Research Article

Variation in bioactive content in *Andrographis paniculata* Nees at different growth stages

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ABSTRACT

An investigation was carried out to study the effect of plant age on different morphological characters and the corresponding changes in the bioactive compound (andrographolide) in the three accessions of *Andrographis paniculata*. The results have shown significant differences and changes in the andrographolide content and yield at various growth stages of the plant. The three accessions with no morphological differences were characterized using RAPD and ISSR profiles and the variation in andrographolide content was estimated by HPLC. A plant age of 80-105 DAT (days after transplantation) may be recommended for harvesting to get a good andrographolide yield though the highest herbage yield may be obtained at 120 DAT. **[Medicinal Plants 2014; 6(1) : 53-61]**

Keywords : *Andrographis*, andrographolide, date of harvesting, HPLC, RAPD

INTRODUCTION

Andrographis paniculata Nees. belongs to the family Acanthaceae, and is commonly known as 'Kalmegh'. *Andrographis* has about 40 species distributed in the tropical Asia and throughout the plains of India. It has an annual habit (perennial if maintained), erect 60 – 100 cm in height, stem quadrangular and much branched; and is a self-pollinated crop. The active bitter principle of Kalmegh is Andrographolide (a diterpene lactone). It is common bitter tonic prescribed for children in liver troubles and also it is used in intermittent and remittent fevers. The extracts protect liver tissue from alcohol induced toxicity and accelerate digestion and absorption of carbohydrates. Decoction of the whole plant is a blood purifier, used to cure jaundice, dermatological diseases, dyspepsia and treating many others ailments.

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Plant growth and development are complex biological phenomena that depend upon genetic and environmental variables (Waller *et al.*, 1978). For majority of the medicinal plants there is no information available about the most favorable temperature, humidity and soil condition for the growth of the plants and the optimal harvesting time for procuring maximum yield of secondary metabolites; the main component giving medicinal property in plants. Plant growth development in relation to season has distinct impact on accumulation of secondary metabolites (Teiz *et al.*, 1998). Tetenyi (1992) suggested when setting objectives, plant breeders should consider the chemical modifications which may take place due to ecological and geographical conditions.

To formulate appropriate breeding strategy for the improvement of a character, it is very important to know the nature of genetic control of that character and to what extent it is influenced by the environment factors and the variations in its yield of bioactive compounds in different micro and macro environments. The present investigation has been undertaken to assess the optimum stage of harvesting of *Andrographis paniculata* for maximum dry foliage yield and quality of bioactive content.

MATERIALS AND METHODS

This experiment was conducted for three consecutive years from 2006 to 2008. Plants were sampled at different age *viz.* 40 DAT to 120 DAT (days after transplantation) at 20 days interval (Fig. 1). The data were collected on various changes in morphological characters, herbage yield plant^{-1} and andrographolide content and andrographolide yield.

There were 15 combination of three accessions and five sampling age at 20 days interval after transplanting *viz.*, 40 DAT, 60 DAT, 80 DAT, 100 DAT and 120 DAT. The Shimadzu HPLC system used for the estimation of andrographolide consists of LC-10AD *VP* pump, Rheodyne sample injector, SPD 10A UV-VIS detector along with Aimil Chromatograph data station for data collection and analysis. With the help of this system, selected phytochemicals (standardization, screening and quantification) were carried out (Fig. 2) at 229 nm wavelength (Chauhan *et al.*, 2000) and the sampling for andrographolide yield and content started from 60 DAT. Since the accessions studied in the present investigation were devoid of distinct phenotypic characters, molecular characterization through RAPD and ISSR were carried out for establishing any differences among the accessions at genetic level. For the RAPD analysis, Polymerase Chain Reaction (PCR) was performed based on the protocol of Williams *et al.* (1990) with minor modifications. Amplification reactions were performed with 2.5µl of 10X PCR buffer, 5 pmole of the primer, 1U of Taq DNA polymerase, 30 ng of genomic DNA. DNA amplification was performed in a Thermal Cycler programmed for 43 cycles. ISSR analysis was performed using ISSR primers. Polymerase Chain Reaction (PCR) was performed based on the protocol of Zietwiecki *et al.* (1994) with some modifications. Field layout was prepared using RCBD

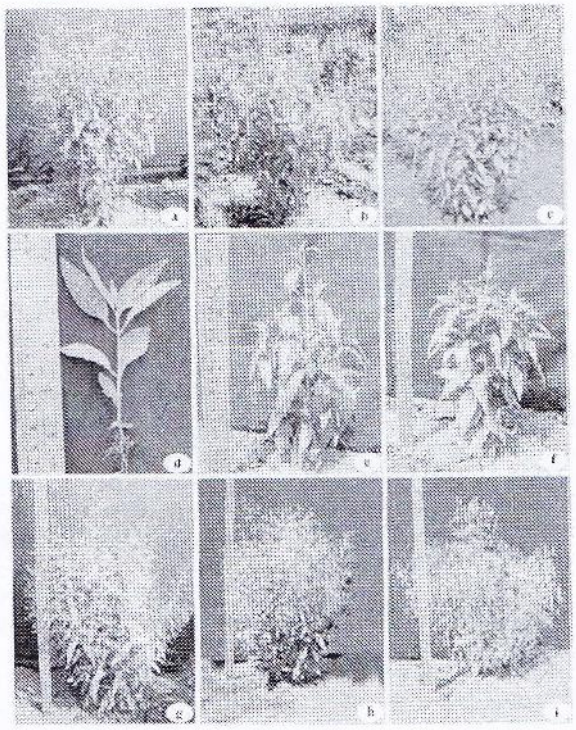

Fig. 1. Plant habit of (a) Ac. 1; (b) Ac. 2; (c) Ac. 3 at 80 DAT, Different growth phases of *A. paniculata*; (d) Seedling at transplanting stage; (e) 40 DAT; (f) 60 DAT; (g) 80 DAT; (h) 100 DAT; (i) 120 DAT

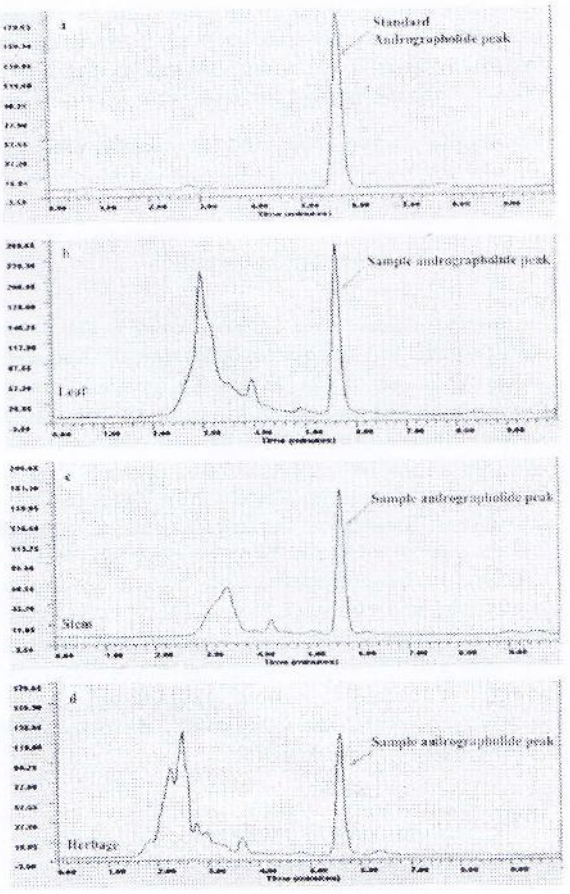

Fig. 2. HPLC graph showing the concentration of andrographolide in (a) Standard (b) Leaf (c) Stem (d) Herbage

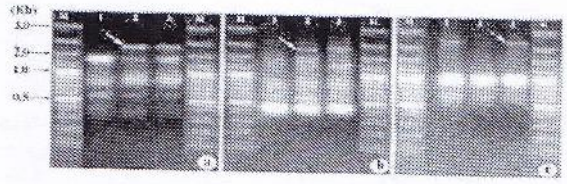

Fig. 3. RAPD banding pattern of *A. paniculata* with (a) OPA 08, (b) OPA 18 and (c) OPN 02 [M-100 bp DNA ladder plus, 1 - Ac 1, 2 - Ac 2 and 3 - Ac 3]

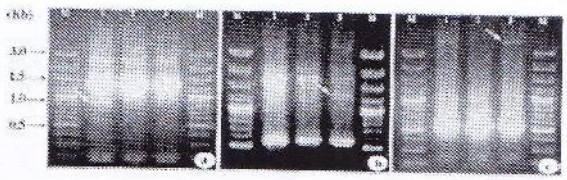

Fig. 4. ISSR banding pattern of *A. paniculata* with (a) (CT) 9G (b) (CT) 8RC and (c) (CA) 8AT [M-100 bp DNA ladder plus, 1 - Ac 1, 2 - Ac 2 and 3 - Ac]

design and the pooled analysis was done in the statistical software MSTATC.

RESULTS AND DISCUSSION

Differentiation of the three accessions at genetic level

Since the accessions studied in the present investigation were morphologically indistinguishable molecular characterization through RAPD and ISSR were carried out for establishing differences among the accessions at genetic level (Figs. 3 and 4). Molecular analysis using Jaccard's coefficient, cophenetic correlation, principal coordinate analysis (PCA) and dendrogram (Fig. 5) of the three accessions also clearly showed the differences and relationship amongst them.

Molecular markers have demonstrated its usefulness to find out genetic similarities and differences between accessions even when a classical morphological description is severely limited. Despite the importance of the crop, very little research has been done to assess the genetic variation of this species using molecular markers (Padmesh *et al.*, 1999).

Morphological characters

The pooled analysis of variance indicated that there were no significance difference of the accessions used but the stages of growth had a profound significant effect on the different parameters recorded. Six morphological characters (plant height, number of primary branches plant^{-1}, number of secondary branches plant^{-1}, plant canopy spread, stem diameter and number of leaves plant^{-1}) were observed and recorded in the three selected accessions at five stages of plant growth.

Analysis of variance (ANOVA) of the pooled data for these characters showed that there was no genotypic difference among the accessions. However stages of plant age had significant influence on all the characters studied. Individual effect of stages of plant age significantly influenced all the morphological characters irrespective of the accessions. They showed lowest at 40 DAT and the highest at 120 DAT in all the accessions (Tables 1a, b).

Ashok *et al.* (2002) have observed that the best harvesting time was at 120 DAT as compared to rest of the harvesting time with regards to achieving higher biomass containing maximum andrographolide content.

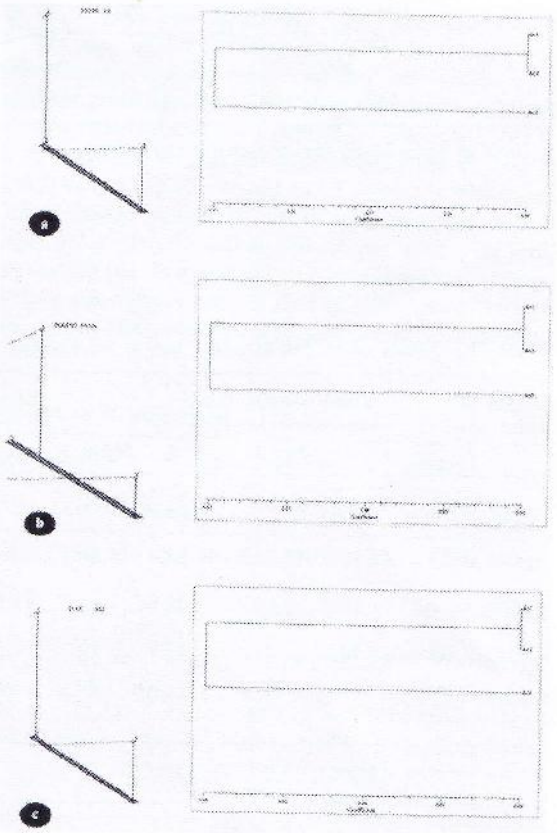

Fig. 5. (A) PCA and Dendrogram of (a) RAPD, (b) ISSR and (c) Combined RAPD-ISSR of the three accessions

Table 1a. Effect of stages of plant height on various morphological characters

Stages of plant age	Plant ht (cm)				No. of primary branch				No. of secondary branch			
	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean
S_1 (40 DAT)	7.19	7.02	7.62	**7.28**	2.64	2.68	2.56	**2.63**	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	**0.00 (0.71)**
S_2 (60 DAT)	17.8	16.93	18.08	**17.60**	14.44	14.06	13.02	**13.84**	2.55 (6.06)	2.34 (5.08)	2.41 (5.33)	**5.49 (2.43)**
S_3 (80 DAT)	29.8	28.95	32.09	**30.28**	22.65	23.03	23.16	**22.95**	8.48 (71.45)	8.83 (77.50)	9.05 (81.50)	**76.82 (8.79)**
S_4 (100 DAT)	44.94	44.92	44.49	**44.78**	33.66	35.27	34.49	**34.47**	14.86 (221.10)	16.20 (262.46)	15.14 (229.16)	**237.57 (15.40)**
S_5 (120 DAT)	48.21	49.72	48.18	**48.70**	38.77	38.81	36.44	**38.01**	18.31 (335.15)	17.65 (311.79)	16.52 (275.64)	**307.53 (17.49)**
	CV_S(%): 6.03				CV_S(%): 5.18				CV_S(%): 8.10			
	Lsd_S (p=.05): 2.339 Ac: NS Lsd_{AcxS} ((p=.05):				Lsd_S (p=.05): 1.513 Ac: NS Lsd_{AcxS} ((p=.05):				Lsd_S (p=.05): 0.948 Ac: NS Lsd_{AcxS} ((p=.05):			

Figures in parentheses denote transformed values

Table 1b. Effect of stages of plant height on various morphological characters

Stages of plant age	Plant canopy (cm)				Stem diameter (mm)				No. of leaves			
	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean
S_1 (40 DAT)	30.6	28.09	27.43	**28.71**	1.57	1.51	1.50	**1.53**	23.47	20.88	21.06	**21.80**
S_2 (60 DAT)	49.33	48.73	46.54	**48.20**	3.36	3.14	3.00	**3.17**	85.07	80.2	77.70	**80.99**
S_3 (80 DAT)	71.47	70.61	71.04	**71.04**	4.80	4.67	4.71	**4.73**	216.16	190.35	189.02	**198.51**
S_4(100 DAT)	97.25	95.71	95.78	**96.25**	5.90	6.09	5.91	**5.97**	418.17	226.29	411.68	**352.05**
S_5 (120 DAT)	113.36	113.19	113.7	**113.42**	6.66	6.59	6.16	**6.47**	437.7	259.54	402.85	**366.70**
	CV_S(%): 3.98				CV_S(%): 121.66				CV_S(%): 13.45			
	Lsd_S (p=.05): 3.712 Ac: NS Lsd_{AcxS} ((p=.05):				Lsd_S (p=.05): Ac: NS Lsd_{AcxS} ((p=.05): NS				Lsd_S (p=.05): 26.71 Ac: S Lsd_{AcxS} ((p=.05): 35.83			

Table 2. Effect of stages of plant age and accessions on fresh weight in *A. paniculata*

Stages of plant age	Leaf Fwt. (gm plant^{-1})				Stem Fwt. (gm plant^{-1})				Herbage Fwt. (gm plant^{-1})			
	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean
S_1 (40 DAT)	1.17	1.09	1.22	**1.16**	0.16	0.18	0.2	**0.18**	1.32	1.27	1.42	**1.34**
S_2 (60 DAT)	4.76	5.74	6.24	**5.58**	2.34	2.93	3.08	**2.78**	7.1	8.67	9.32	**8.36**
S_3 (80 DAT)	21.38	21.66	21.42	**21.48**	6.99	6.99	7.34	**7.11**	28.37	28.65	28.76	**28.59**
S_4 (100 DAT)	30.33	40.80	39.59	**36.91**	22.65	26.23	23.28	**24.06**	52.99	67.04	69.66	**63.23**
S_5 (120 DAT)	28.6	22.18	27.02	**25.93**	41.3	29.41	39.83	**36.85**	69.90	51.59	66.85	**62.78**
	CV_S(%): 15.53				CV_S(%): 23.71				CV_S(%): 16.71			
	Lsd_S (p=.05): 4.768 Ac: NS Lsd_{AcxS} ((p=.05): 3.693				Lsd_S (p=.05): 5.664 Ac: NS Lsd_{AcxS} ((p=.05): 4.388				Lsd_S (p=.05): 9.121 Ac: S Lsd_{AcxS} ((p=.05): 7.065			

Subsequently, Maheshwari *et al.* (2002) have reported that highest herbage yield was obtained with planting on 16th July in both the years (1999 and 2000) and harvesting on 16th November which was 120 DAT as observed in the present case.

Yield Partitioning

The results showed that the stages of plant age significantly influenced the yields of fresh leaves, dry leaves, fresh stems, dry stems and fresh and dry herbage; which were very low in the initial stages of plant age up to 60 DAT and thereafter, progressive increase in weight was observed irrespective of the accessions. Significantly highest fresh and dry leaf yields were noted at plant age 100 DAT (36.91 g plant^{-1}, 8.74 gm plant^{-1}). The fresh and dry stem yield was negligible at early stages of growth but they attained the highest at 120 DAT (36.85g plant^{-1} and 14.13 g plant^{-1}). Maximum fresh herbage weight per plant was observed in 100 DAT (63.g plant^{-1}) which was at par with 120 DAT harvesting (62.78 g plant^{-1}). The maximum dry herbage weight was observed at 120 DAT (22.24 g plant^{-1}) which was at par with 100 DAT (20.36 g plant^{-1}). Interaction

Table 3. Effect of stages of plant age and accessions on dry weight in *A. paniculata*

Stages of plant age	Leaf Fwt. (gm plant^{-1})				Stem Fwt. (gm plant^{-1})				Herbage Fwt. (gm plant^{-1})			
	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean	Ac. 1	Ac. 2	Ac. 3	Mean
S_1(40 DAT)	0.28	0.29	0.30	**0.29**	0.04	0.04	0.05	**0.04**	0.32	0.33	0.34	**0.33**
S_2(60 DAT)	1.21	1.43	1.47	**1.37**	0.33	0.44	0.41	**0.39**	1.54	1.87	1.87	**1.76**
S_3(80 DAT)	5.29	5.09	4.68	**5.02**	1.89	1.85	1.60	**1.78**	7.17	6.94	6.28	**6.80**
S_4(100 DAT)	7.80	9.78	8.65	**8.74**	8.13	8.96	8.80	**8.63**	15.93	18.73	26.42	**20.36**
S_5(120 DAT)	7.53	7.72	9.07	**8.10**	15.65	11.08	15.67	**14.13**	23.18	18.79	24.73	**22.24**
	CV_S(%): 23.86				CV_S(%): 25.74				CV_S(%): 22.77			
	Lsd_S (p=.05): 1.922 Ac: NS Lsd_{AcxS} ((p=.05): 1.489				Lsd_S (p=.05): 2.167 Ac: NS Lsd_{AcxS} ((p=.05): 1.678				Lsd_S (p=.05): 3.749 Ac: NS Lsd_{AcxS} ((p=.05): NS			

Table 4. Total Plot yield of different accessions (Kg Ha^{-1})

Plot yield	Fresh leaf yield (Kg/Ha)	Fresh stem yield (Kg/Ha)	Fresh herbage yield (Kg/Ha)	Dry leaf yield (Kg/Ha)	Dry stem yield (Kg/Ha)	Dry herbage yield (Kg/Ha)
Ac. 1	2149.26	3441.76	5591.02	696.2	1401.11	2097.32
Ac. 2	2230.46	3132.22	5362.69	819.63	1202.22	2021.85
Ac. 3	2251.67	3318.89	5570.56	755.46	1305.46	2060.93
Mean	**2210.46**	**3297.62**	**5508.09**	**757.1**	**1302.93**	**2060.03**
CV%	31.32	28.59	29.06	39.96	29.56	32.70
Lsd_{AC} (p=.05)	1569.00	2137.00	3628.00	685.80	873.00	1527.00

of sampling time and accessions affected the yield of fresh leaves, dry leaves, fresh stems, dry stems and fresh herbage plant^{-1}. Accession 2 sampled at 100 DAT showed highest weight of fresh leaves (40.80 gm plant^{-1}), which was at par with accession 3 at plant age 100 DAT (39.59 gm plant^{-1}). Leaf dry weight was maximum in accession 2 sampled at 100 DAT (9.78 gm plant^{-1}) which was at par with sampling of accession 3 at 120 DAT (9.07 gm plant^{-1}) and accession 3 at 100 DAT sampling (8.65 g plant^{-1}). The highest weight of fresh stem (41.30 g plant^{-1}) in accession 1 was observed at plant age 120 DAT which was at par with that of accession 3 at plant age 120 DAT (39.83 g plant^{-1}). Similarly, higher yield of the dry stem (15.67 g plant^{-1}) was recorded in accession 3 at plant age 120 DAT which was at par with accession 1 at plant age 120 DAT (15.65 g plant^{-1}). Highest fresh herbage plant^{-1} (69.90 g) was harvested in accession 1 at plant age 120 DAT which was at par with that of accession 3 at 100 DAT (69.66 g plant^{-1}), accession 2 at 100 DAT (67.04 g plant^{-1}) and accession 3 at 120 DAT (66.85 g plant^{-1}) (Tables 2 and 3). Total plot yield of leaf, stem and herbage were not significantly different in all the accessions (Table 4).

Nemade *et al.* (2003) carried out experiments on effect of planting dates at Akola, Maharashtra and concluded that planting in first week of July and harvesting in second week of November (120-130 DAT) produced maximum fresh and dry foliage yield in *A. paniculata* which is in accordance with the present finding.

The yield partitioning was done on per plant basis at 20-day interval taking data of leaf and stem weight separately. The yield partitioning of the total herbage into leaf and stem showed a significant trend in all the three accessions. The trend was visible in the fresh as well as the dry weight of the plants.

In all three these accessions, the results of fresh weight yield showed that leaf weight contributed maximum towards total biomass production up to plant age 100 DAT whereas stem weight contributed maximum towards total biomass production at plant age 120 DAT. In accession 1, the contribution of leaf towards total biomass production was highest (88%) at plant age 40 DAT which gradually declined to 57 per cent at plant age 100 DAT and 41 per cent at plant age 120 DAT. In accession 2, the contribution of leaf towards total biomass production was marginally lower than accession 1 (86%) at plant age 40 DAT which was reduced to 61 per cent at plant age 100 DAT and 43 per cent at plant age 120 DAT. Similar trend was also noticed in case of accession 3, the contribution of leaf towards total biomass production was 86 per cent at plant age 40 DAT which was gradually declined to 63 per cent at plant age 100 DAT and 40 per cent at plant age 120 DAT.

Similar trend was observed for dry weight yield, the major contribution towards total dry biomass yield was from dry leaf weight during the initial growth phases which was changed to dry stem weight at the older stages of growth in all the three accessions. The contribution of dry leaf towards total dry biomass production was 87 per cent in accession 1 at plant age 40 DAT and it declined progressively to 74 per cent at plant age 80 DAT, 49 per cent at plant age 100 DAT and 39 per cent at plant age 120 DAT. Trend although was similar in case of accession 2 and accession 3 but actual values differed. The contribution of dry leaf towards total dry biomass production in accession 2 was 87 per cent at plant age 40 DAT, 52 per cent at plant age 100 DAT and 41 per cent in plant age 120 DAT. In case of accession 3, these values were 86 per cent at plant age 40 DAT, 53 per cent at plant age100 DAT and 37 per cent at plant age 120 DAT (Fig. 6).

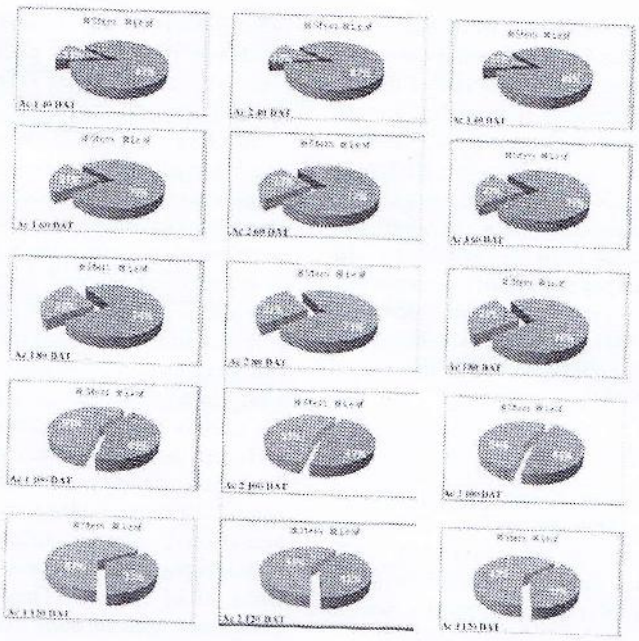

Fig. 6. Yield partition of dry biomass production in the three accessions at different plant ages

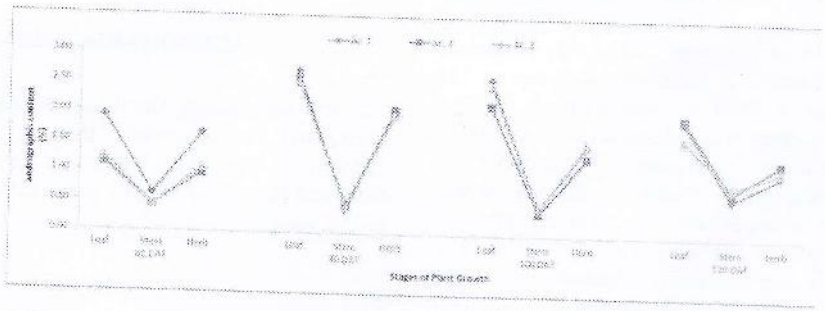

Fig. 7. Changes in andrographolide content in leaves, stem and herbage in three accessions of *A. paniculata* at different stages of plant age

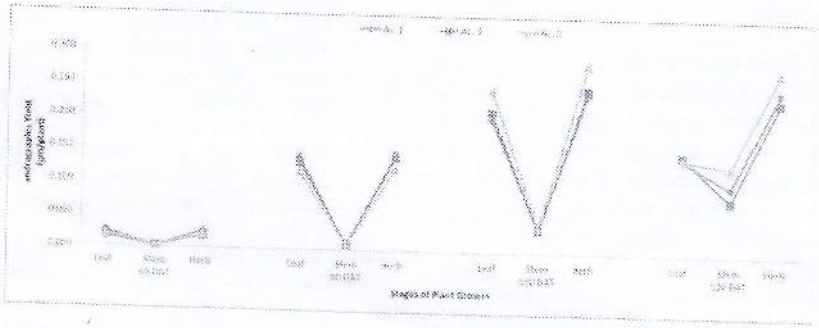

Fig. 8. Changes in andrographolide yield in leaves, stems and herbage in three accessions of *A. paniculata* at different stages of plant age

Similar observations were reported by Ashok *et al.* (2002) who revealed that the best harvesting time was observed at 120 days after sowing as compared to rest of the harvesting time with regards to achieving higher biomass containing maximum andrographolide content. Bhan *et al.* (2006) revealed that among different harvesting times, last week of October was found to be optimum time for obtaining maximum dry herbage (931.3 kg ha^{-1}) and total andrographolide yield (61.83 kg ha^{-1}), respectively. Further, it was observed that on the basis of leaf/stem ratio the relative estimates of total andrographolide yield was recorded higher in October month than that of recorded in the months of September or November.

Andrographolide content

Andrographolide content was estimated in plants of different age from the three accessions at 20-day interval starting from plant age 60 DAT to 120 DAT. The study showed that andrographolide content increased at early age but after a certain point it started declining.

Andrographolide content in the leaves was 1.42 % at plant age 60 DAT which rose to maximum at plant age 80 DAT (2.59 %), and thereafter progressively reduced to 1.85 % at plant age 120 DAT. Maximum andrographolide content in stem was observed at 120 DAT of plant age (0.76%) whereas aximum herbage andrographolide content was estimated at plant age 80 DAT (2.03%) and minimum at plant age 120 DAT (1.15 Maximum andrographolide content in the leaves was estimated in case of accession 1 at plant age 80 DAT (2.69 %) which was at par with that of the 100 DAT sampling of the same accession. Maximum herbage andrographolide content was in accession 1 at plant age 80 DAT (2.08 %) which was as at par (Fig. 7) with the accession 2 at plant age 80 DAT (2.03%).

Andrographolide yield

There was no significant effect of the accessions on the andrographolide yield of leaves, stem and herbage in all the stages of plant growth. Sampling time significantly influenced the andrographolide yields of leaf, stem and herbage irrespective of the accessions. The lowest leaf andrographolide yield was observed at plant age 60 DAT (0.02 g $plant^{-1}$) and the highest was at plant age 100 DAT (0.22 g $plant^{-1}$). The lowest andrographolide yield of stem was recorded at plant age 60 DAT (0.002 g $plant^{-1}$) whereas highest at plant age 120 DAT (0.106g $plant^{-1}$). The lowest total herbage andrographolide yield was found at plant age 60 DAT (0.02 g $plant^{-1}$). Significantly highest andrographolide yield was recorded at plant age 100 DAT (0.26 gm $plant^{-1}$) which was at par (Fig. 8) with the yield recorded at plant age 120 DAT (0.25 gm $plant^{-1}$).

Sharma *et al.* (1992) standardized Kalmegh (*Andrographis paniculata*) by high pressure liquid chromatographic determination of its major active constituent of andrographolide. The leaves of this herb were found to contain the highest amount (2.39% w/w) of andrographolide and the the lowest. In the present investigations higher andrographolide content has been obtained in leaves as compared to stem. However, leaf and stem ratio of the total herbage would regulate the total yield of andrographolide in the produce. Bhan *et al.* (2006) reported estimated total andrographolide yield on the basis of leaf/stem ratio.

Accession 1 and accession 3 were better in producing total andrographolide yield compared to accession 2; similar difference in the production of andrographolide in accessions was also reported by Bhan *et al.* (2005). They observed that out of 10 accessions used in their experiments, Ac. 1 and Ac. 9 have been identified as the best sources for obtaining higher drug yield.

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