. At maturity most of the accessions showed conspicuous changes in colour. For example, Ac-1, which had dark green young leaves, turned completely to reddish brown at the time of flowering. This change in leaf colour can be used as an indication to maturity.

Inflorescence in kalmegh was reported to be as a cymose panicle (Bentley and Trimen, 1890), raceme (Hooker, 1892), raceme to subpaniculate

(Gamble, 1921), and pyramidal panicle (Kirtikar and Basu, 1935). Panicles observed in this study were both terminal and axillary in position among all the accessions, though branching of inflorescences varied. Inflorescence characters like inflorescence length, peduncle length and pedicel length agreed with the description of Hooker (1892) and Aiyar and Kolammal (1962). Similarly floral characters agreed with description by Bentley and Trimen (1890), Hooker (1892) and Datta and Mukerji (1952). However, the purple patches in the lower lip of corolla showed variation in their pattern (Plate IV).

Fruit and seed characters were also uniform among the accessions and were in agreement with the descriptions of Mathew (1983) and *Warrier et al.* (1996). But fruit colour at maturity varied among the accessions. It was observed that accessions, which had reddish brown leaves on maturity, also had capsules with reddish tinge.

5.2 Herbage yield

In the Indian systems of medicine the whole plant of Kalmegh is often harvested, dried and used (Aiyar and Kolammal, 1962 and Farooqi *et al.*, 1999). In the present study total plant dry weight had been observed at various months after transplanting (1, 2, 3 and 4 MAT). The various dates of harvest had significant effect on the herbage yield. But it was exceptionally pronounced at 3 MAT compared to 2 MAT. Though certain accessions namely Ac-3 and Ac-8 produced significantly high yields at 4 MAT the optimum stage of harvest for kalmegh can be fixed as 3 MAT, i.e. 90 days after transplanting. Quantum increase in foliage up to 3 MAT was earlier reported in Kalmegh (Anon., 1998). Initial slow growth of Andrographis had been noted by Prasad and Joseph (1997) and Joy *et al.* (1998). Sudden increase in growth after a period of slow growth has been reported in *Phyllanthus niruri* (Phadnis, 1994).

The various accessions showed significant variability on all dates of harvest except at 4 MAT. However, the ranking of these accessions was not

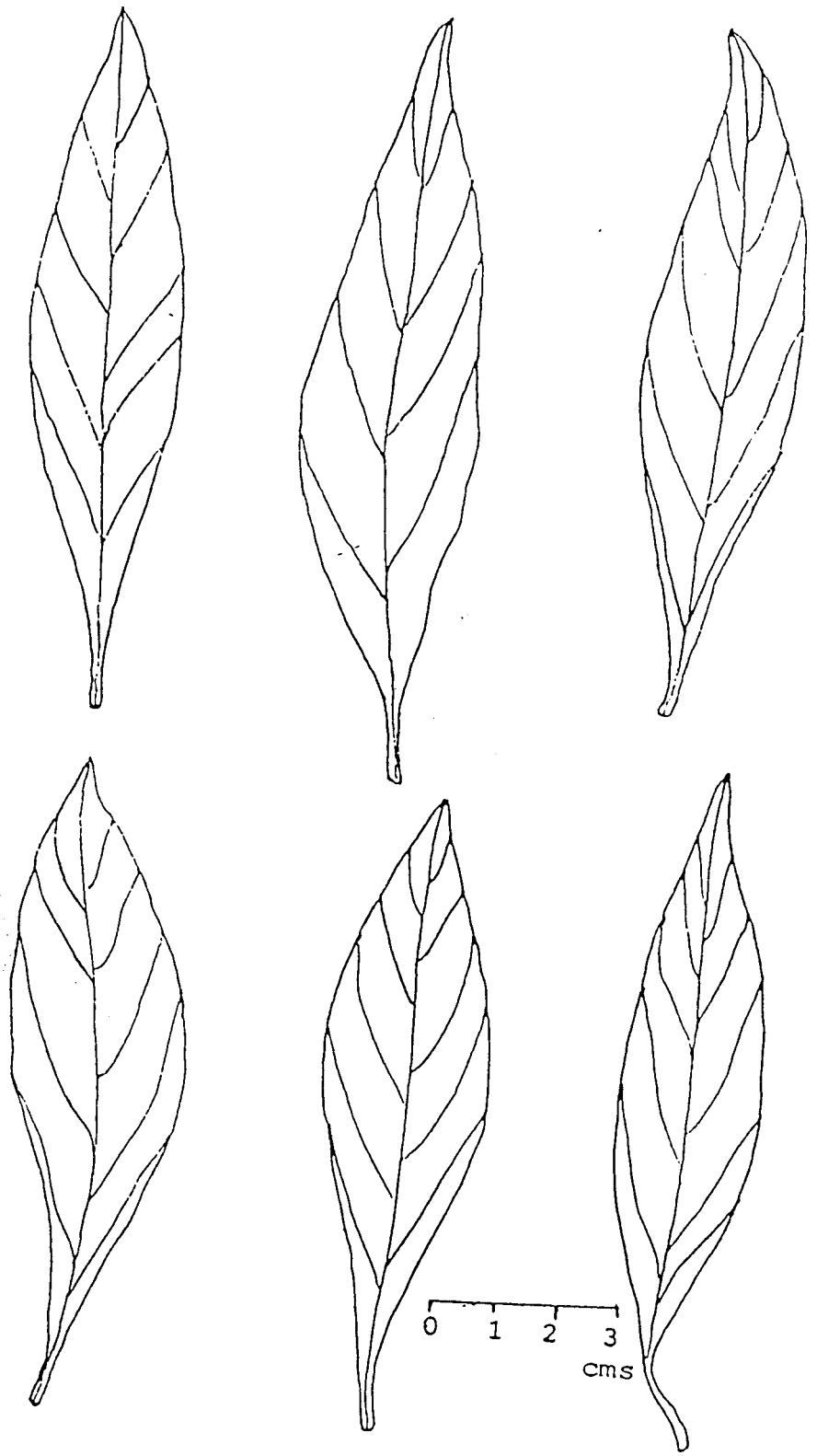


Fig. 20. Leaf outlines of Andrographis paniculata

consistent throughout as suggested also by their significant association with the dates of harvest. At the optimum stage of harvest, i.e. 3 MAT, the highest herbage yield was given by Ac-4, which was collected from Aluva, Ernakulam District. Increase in plant height also slackened after the third month. This coupled with leaf loss after third month render retaining of plants beyond this period uneconomical. At 3 and 4 MAT, the total plant dry weight was mainly contributed by stem (60%) followed by leaves (30%). Similar results were reported in *Phyllanthus niruri* (Phadnis, 1994).

During the first two months after transplanting, dry matter allocation was more in leaves than in stem and roots. After 2 MAT, assimilates were accumulated more in stem than in other plant parts. Translocation of assimilates to roots was only marginal. Similar results are observed in cassava (Ramanujan and Birdar, 1987) and plumbago (Menon, 1999). In short, according to this study, optimum stage for harvest in kalmegh in terms of fresh weight as well as dry weight (stem, root, leaf and total) is at 3 MAT. Though there was increase in dry weight and fresh weight at 4 MAT also, that increase was not significant. Similar results were reported in periwinkle (Pareek *et al.*, 1981).

Herbage yield was influenced also by the days taken to reach 50% flowering. For example Ac-1 and Ac-7 which came into flowering at the earliest period (approximately 100 days after sowing) produced lowest herbage yield on the final dates of harvest. Considerable variation was observed among the accessions for flowering (95 to 165 days).

In terms of fresh weight, the increase in herbage yield beyond 3rd month was even less. This can be attributed to the leaf loss and low rate of leaf production after flowering. Proportionate contribution by leaves to the plant weight is higher in case of fresh weight than dry weight. Kalmegh is used for many drug preparations in the fresh state also. Hence it is advisable to harvest the plant at 3 MAT to get maximum yield in terms of fresh weight.

5.3 Physiological parameters

Leaf area was comparable to the values recorded in the crop by Alagesaboopathi (1993). Leaf area showed significant increase up to 3 MAT but it was followed by a decline beyond this stage. Marked decline in leaf area at 4 MAT can be attributed to leaf loss during this period.

In *Holostemma annulare*, Meera (1994) reported a progressive increase in leaf area up to 12 months after transplanting and a decline thereafter. Similar pattern of leaf area development was reported also in colocassia (Mohankumar and Sadanandan, 1989).

Variation in LAI was similar to the pattern of leaf area. Plants at 3 and 4 MAT produced higher LAI than at earlier dates. High LAI was associated with high leaf area and leaf dry weight. Variation for LAI at different periods of growth has been reported in pigeonpea (Mehra *et al.*, 1987) and in *Plumbago* spp. (Menon, 1999).

Leaf area development per unit weight of dry matter accumulation per plant (LAR) showed a significant decrease from 1 MAT to 4 MAT. As dry matter accumulation was more in leaves during the first two months, LAR was also observed high at the earlier dates. At 3 and 4 MAT, contribution of leaves to total dry matter decreased, and this was the reason for a lower LAR at later stages. Significant difference for LAR among accessions at different stages of crop growth was reported in colocassia (Chowdhury, 1995).

Dry weight increase in a time interval in relation to the initial weight (RGR), showed a continuous decrease among the accessions. It is because the difference in dry weight among the accessions at earlier stages was high compared to the later stages. In addition, the initial weight was significantly lower at earlier dates.

RGR and NAR were also higher in the initial stages. This shows an increase in the efficiency of available leaf and relatively high dry matter production at the earlier periods. Negative values obtained for CGR, RGR and NAR in stage 3, might be due to the fact that leaves lost during this stage was not reckoned into the determination of these parameters. It may partly be explained by sampling variation also. Negative values are reported also in other crops for example castor (Reddy *et al.*, 1997) and plumbago (Menon, 1999).

5.4 Heritability

Variability among the accessions when expressed as a ratio to the mean (PCV) was inconsistent on various dates of harvest. It was generally low on the later dates. This was because phenotypic standard deviation did not increase commensurate with increase in the mean expression at later stages. Heritability (broad sense) indicates to what extent the observed phenotypic differences are due to differences in genotype. As in the case of PCV, heritability also showed inconsistency at various dates of harvest. However, leaf dry weight showed consistently high heritability at all months. At 3 MAT, which is the optimum stage of harvest all the characters relating to herbage yield were highly heritable. Such high heritability suggests that the identification of superior types of kalmegh in a locality by their phenotypic performance would be effective.

Physiological parameters generally showed high heritability at the optimum stage of harvest (3 MAT). This is supported by the findings in soybean (Mahajan *et al.*, 1994) and periwinkle (Dwivedi *et al.*, 1999).

5.5 Biochemical studies

5.5.1 Total phenol content

Presence of polyphenols and many other phenolic compounds have been reported in kalmegh (Asolkar *et al.*, 1992). It varied considerably among the accessions ranging from 28.2 to 83.4 mg g^{-1}. Though presence of pehnol was

reported in different species of Andrographis (Alagesaboopathi and Balu, 1996c), no work had so far been done to assess the variability among the accessions in any of the Andrographis species. However, such variability had been studied in several crops for instance black pepper (Dagade, 1999) and tomato (Bose, 1999).

5.5.2 Andrographolide

Based on the absorbance of plant samples at 226 nm for various accessions of Kalmegh (Fig.18) we can conclude that

1) A straight-line common up to 0.14 per cent was noticed in general, for all the accessions. It is an indication that active ingredient is constantly increasing, even though concentration of active ingredient for each accession is varying.
2) Variation for active ingredient is explicit from the colorimetric and graph method.
3) Effect of expression is directly proportional to concentration of sample.

Wide variation in Andrographolide was observed among accessions (0.77 to 4.29%) as in agreement with earlier reports. The lowest value obtained in this study is comparable to those by Moktader and Sircar (1939), Datta and Mukerji (1952), Maiti *et al.* (1959), Dutt *et al.* (1999). The maximum value agreed with the results of Datta and Mukerji (1952), Alagesaboopathi (1993) and Rajesh (1994).

5.5.3 Flavonoid content

Flavonoids contain conjugated aromatic systems and thus show intense absorption bands in the UV and visible regions of the spectrum. Out of the different classes of flavonoids recognized, flavonoles and flavones give yellow to yellowish green fluorescent spots under UV light (Harborne, 1973). Running solvent used in this study was a mixture of hexane and ethyl acetate. Running solvent when tried at various ratios (Hexane: ethyl acetate at 3:1, 3:2, 1:1, 1:2, 1:3 and 1:4) showed yellow to pale green fluorescent spots, indicating the presence of

flavonoids in the plant samples. Among the various ratios tried 1:2 and 1:3 gave better results. In both cases it could be noticed that concentration of ethyl acetate is more. As the polarity of ethyl acetate is more than hexane, the polarity of running solvent system at 1:2 and 1:3 should also be high. This indicates the polar nature of fluorescent compounds present inside the plant samples. However accessions such as Ac-2, Ac-6. Ac-8 and Ac-10 failed to separate into distinct spots. It can be attributed to the failure of the running solvent system used in this study to separate the flavonoids present in these accessions. Also these accessions may contain flavonoids (other than flavones and flavonoles), which cannot give characteristic fluorescent spots under UV light. When the concentration of ethyl acetate was further increased (1:4) most of the spots accumulated at the solvent front. In short the combination of hexane: ethyl acetate at 1:3 was the best as it gave a distinct profile of the flavonoid spots, which could be used for further characterization studies.

5.5.4 Comparative evaluation of biochemical compounds among kalmegh accessions

The results revealed that (Table 5, 9, 20 and 23) moisture content is positively related to andrographolide content. For example accession Ac-2 which gave maximum andrographolide (4.29%) also recorded maximum moisture content (70.7%). Whereas phenol content varied with accessions to a limited extend. Any way phenol content was always low in those accessions in which high andrographolide is recorded. Phenol content always depends on both genetic and environmental factors. Phenol can be converted to other secondary products like andrographolide especially in kalmegh. It can also be inferred that total dry weight and phenol percentage have a direct relation.

This can be schematically represented as shown below

1	Moisture content ↑	Andrographolide ↑	e.g.Ac-2, Ac-5
2	Dry weight ↑	Phenol ↑	e.g.Ac-4, Ac-10
3	Phenol ↑	Andrographolide ↓	e.g.Ac-4, Ac-6

Above statements can also be supported with the grouping of the kalmegh accessions studied. The accessions are classified into three groups such as low, medium and high. Details are furnished in Table 25.

Table 25. Biochemical characterization of kalmegh accessions

Accession	*Andrographolide (g / plant)	#Phenol (g / plant)	$Dry weight (g)	&Moisture content (%)
Ac-1	Low	Low	Low	High
Ac-2	High	Medium	Medium	High
Ac-3	Medium	Medium	Medium	Low
Ac-4	Low	High	High	Low
Ac-5	High	Medium	High	High
Ac-6	Low	Medium	Medium	High
Ac-7	Medium	Low	Low	Medium
Ac-8	Low	Low	High	Low
Ac-9	High	Low	Medium	Low
Ac-10	Medium	High	High	High

*Low: <0.5 Medium: 0.5-1.0 High: >1.0

#Low: <1.5 Medium: 1.5-3.0 High: >3.0

$Low: <30 Medium: 30-35 High: >35

&Low: < 60 Medium: 60-65 High: >65

This grouping is confirming our interpretation of high andrographolide in accessions having high moisture content. The results also reveal that medium dry weight and phenol may have low or high andrographolide content. Same is the trend with low phenol and low dry weight. It indicated that when dry matter and phenol contents are in the same level, plants showed fluctuation in the andrographolide content.

In the light of the above observations, any accession having higher moisture content with same level of phenol content and dry weight can be considered as a superior type and recommended for rapid multiplication.

Summary

SUMMARY

The accessions of kalmegh studied originated from various parts of Kerala and neighbouring states. Wide variation observed among these accessions could be attributed to genetic and\or locational differences. These accessions were grown under a uniform set of conditions in an appropriate field design to assess morphological variability, growth pattern and the optimum stage for maximum herbage yield. It was also aimed for analyzing variation in biochemical compounds.

Description of the various accessions with respect to morphological features such as root, inflorescence, flower and fruit characters did not reveal much variation. However limited variation for habit, stem and leaf characters existed among most of the accessions.

In respect of characters relating to herbage yield and physiological parameters, accessions showed lot of variability at the phenotypic level. Heritability for the characters when measured on various dates of harvest was inconsistent. It was generally the highest at 3 months after transplanting for yield and yield components. On the basis of growth pattern, the optimum stage of harvest can be fixed as 3 months after transplanting. Ranking of accessions for herbage yield and its components showed inconsistency over various dates of harvest. At The optimum stage of harvest, the maximum yield was produced by Ac-4, which was collected from Aluva (Ernakulam district). Physiological parameters namely leaf area and LAI increased steadily up to 3 MAT and thereafter it declined. These parameters maintained high heritability at all the dates of harvest. Parameters such as LAR, RGR, CGR, and NAR were high on the earlier dates of harvest.

Biochemical compounds namely total phenol and andrographolide showed considerable variation among the accessions. Accessions having higher

moisture content recorded higher andrographolide. Total phenol content and dry weight were directly related. However accessions having high phenol content generally showed low andrographolide content. Accession Ac-2 (collected from Kanchikode, Palakkad) recorded the maximum andrographolide content on dry weight basis. Whereas Ac-5 (collected from Chevakkadu, Tamil Nadu) showed the maximum value on per plant basis. Maximum Phenol content was observed in Ac-10 (collected from Murukumpuzha, Thiruvananthapuram) on both dry weight and per plant basis.

References

REFERENCES

Abdul-Baki, A. and Anderson, J.D. 1973. Vigour determination in soybean seed by multiple criteria. *Crop Sci.* **13**:630-633

*Ahmed, M. and Talukder, S.A. 1977. Studies on the hypoglycaemic activity of kalmegh (*Andrographis paniculata* Nees.) on the blood sugar level of rats. *Bangladesh Pharm. J.* **6**(2):21

*Ahmedullah, M. and Nayar, M.P. 1986. *Endemic Pl. Indian Region.***1**:143-146

Aiyar, K.N. and Kolammal, M. 1962. *Pharmacognosy of Ayurvedic Drugs 1(5).* Department of Pharmacognosy, University of Kerala, Trivandrum, pp.63-69

Alagesaboopathi, C. 1993. Studies on the biology of some medicinally potential species of Andrographis Wall. of the Shevaroy hills of Tamil Nadu, India. Ph.D. thesis, Bharathidasan University, Tiruchirapalli, Tamil Nadu.

Alagesaboopathi, C. and Balu, S. 1995. Mass multiplication of *Andrographis alata* Nees by stem cuttings. *J. Econ. Tax. Bot.* **19**:687-689

Alagesaboopathi, C. and Balu, S. 1996a. Seed germination studies on *Andrographis paniculata* Nees. *J. Living World* **3**(1):46-49

Alagesaboopathi, C. and Balu, S. 1996b. Seed germination studies on *Andrographis echioides* Nees. *Crop Res.* **12**(3):383-386

Alagesaboopathi, C. and Balu, S. 1996c. Phytochemical observations on some species of Andrographis Wall. *Ancient Sci. Life* **15**(4):293-295

Alagesaboopathi, C. and Balu, S. 1998. Abnormal leaves in *Andrographis paniculata* Nees (Acanthaceae). *J. Econ. Tax. Bot.* **22**(3):627-628

Alagesaboopathi, C. and Balu, S. 1999. Ethnobotany of Indian Andrographis Wallich ex Nees. *J. Econ. Tax. Bot.* **23**(1):29-32

*Allison, A.J., Butcher, D.N., Connolly, J.D. and Overton, K.H. 1968. Paniculides A, B and C bisabolenoid lactones from tissue cultures of *Andrographis paniculata. Chem. Commun.* p.1493

Anonymous. 1948. *The Wealth of India - Raw Materials (Vol.I).* Publications and Information Directorate, CSIR, New Delhi, pp.77-78

Anonymous. 1966. *Pharmacopoeia of India*. Government of India Ministry of Health Publication, New Delhi, pp.385-387

Anonymous. 1985. *The Wealth of India - Raw Materials (Vol.I)*. Publication and Information Directorate, CSIR, New Delhi, pp.264-266

Anonymous. 1996. *Trial Data Report*. Eleventh All India Workshop of AICRP on Medicinal and Aromatic Plants at RAU, Udaipur Campus. p.76

Anonymous. 1998. *Trial Data Report*. Twelfth All India Workshop of AICRP on Medicinal and Aromatic Plants at CCS, Haryana Agricultural University. pp.192-198

Arya, V.P. 1962. The structure and stereochemistry of andrographolide. *J. Sci. Ind. Res.* **21B**(6):281-283

Asolkar, L.V., Kalkar, K.K. and Chakre, D.J. 1992. *Glossary of Indian Medicinal Plants with Active Principles*. Publication of Information Directorate, New Delhi, pp.65-66

Balu, S., Alagesaboopathi, C. and Elango, V. 1993.Antipyretic activities of some species of Andrographis Wall. *Ancient Sci. Life* **12**(3&4):399-402

Balu, S. and Alagesaboopathi, C. 1995. Propagation of *Andrographis lineata* Nees by stem cuttings. *Ancient Sci. Life* **14**(4):235-239

Balu, S. and Alagesaboopathi, C. 1996. Introduction and mass multiplication of *Andrographis paniculata* Nees by stem cuttings in Shevaroy hills, Tamil Nadu. *J. Econ. Tax. Bot.* **20**(1):107-109

*Banerjee, P.K. and Datta, P.C. 1991.Effect of seasons, age, phase and hormones on the medicinal compound accumulation in plants. *Recent Advances in Medicinal, Aromatic and Spice Crops*. (Ed.Choudhari, R.S.P.). Today and Tomorrow's printers and publishers, New Delhi, p.436

Bentley, R. and Trimen, H. 1880. *Medicinal Plants (Vol.III)*. J and A Churchil, London, pp.197-199

Bhardwaj, D.K., Gupta, A.K., Chand, R. and Jain, K. 1981. Syntheses of *Andrographis paniculata* flavones. *Curr. Sci.* **50**(17):750-753

*Blackman, V.H. 1919. The compound interest law in plant growth. *Ann. Bot.* **33**:353-360

Bose, S.S.C. 1999. Screening and biochemical characterization of tomato genotype for resistance to bacterial wilt. M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, pp.

*Chauhan, S.K., Singh, B.P., Kimothi, G.P. and Agrawal, S. 1999. Determination of andrographolide in *Andrographis paniculata* by high performance thin layer chromatography. *Indian Drugs* **36**(2):130-132

Chakravarthi, D. and Chakravarthi, R.N. 1952. Andrographolide I. *J. Chem. Soc.* p.1697

*Chen, J. and Jiang, R. 1980. Morphological and histological studies on *Andrographis paniculata* (Burm, F.) Nees. and comparison with four other Acanthaceae herbs. *Acta. Pharm. Sin.* **15**:750-760

Chopra, R.N., Chopra, I.C., Handa, K.L. and Kapur, L.D. 1958. *Chopra's Indigenous Drugs of India*. U.N. Dhur and Sons Pvt. Ltd., Calcutta, p.278

Chowdhury, S.R. 1995. Leaf area development in colocassia and its relationship with yield. *Indian J. Pl. Physiol.* **38**(4):305-308

Dagade, S.B. 1999. Anatomical and biochemical investigations on Phytophthora foot rot disease reaction in Piper spp. M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, pp.58 and 93

*Das, S. and Das, A.P. 1989. Angiospermic Wall flora of Midnapore town. *Proc. 76th session of the Indian Sci. Cong.* Madurai, India, p.59

Datta, S.C. and Mukerji, B. 1952. *Pharmacognosy of Indian Leaf Drugs (Vol.II)*. Govt. of India, Ministry of Health, Calcutta, pp.66-68

Dua, V.K., Ojha, V.P., Biswas, S., Valecha, N., Singh, N. and Sharma, V.P. 1999. Antimalarial activity of different fractions isolated from the leaves of *Andrographis paniculata*. *J. Med. Arom. Pl. Sci.* **21**:1069-1073

Dutt, B., Srivastava, L. and Chand, R. 1999. Chemical screening of chirata (*Swertia chirayita* Karst.) collections from Himachal Pradesh for bitter content variability. *Ancient Sci. Life* **18**(3&4):259-263

Dwivedi, S., Singh, M., Singh, A.P., Sharma, S., Uniyal, G.C. and Kumar, S. 1999. Genetic variability, heritability and genetic advance for alkaloid yield attributing traits in 26 genotypes of periwinkle (*Catharanthus roseus*). *J. Med. Arom. Pl. Sci.* **21**:320-324

Dymock, W. 1890. *Pharmacographia India (Vol.III)*. Periodical Experts, New Delhi, pp.46-48

Farooqi, A.A., Khan, M.M. and Vasundhara, M. 1999. *Production Technology of Medicinal and Aromatic Crops*. Natural Remedies Pvt. Ltd., Bangalore, pp.55-56

*Fujitha, T., Fujitani, R., Takeda, Y., Takaishi, Y., Yamada, T., Kido, M. and Miura, I. 1984. On the diterpenoids of *Andrographis paniculata* X-ray crystallographic analysis of andrographolide and structural determination of new minor diterpenoids. *Chem. Pharm. Bull.* **32**:2117-2125

Gaind, K.N., Dar, K.N. and Kaul, R.N. 1963. Spectrophotometric estimation of andrographolide. *Indian J. Pharmacy* **25**(7):225-226

Gamble, J.S. 1921. *Flora of the Presidency of Madras Vol.IV.*(Reprint 1986)Bishen Singh,Mahendra Pal Singh, Dehradun, pp.1045-1051

Ghose, S.S. 1984. *Drugs of Hindoosthan.* 9th Ed. Hahnemann Publishing Co. Pvt. Ltd., Calcutta, pp.62-65

*Girach, R.D., Aminuddin and Khan, S.A. 1994. *Andrographis paniculata* - A possible substitute for *Swertia chirayita* in south eastern India. *Int. J. Pharmacognosy* **32**(1):95-97

Govindachary, T.R., Pai, B.R., Srinivasan, M. and Kalyanaraman, P.S. 1969. Chemical investigation of *Andrographis paniculata. Indian J. Chem.* **7**:306

Govindamenon, K. 1931. *Bharathiya Oushadangal (Malayalam).* Neethi Printers, Cochin, pp.290-291

Gupta, K.K., Taneja, S.C., Dhar, K.L. and Atal, C.K. 1983. Flavonoids of *Andrographis paniculata* Nees. *Phytochemistry* **22**:314-315

Gupta, K.K., Taneja, S.C. and Dhar, K.L. 1996. Flavonoid glycosides of *Andrographis paniculata. Indian J. Chem.* **35B**(5):512-513

*Handa, S.S. and Sharma, A. 1990. Hepatoprotective activity of andrographolide against galactosamine and paracetamol intoxication in rats. *Indian J. Med. Res.* **92**:284-292

Hammer, K., Junghanns, W. and Pank, F. 1996. Evaluation of a variable collection of *Ocimum* spp. *Proc. of Int.symp.Breeding Res.on Med. and Arom. Pl., Germany,* **2**(1): 41-44

Harborne, J.B. 1973. *Phytochemical Methods.* Chapman and Hall, London, pp.39-86

Hayashi, H., Yasuma, M., Hiraoka, N., Ikeshiro, Y., Yamamoto, H., Yesilada, E., Sezik, E., Honda, G. and Tabata, M. 1995. Flavonoid variation in the leaves of *Glycyrrhiza glabra. Phytochemistry* **42**(3):701-704

Hooker, J.D. 1892. *Flora of British India (Vol.IV).* Periodical Expert Book Agency, New Delhi, pp.501-506

*Hu, C.Q. and Zhou, B.N. 1982. Isolation and structure of two diterpenoid glucosides from *Andrographis paniculata* Nees. *Acta Pharm. Sin.* **17**:435-440

Hussain, A., Virmani, O.P., Popli, S.P., Misra, L.N., Gupta, M.M., Srivastava, G.N., Abraham, Z. and Singh, A.K. 1992. Dictionary of Medicinal Plants. CIMAP, Lucknow, pp.32-33

*Indrayanto, G., Cholies, N. and Wahyudi. 1985.Influence of fruit size of *Solanum wrightii* on its solasodine content. *Planta Med.* **5**:470

Jamwal, M. and Kaul, B.L. 1997. Comparitive morphological studies in three different population samples of kalmegh (*Andrographis paniculata*). *Crop Improvement* **24**(1):65-68

*John, D. 1984. One hundred useful raw drugs of the Kani tribes of Trivandrum forest division, Kerala, India,*Int. J.Crude Drug Res.***22** (1):17-39

Joy, P.P., Thomas, J., Mathew, S. and Skaria, B.P. 1998.*Medicinal Plants.*Kerala Agricultural University, Aromatic Plant Research Station, Odakkali, pp.48-49

*Kanniappan, M., Mathuran, L.M. and Natarajan, R. 1991. Study of the antipyretic effect of Chiretta (*Andrographis paniculata*). *Indian Veterinary J.* **68**:314-316

Kapur, P. 1997. Dry matter allocation in *Andrographis paniculata* (Burm.f.) Wall ex Nees with varying light. *Adv. Pl. Sci.* **11**(2):171-176

Kamboj, V.P. 2000. Herbal medicine. *Curr. Sci.* **78**(1):35-39

Kareem, M.A. 1996. Treasure of Malabbar in danger. *Amruth* **4**(10):3-7

Kirtikar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants (Vol.III)*. International Book Distributors, Dehradun, pp.1884-1886

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

*Kuroyanagi, M., Sato, M., Ueno, A, and Nishi, K. 1987. Flavonoids from *Andrographis paniculata. Chem.Pharm.Bull.* **35**(2):4429-4435

*Lawrence, G.H.M. 1969. *Taxonomy of Vascular Plants*. Oxford and IBH, New Delhi, pp.707-709

*Madav, S., Tandan, S.K., Lal, J. and Tripathi, H.C. 1996. Antinflammatory activity of andrographolide. *Fitoterapia* **67**(5):452-458

Mahajan, C.R., Patil, R.A., Mehetre, S.S. and Ghatge, R.D. 1994. Genotypic and phenotypic variability and heritability of some quantitative and qualitative characters in soybean (Glycine max (L) Merill). *Ann.Agric.Res.* **15**(1):41-44

Maiti, P.C. 1964. Andrographolide: the active principle of Kalmegh. *Bull. Bot. Sur. India* **6**(1):63-65

Maiti, P.C., Kanji, S.K. and Chatterjee, R. 1959. Studies in kalmegh extracts. *Indian J. Pharmacy*. **21**(6):169-171

Mathew, K.M. 1983. *Flora of the Tamilnadu Carnatic (Vol.III)*. The Rapinat Herbarium, Tiruchirappilli. pp.1141-1453

Meera, N. 1994. Standardization of propagation and stage of harvest in adakodien (*Holostemma annulare* K.Schum). M.Sc. (Hort) thesis, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.84

Mehetre, S.S., Shinde, R.B., Borle, U.M. and Surana, P.P. 1998. Studies on variability, heritability and genetic advance for some morpho-physiological traits in soybean [*Glycine max* (L.) Merrill]. *Adv. Pl. Sci.* **11**(1):27-31

Mehra, R.B., Singh, S.P. and Das, P.K. 1987. Analysis of components of growth and their interrelationship in pigeon pea [*Cajanus cajan* (L.) Millsp.]. *Indian J. Pl. Physiol.* **30**(1):78-86

Menon, J.S. 1999. Analysis of growth, flowering and quality in 'Koduveli', plumbago spp. Ph.D. (Hort) thesis,Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, p.101-116

Misra, H.O., Sharma, J.R., Lal, R.K. and Sharma, S. 1998. Genetic variability and path analysis in aswagandha (*Withania somnifera*). *J. Med. Arom. Pl. Sci.* **20**(3):753-756

*Mohankumar, C.R. and Sadanadan, N. 1989.Growth and dry matter accumulation in taro (Colocassia esculenta. Schott.) as influenced by NPK nutrition. *J. Root Crops* **15**:103-108

Moktader, A. and Sircar, S.G. 1939. On the bitter principle from *Andrographis paniculata* Nees. *J. Indian Chem. Soc.* **16**:333-338

*Moreno, B.G.V., Tomayo, C.C.H. and Estrada, E.I. 1987. Basic morphology of 17 introductions of the genus Ocimum in the Department of Valle. *Acta Agronomica* **37**(3):34-42

Nadkarni, A.K. 1954. *Indian Materia Medica Vol.I.* 3rd Ed. Popular Book Depot, Bombay, pp.101-103

*Nair, K.U., Nair, A.R. and Nair, C.P.R. 1992. Techno/economic data and cultivation and preservation of some south Indian medicinal plants. *Aryavaidyan* **5**:238-240

*Nizamudeen, S.K., Ramasamy, S. and Kameswaran, L. 1978. Effect of *Andrographis paniculata* on Snake Venom induced death and its mechanism. *Indian J. Pharm. Sci.* **40**:132-133

*Ojha, T.N. 1983. Chemical constituents of *Andrographis paniculata* Nees Part-II. *Acta Cienc Indica.* **9c**:239-240

Padmesh, P., Sabu, K.K. and Seeni, S. 1999. Analysis of genetic variations in *Andrographis paniculata* using isozyme and random amplified polymorphic DNA markers. *Proc. of the Eleventh Kerala Sci. Cong., Kasaragod*, pp.95-100

Pandita, K., Bhatia, M.S., Tappu, R.K., Agarwal, S.G., Dhar, K.L. and Atal, C.K. 1983. Seasonal variation of alkaloids of *Adhatoda vasica* and detection of glycosides and N. Osides of vasicine and vasicinone. *Planta Med.* **48**:81-82

*Panthong, A., Kanjanapothi, D. and Taylor, W.C. 1986. Ethnobotanical review of medicinal plants from Thai traditional books, part I. Plants with anti-inflammatory anti-asthmatic .and anti-hypertensive properties. *J. Ethnopharmacol.* **18**(3):213-228

Pareek, S.K., Maheswari, M.L. and Gupta, R. 1980. Domestication studies on *Ocimum sanctum* for high oil and eugenol content. *Indian Perfumer* **24**(2) 93-100

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

Phadnis, A.D. 1994. Study of growth cycle of Bhuiavli (*Phyllanthus niruri*) and its phytochemistry during growth. *Ancient Sci. Life* **13**(3&4):274-279

*Prakash, V.1998. Indian medicinal plants: Current status. Proc. 68th Annual Session of the National Academy of Sciences, CDRI, Lucknow, India. pp 31-32

*Prasad, P.P. and Joseph, B. 1997. Oushada Sasyengalum Avayude Krishi-reethikalum (Malayalam). Nagarjuna Research Foundation, Thodupuzha, India, pp.39-40

Radford, A. E. 1986. Fundamentals of Plant Systematics. Harper and Row Publishers, Inc. New York.

Rahman, N .N. N. A., Furuta, T., Kojima, S., Takame, K. and Ali Mohd., M. 1999. Antimalarial activity of extracts of Malaysian medicinal plants. J. Ethnopharmacol. **64**(3):249-254

Rajesh, P.M. 1994. Standardization of cultural practices in Kalmegh (*Andrographis paniculata* Nees). M.Sc.(Ag.) thesis, UAS, Bangalore.

Ramanujam, T. and Biradar, R.S. 1987.Growth analysis in cassava (*Manihot esculenta* Crantz). *Indian J. Pl. Physiol.* **30**(2):144-153

*Rana, A.C. and Avadhoot, Y. 1991. Hepatoprotective effects of *Andrographis paniculata* against carbon tetrachloride induced liver damage. *Archives Pharmacol. Res.* **14**:93-95

*Randa, S.S. and Sharma, A. 1990. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbon tetrachloride. *Indian J. Med. Res.* **92**(4):276-283

Rangaswami, S. and Rao, V.S. 1951. Andrographolide and some of its derivatives. *J. Sci. Ind. Res.* **10B**:201-204

Rastogi, R.P. and Mehrotra, B.N. 1993. *Compendium of Indian medicinal Plants (Vol.III)*. Publications and Information Directorate, New Delhi, pp.41-42

Rastogi, D. and Srivastava, L.J. 1995. Studies on correlation and path analysis for selected bitter principle contributing parameters in *Swertia chirayita* Karst. *Indian J. Forestry* **18**(3):196-200

*Reddy, M.B., Reddy, K.R. and Reddy, M.N. 1988. A survey of medicinal plants of Chenchu tribes of Andra Pradesh, India. *Int. J. Crude Drug Res.* **26**(4):189-196

*Reddy, R.K. 1988. Folk medicine from Chittoor district, Andra Pradesh, India, used in the treatment of Jaundice. *Int. J. Crude Drug Res.* **26**:137-140

Reddy, Y.V., Singh, B.G. and Reddy, S.N. 1997. Growth analysis in castor (*Ricinus communis* L.). *Indian J. Pl. Phyhsiol.* **2**(1):87-89

*Redford, P.J. 1967. Growth analysis formulae their use and abuse. *Crop Sci.* **7**:171

*Roy, K.S. and Datta, P.C. 1987.Andrographolide content in cytotypes of *Andrographis paniculata.Indian Drugs* **26**(1):7-9

Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods* 2nd Ed. New Age International Pvt. Ltd., Coimbatore. pp.193-194

Sarma, V.V.L.N. 1998. Chemotaxonomy of the Acanthaceae - tribe Justiceae. *Adv. Pl. Sci.* **11**(1):11-18

Seeni, S., Sabu, K.K. and Padmesh, P. 1998. An introduction to intra-specific variation in medicinal plants. *Amruth* 6(**6**):3-8

*Sen, S. and Datta, P.C. 1986. Alkaloid quantity of some Apocynaceous leaf drugs in relation to season. *Acta Hort.* **138**:177-186

*Shahid, A. 1985. Anti-inflammatory activity of *Andrographis paniculata* Nees (Chirayata). *Nagarjun* **29**:10-11

Sharma, J.R. 1999. PGR issues: National concern and global conflicts with special reference to medicinal and aromatic plants. *J. Med. Arom. Pl. Sci.* **21**:1111-1118

Singh, C., Jamwal, V., Gupta, G.K., Sharma, A.K. and Singh, P. 1999. Comparitive growth, herbage yield, asiaticoside and madecassocide composition in Brahamamanduki (*Centella asiatica*). *J. Med. Arom. Pl. Sci.* **21**:1048-1050

Sivarajan, V.V. and Balachandran, I. 1994. *Ayurvedic Drugs and their Plant Sources*. Oxford and IBH Publ. Co. Pvt. Ltd., New Delhi, pp.245-247

Srivastava, S.K., Bose, P.C., Ray, G.K. and Mukherji, B. 1959. Estimation of Andrographolide in Kalmegh. *Indian J. Pharmacy* **21**(8):229-230

Subba Rao, V. 1962. Estimation of andrographolide in kalmegh. *Indian J. Pharmacy* **24**(6):134

Talukdar, P.B., Banerjee, S., Chatterjee, P.K. and Datta, A.K. 1968. Studies on the estimation of Andrographolide in *Andrographis paniculata* Nees (Kalmegh). *Indian J. Chem.* **6**(7):359-363

*Trivedi, V. and Rawal, V.M. 1998. Effect of aqueous extract of *Andrographis paniculata*. *Indian J. Pharmacol.* **30**(5):318-322

*Trofimova, I.A. 1994. Early post-embryonic stages in ontogeny of cultivated species of the family Acanthaceae. *Byulleten-Glavnogo-Botanicheskogo-Sao* **170**:72-85

*Tuu, M.V., Huong, N.K. and Hong, T.N. 1984. Chemical component of Xugen Tam lein trees in Ho Chi Minn city. *Tap. Chi. Duoc Hoc.* **2**:7-8

*Vijaya, S.B. and Nanavati, D.E. 1978. *Andrographis paniculata* (Burm) Wall ex Nees (kalmegh). *Indian Drugs* **15**(10):187-190

*Wahi, P. 1978. Ecology of *Andrographis paniculata* phenology and growth habit. *J. Ecol.* **5**:43-47

*Wahi, P. 1980. Pattern of energy accumulation in *Andrographis paniculata* as influenced by various levels of light intensity. *Indian J. Ecol.* **7**:105-113

Warrier, P.k., Nambiar, V.P.K. and Ramankutty, C. 1996. *Indian Medicinal Plants-A compendium of 500 species (Vol-1).* Orient Longman Ltd., Madras, *pp.245-247*

*Watson, D.J. 1952. The physiological basis of variation in yield. Adv. Agron. **4**:101-145

*Whitehead, F.H. and Mycersough, P.J. 1962. Growth analysis of plants-ratio of mean relative growth rate to mean growth rate of increase of leaf area. *New Physiologist* **61**:314-321

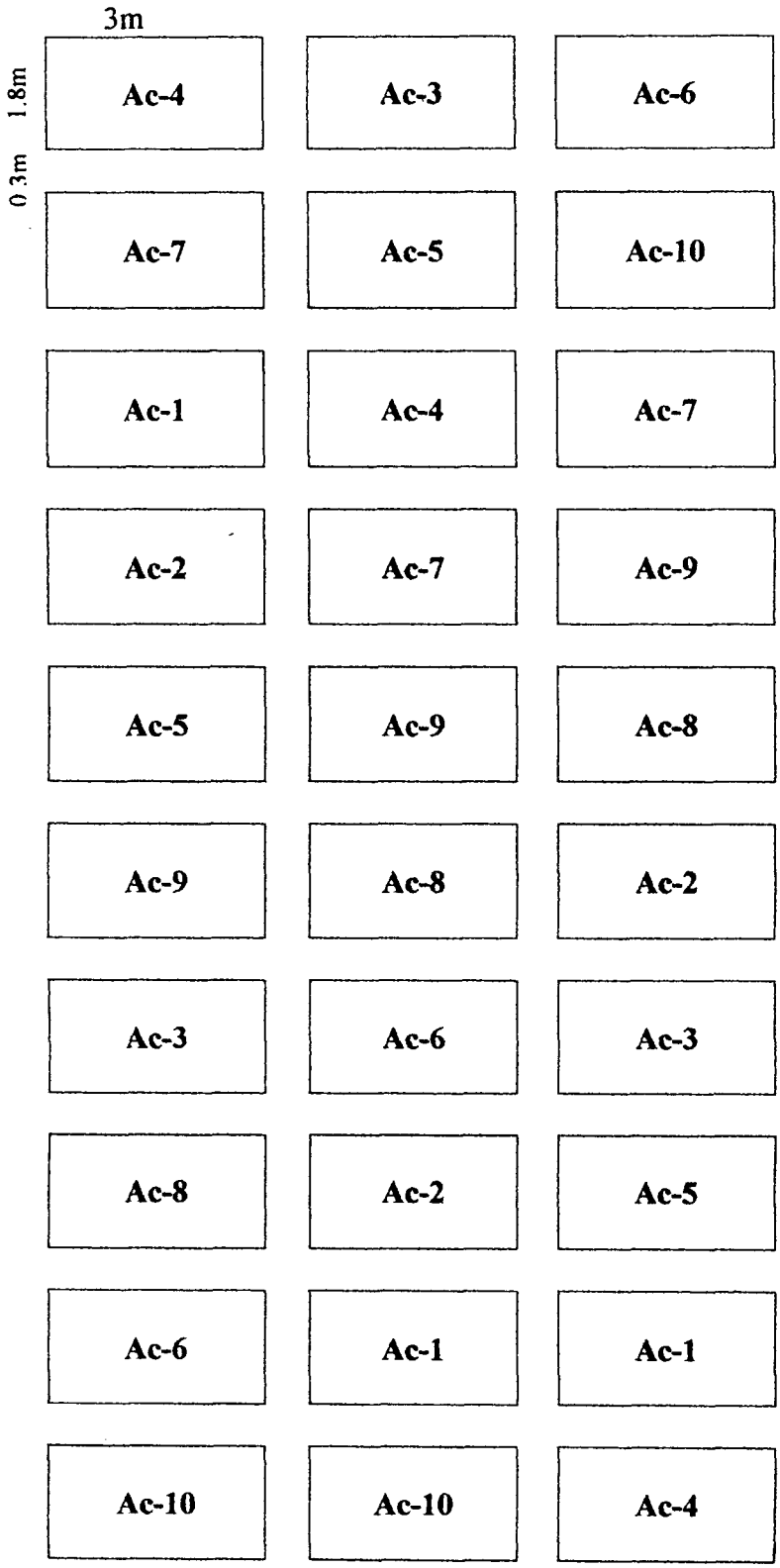
*Xianglin, L., Shuliang, Z. and Zhongshan, W. 1981. Analysis of andrographolide compounds I: Iron pair high performance liquid chromatographic analysis of andrographolide derivatives. *Acata Pharm. Sin.* **16**:182-189

*Originals not seen

Appendices

APPENDIX-1

LAYOUT PLAN



APPENDIX - II

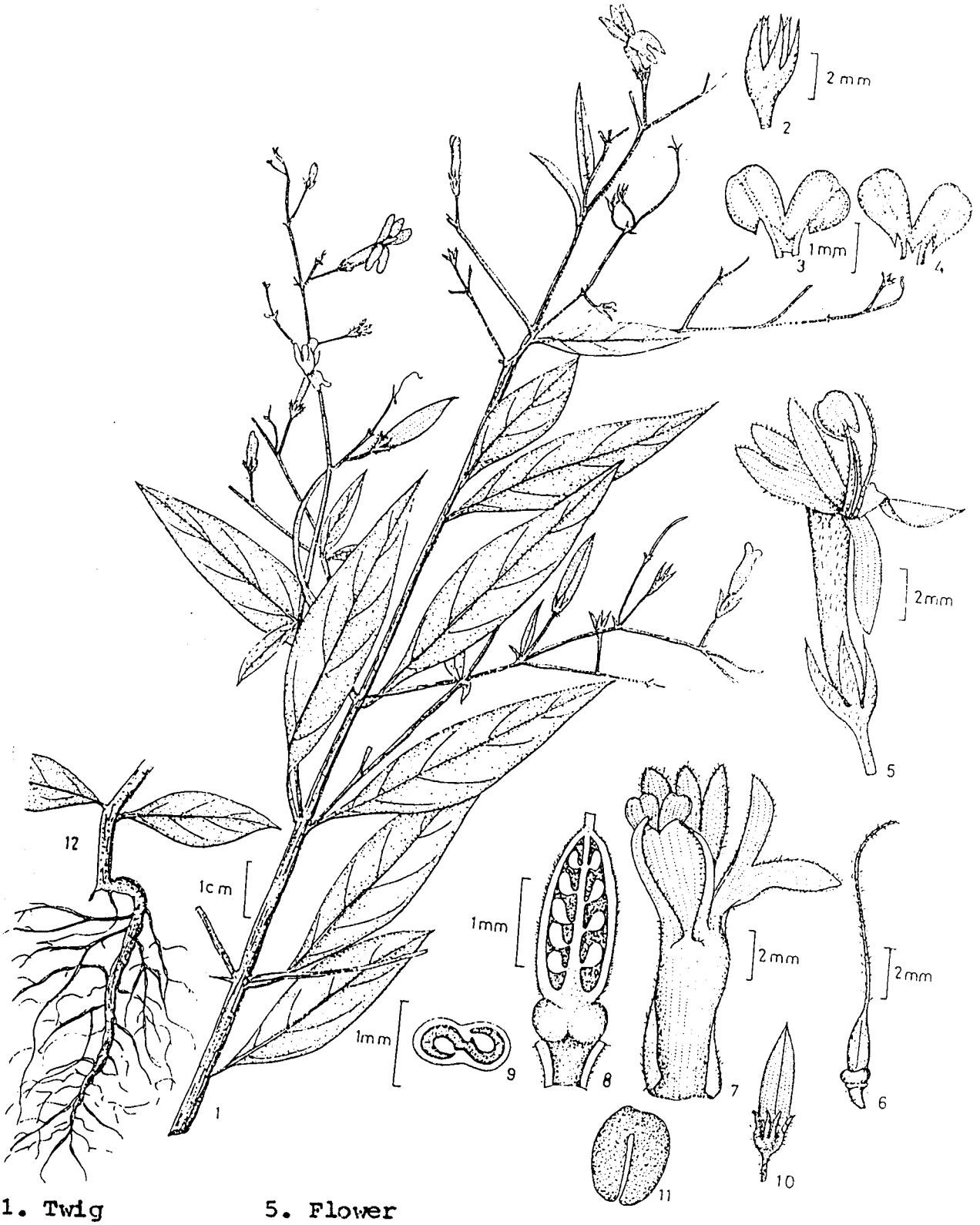
Weekly rainfall (mm), evaporation (mm), surface air temperature (°C), relative humidity (%) and sunshine hours (h day^{-1}) at COH, Vellanikkara from January to December 1999 (Latitude 10°31`N, Longitude 76°13` and Altitude 40.29 MSL)

Week No.	Rainfall		Evaporation (mm)	Surface air temperature (°C)			Relative Humidity (%)		Sunshine Hours (h day^{-1})
	Amount (mm)	NRD		Max.	Min.	Mean	Morning	Evening	
1	0.0	0	39.5	31.9	21.8	26.9	75	45	9.4
2	0.0	0	34.8	32.5	21.9	27.2	79	43	9.5
3	0.0	0	48.2	32.2	22.8	27.5	70	40	10.0
4	0.0	0	41.2	32.5	19.5	26.0	74	32	7.9
5	0.0	0	31.5	33.9	22.1	28.0	83	39	10.1
6	22.8	1	35.8	34.0	23.4	57.4	80	44	9.2
7	0.0	0	43.9	34.7	23.2	29.0	79	39	10.0
8	0.0	0	53.0	34.2	24.5	29.4	70	33	6.9
9	0.0	0	53.4	36.4	22.2	29.3	74	33	10.4
10	0.0	0	40.6	36.5	23.8	30.2	92	34	9.9
11	0.0	0	34.2	35.2	25.0	30.1	89	54	8.4
12	0.0	0	31.7	34.8	25.0	29.9	91	55	8.4
13	0.0	0	34.3	34.9	25.1	30.0	89	54	7.5
14	26.2	2	39.6	34.9	24.5	29.7	90	55	7.8
15	0.0	0	32.3	33.2	25.8	29.5	86	59	7.4
16	7.6	1	27.0	33.1	26.2	29.6	89	62	4.6
17	5.2	1	25.1	32.0	25.9	29.0	90	59	4.2
18	35.0	1	30.7	33.6	25.8	29.7	89	59	6.3
19	37.0	3	21.6	31.0	25.2	28.1	90	66	6.4
20	51.6	4	22.6	30.4	25.1	27.8	88	74	5.5
21	221.2	6	20.1	29.0	23.8	26.4	95	85	2.6
22	143.2	7	21.5	29.8	23.5	26.7	96	75	5.0
23	134.7	6	22.7	29.1	22.8	26.0	94	81	4.8
24	170.9	7	17.3	28.4	22.7	25.5	95	81	1.8
25	114.8	6	20.6	29.6	23.2	26.4	95	76	5.1
26*	21.6	1	26.4	30.9	23.0	27.0	92	67	8.9
27*	114.7	6	20.5	29.6	23.1	26.4	95	80	3.7
28*	124.6	7	18.0	29.0	22.9	26.0	96	76	3.1
29*	326.5	7	12.6	26.9	22.8	24.9	97	92	3.2
30*	182.8	7	13.0	27.7	22.7	25.2	95	83	1.1
31*	194.1	6	17.1	28.7	23.3	26.0	95	84	2.7
32*	121.5	5	20.8	29.5	23.7	26.6	95	74	5.2
33*	8.9	1	24.7	30.6	24.1	27.4	93	69	7.5
34*	3.2	1	25.1	30.0	23.6	26.8	93	69	6.9
35*	7.1	0	20.6	30.0	23.6	26.8	93	71	5.3
36*	18.3	1	18.7	30.0	23.2	26.6	92	67	4.9
37*	10.1	1	27.6	31.0	23.0	27.0	90	65	8.1
38*	0.0	0	31.0	32.6	23.4	28.0	90	56	8.5
39*	0.0	0	28.2	32.9	23.8	28.4	93	60	6.4
40*	80.5	4	19.6	30.5	23.1	26.8	95	71	4.8
41*	185.7	2	23.8	31.5	23.6	27.6	95	75	6.8
42*	161.6	5	16.8	29.5	23.3	26.4	95	80	2.9
43*	38.8	1	19.6	31.3	23.5	27.4	93	74	5.5
44*	41.9	3	20.3	29.6	22.7	26.2	96	73	6.2
45*	2.8	1	22.4	31.4	22.1	26.8	87	62	7.8
46*	0.0	0	26.6	31.1	22.1	26.6	74	46	10.1
47*	4.0	0	29.4	31.1	23.5	27.3	79	62	6.3
48*	0.0	0	36.4	31.9	23.7	27.8	76	55	8.7
49*	0.0	0	34.3	31.8	21.6	26.7	79	49	9.4
50*	0.0	0	31.5	31.8	22.6	27.2	78	50	8.1
51*	0.0	0	44.8	31.4	22.6	27.0	72	47	8.7
52*	0.0	0	49.0	31.4	23.4	27.4	68	43	8.8
Total/ Mean	**2618.9**	**104**	**1502**	**31.57**	**23.44**	**28.07**	**87.23**	**61.09**	**6.71**

* **Crop period**

APPENDIX - III

Andrographis paniculata (Burm.f.) Wallich ex Nees.



1. Twig
2. Calyx
3& 4 Anther
5. Flower
6. Pistil
7. Corolla split open
8& 9 Ovary L.S. & T.S.
10. Capsule
11. Seed
12. Root

APPENDIX - IV

Literature of Andrographis paniculata in Hortus Malabaricus

CARA-CARINAM.

TABULA LVI.

SEcunda ſpecies eſt *Caniram*; Bramannicè *Boin-Caro* vocatur, planta eſt altitudine duum pedum, arenoſa amans, florens tempore pluvioſo. *Radix* plurimis conſtat fibris lignoſis, capillatis, nigricantibus, cujus cortex maarus. *Caules* qui vel ſimplices, vel duo tresve ex radice exſurgunt, virides, quadrangulati, in nodos diſtincti, cauliculos paſſim binos, qui ſe mutuo decuſſant, emittentes. *Folia* è nodis caulium petiolis brevibus bina & bina proveniunt, oblongo-anguſta, inferius contractiora, tenuia, mollia, ſuperficie plana, coloris viridi-fuſci, ſaporis amariſſimi; nervulus in utraque parte eminet, magis autem in adverſa. *Flores* in petiolis tenuibus, viridibus, quadrangulatis, interdum etiam nonnihil ramoſis videntur, qui è caulium nodis, ſupra originem foliorum, paſſim bini & bini ex adverſo oriuntur, iiſque pedunculis brevibus, tenuiſſimis, hinc inde, præſertim in ſuperioribus parvibus adhærent: bipetali ſunt, uno anguſto & reflexo, in totùm albo; altero latiori, ſurrecto, nonnihil reflexo, ac in tres cuſpides æquè altè emicantes diviſo, quod etiam album, & circa oras duobus, rubro obſcuris radiis, qui ad duos extremos cuſpides excurrunt, variegatum eſt, & in medio macula rubra notatum. *Calyx* laxus eſt, in quinque anguſta & piloſa folia diviſus, cui tenuiori pede inſident. *Stamen* habent longiuſculum, albicans & piloſum. *Stylus* tenuis eſt & aliquantulum rubeſcens, ex germine capſularum ſeminalium ortum trahens. *Capſulæ ſeminales* anguſtæ, quadrangulatæ, compreſſæ, planæ & duriolæ, in longum ſulco ſtriatæ, interſepimento ex ſulcis excurrente, in duas quaſi camerulas ſeminales oblongo anguſtas diſtinctæ. *Semina* quæ in ſingulis camerulis tria reperiuntur quatuorve, parum

oblonga, primò viridia, dein albicantia, ſicca ruffa ceu ex flavo rubeſcentia, ſubamara. Tota planta cum infuſione *Oryzæ* macerata morſui virulento ſerpentis, Copra Capella, dicti, medetur, epota nempe, quod & peruncta præſtat.

TAB. 56.

Quod hæc planta alterum genus eſſet *Caniram*, cujus ſpecies parte *prima*, *ſeptima*, *octava* hujus Operis ſunt deſcriptæ, error eſt, quia hic ſolum nomen ſine convenientia in genere reperitur; pertinet enim inter *plantas flore papilionaceo ſiliquoſas ſeu leguminoſas*; cum etiam ſiliqua, ſecundum delineationem aliquo modo veſicalis videatur (quamvis hoc ex deſcriptione non pateat) referri poſſet inter plantas *Crotolarias* dictas, præcipue cum ſemina illarum ſeminibus aſſimilentur; cæteræ vero *Caniram* omnes plantæ pomiferæ ſunt vel bacciferæ; atque ideo planta hæc ſui generis eſt.

APPENDIX - V.

ANOVA,(Mean squares) for plant characters in kalmegh accessions

Source	df	Mean sum of squares															
		Total plant fresh weight				Stem dry weight				Root dry weight				Leaf dry weight			
		1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Replication	2	0.53	19.45	933.41	15.19	0.000012	0.253	21.598	4.837	0.000073	0.027	0.220	0.575	0.001	0.250	1.942	4.190
Accession	9	0.86*	162.11	911.32**	1204.99*	0.000556**	1.735**	41.981**	43.352	0.000285**	0.2.03**	0.601**	0.775	0.032**	4.358**	19.638**	19.115**
Error	18	0.29	101.21	191.85	428.39	0.000095	0.209	6.155	47.897	0.0000454	0.047	0.078	0.538	0.003	0.218	1.117	2.208

APPENDIX V. (continued)

Source	df	Mean sum of squares															
		Total plant dry weight				Shoot length				Root length				Dry weight/ Fresh weight			
		1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Replication	2	0.002	0.738	35.000	15.388	4.73	9.62	197.67	55.46	0.08	6.30	1.17	21.05	0.007	0.001	0.002	0.002
Accession	9	0.043**	10.623**	98.498**	92.620	7.47**	146.94**	159.08**	90.35**	8.52**	9.79	10.61	30.50**	0.003	0.005	0.005	0.008**
Error	18	0.003	0.667	10.137	62.003	1.61	17.78	23.92	23.99	1.49	7.39	18.31	5.18	0.003	0.004	0.002	0.002

* Significant at 5% level
** Significant at 1% level

APPENDIX - VI.

ANOVA (Mean squares) for physiological parameters in kalmegh accessions

Source	df	Mean sum of squares											
		Leaf area				LAI				LAR			
		1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT	1 MAT	2 MAT	3 MAT	4 MAT
Replication	2	21.7	4424.5	34335.1	74089.6	0.00006	0.012	0.095	0.206	7.39	10.13	13.35049.89	7.41
Accession	9	567.6**	77071.8**	347269.3**	338031.2**	0.00158**	0.214**	0.965**	0.939**	112.23**	149.89**	443.06**	115.22
Error	18	50.0	3855.9	19747.1	39043.0	0.00014	0.011	0.055	0.108	36.29	20.50	61.81	68.79

APPENDIX - VI. (Continued)

Source	df	Mean sum of squares								
		RGR			NAR			CGR		
		Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
Replication	2	25.53	128.59	40.37	0.03	0.02	4.00	0.002	0.13	0.09
Accession	9	212.75**	231.13**	129.64*	0.19**	0.49*	7.97	0.03**	0.24**	0.32
Error	18	50.53	33.258	40.95	0.02	0.17	3.68	0.002	0.03	0.16

* Significant at 5% level
** Significant at 1% level

Stage 1,stage 2 and stage 3 refer to periods of growth between 1st and 2nd , 2nd and 3rd and 3rd and 4th MAT respectively

VARIABILITY IN MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERS IN KALMEGH

(*Andrographis paniculata* Nees.)

By

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ABSTRACT OF THE THESIS

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Faculty of Agriculture

Kerala Agricultural University

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2000

ABSTRACT

An experiment in kalmegh (*Andrographis paniculata* Nees.) was conducted in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1998-2000, with the objectives of understanding the morphological variability, growth pattern, optimum stage of harvest and the variation in different biochemical compounds among the accessions. The accessions were collected from Kerala and neighbouring states.

Ten accessions were compared based on 50 morphological, 16 physiological and 3 biochemical characters. In general, accessions showed uniform root, inflorescence, flower and fruit characters. However a limited variation for habit, stem and leaf characters existed among most of the accessions.

In general, accessions showed variability for characters namely total plant dry weight, stem dry weight, leaf dry weight and root dry weight when observed at 1, 2, 3 and 4 months after transplanting. Ranking of the accessions was not consistent on the various dates of harvest. Optimum stage of harvest is recommended as 3 months after transplanting. At this stage maximum herbage yield was recorded by accession Ac-4. Physiological parameters namely Leaf area and LAI showed steady increase up to three months after transplanting and thereafter it declined. LAR, RGR, CGR and NAR recorded higher values at earlier dates.

Heritability (broad sense) of characters showed inconsistency at various dates of harvest. However leaf dry weight showed higher heritability on all dates. At 3 months after transplanting i.e. the optimum stage of harvest, heritability was high for all the characters relating to herbage yield and most of the physiological parameters.

Biochemical studies revealed that the total phenol content and andrographolide content varied considerably among the accessions. Phenol content in the plant increased with an increase in dry weight while Andrographolide content increased with a proportionate increase in moisture content.