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Growth Behavior, Biomass and Diterpenoid Lactones Production in Kalmegh (*Andrographis Paniculata* Nees.) Strains at Different Population Densities

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Abstract: About 4 strains of kalmegh (*Andrographis paniculata* Nees.), CIMAP/AP-3, CIMAP/AP-10 and CIMAP/AH-89 and local check were evaluated at three population densities (74,074, 111,111 and 222,222 plants ha^{-1}) for growth behavior, biomass and diterpenoid lactones production at the Central Institute of Medicinal and Aromatic Plants, Lucknow, India during July to November, 2004 and 2005. The strain CIMAP/AP-3 was found to possess desired agronomic traits viz., 60 cm plant height, 45 cm plant spread, 28 branches per plant and 60° branching angle from main shoot. The advantage in biomass and diterpenoid lactones (andrographolide and neo-andrographolide) production with CIMAP/AP-3 over other strains was 33.4-79.4 and 31.6-70.7%, respectively. Planting at a closer spacing of 30×15 cm accommodating 2,22,222 plants ha^{-1} produced maximum dry biomass and andrographolide and neoandrographolide yield under the subtropical climate of North India.

Keywords: *Andrographis paniculata*, strain, plant population, biomass yield, andrographolide, neo-andrographolide

INTRODUCTION

Kalmegh (*Andrographis paniculata* Nees.) an annual herb (Family Acanthaceae) has been in use in Indian system of medicine; Ayurveda, Yunani, Shiddha and Homeopathy (AYUSH). Its distribution is recorded in India, Sri Lanka, Malaya Peninsula, China and Thailand. In India, it occurs through out in the plains and also in forests as undergrowth. It is grown as a snake-repellant in households. This species is globally distributed in Indo-Malesia. Within India, chiefly found in the plains throughout India from Himachal Pradesh to Assam and Mizoram and all over Peninsular India.

The plant has been reported to possess antipyretic, antihepatotoxic, antihistamic, analgesic, antibacterial, anti-inflammatory, antifertility and immunosuppressive properties due to its bitter andrographolide content (Anonymous, 1989, 1990; Murugaian *et al*., 1995; Saxena *et al*., 2000; Srivastava *et al*., 1995; Tripathi and Tripathi, 1991).

Kalmegh is also an important herb for the treatment of dysentery, diarrhea, enteritis, fever, cough, sore throat, tonsillitis, bronchitis, arthralgia, menstrual and postpartum haematometra; hypertension and snake bite (Gupta and Srivastava, 1994; Madav *et al*., 1995; Matsuda *et al*., 1994). At present, price of dried herb in Indian market ranges from 15-20 Rs kg^{-1}. To ensure regular supply of herb with consistent quality, it is essential that an identified variety with desired chemical constituents be brought under cultivation. Under its crop improvement program, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India, selected three strains of kalmegh; AP-3, AP-10 and AH-89. To identify the best strain with respect to agronomic traits, herb and alkaloid production, the selected strains were evaluated against a local check at three population densities so as to select best strain for its commercial cultivation in the country.

MATERIALS AND METHODS

Experimental site: The evaluation trial was conducted during 2004 and 2005 (July to November) on sandy loam soil (pH 7.8) at the research farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow located at 26.5°N, 80.5°E with an elevation of 120 m above mean sea level.

Treatments and experimental design: About 3 new strains of kalmegh (*Andrographis paniculata* Nees.) CIM./AP-3, CIM./AP-10 and CIM./AH-89 were evaluated against a local check at three spacing, 45×30 cm and 30×15 cm, accommodating 74,074, 111,111 and 222,222 plants ha^{-1}, respectively. The experiment was conducted during July to November, 2004 and 2005 in a factorial randomized block design with three replications and each gross and net plot measuring 2.7×1.8 m and 1.8×1.2 m, respectively.

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Cultural operations: About 35 days old seedlings were transplanted at spacing as per treatments in first fortnight of August, 2004 and 2005. The crop received a basal dose 40 kg ha^{-1} each of N, P_2O_5 and K_2O before transplanting on 14 August. Nitrogen 40 kg ha^{-1} was applied as topdressing 30 Days After Transplanting (DAT). Symptoms of iron deficiency were noted on the foliage at about 40 DAT. Spraying of $FeSO_4$ 0.25% + citric acid 0.25% was done at 42 DAT as a corrective measure to iron deficiency. About 2 manual weeding were done at 30 and 60 DAT to minimize the weed competition. The crop received two irrigations during rain free periods in the 3rd week of October and second week of November. The crop was harvested at 105 DAT in end of November, 2004 and 2005.

Plant growth and biomass production: Observations on growth and yield attributes viz; plant height, plant spread, branch emergence, number of branches, branching angle from main shoot were recorded before harvest at the beginning of seed setting stage from 10 randomly selected plants from each plot. Data on dry biomass accumulation were recorded at pre-flowering, flowering and beginning of seed setting stage at 80, 92 and 105 DAT, respectively from 2 m row length in each plot.

Estimation of andrographolide and neo-andrographolide contents and yield: Andrographolide and neo-andrographolide contents (w/w %) in dry shoot biomass were estimated at three different growth stages. The contents of andrographolide, neo-andrographolide and the total diterpenoid lactones (Andrographolide + neo-andrographolide) were multiplied with dry biomass yield of respective treatments and the effect of treatments and the stage of harvest on their production. Andrographolide and neoandrographolide contents in plant samples were analysed as per the method reported earlier (Jain *et al*., 2000).

Sample preparation: About 1.0 g air dried (50°C for 24 h) and powdered shoots of *A. paniculata* plants were extracted using methanol (3×10 mL, 12 h at room temp. 20±5°C), filtered and the combined extract was concentrated under vacuum till dryness. To the extract, thus obtained 5 mL methanol was added and filtered through Millipore filter (0.45 μm) and a known amount was subjected to HPLC analysis.

HPLC analysis: A Shimadzu (Japan) LC-10A gradient high performance liquid chromatography instrument equipped with two LC-10AD pumps a model CBM-10 interface, a model 7725i manual injector (Rheodyne) a 20 μL sample loop a PDA detector (SPD-M10A), LC-10 workstation equipped with a Pentium Computer and HP-deskjet printer have been used for the analysis of andrographolide and neoandrographaolide content and HPLC finger print. Experiment was peformed on a C_{18} water make μ Bondpak (300×3.9 mm, I.D. 10 μm); mobile phase, acetonitrile water (30:70) flow rate 1.0 mL min^{-1}; UV detection 230 nm where 10 μL of standard andrographalide and neoandrographolide solution in methanol (1 mg mL^{-1}) and sample solution in methanol as prepared above were injected separately and the percent contents of andrographalide and neoandrographolide were estimated by the area count of andrographalide and neoandrographolide peak in standard and sample tracks.

Statistical analysis: Data on observed parameters were statistically analyzed using ANOVA and treatment differences were separated using LSD at 5% level of probability.

RESULTS AND DISCUSSION

Growth and yield attributes: Observations on various growth and yield attributes (Table 1) indicated that plants of AP-3 were taller (60 cm), having more number of branches (28) and plant spread (45 cm) than remaining strains. Considerable differences in the branching angle

Table 1: Growth and yield attributes of kalmegh strains (mean of 2004 and 2005)

Treatments	Plant height (cm)	Plant spread (cm)	Branch emergence from ground (cm)	No. of branches/ plant	Branching angle from main shoot (°)
Strains					
AP-3	60.0	45.0	1.5	28.0	60
AP-10	53.0	30.0	4.5	16.0	45
AH-89	50.0	36.0	4.5	20.5	45
Local	55.0	40.0	3.2	25.5	45
LSD (p = 0.05)	4.0	3.2	0.5	2.8	5
Spacing (cm)					
45×30	53.5	45.0	4.5	25.1	48
30×30	55.0	37.0	4.4	22.5	45
30×15	56.5	32.0	4.5	19.8	43
LSD (p = 0.05)	NS	2.7	NS	2.5	NS

Table 2: Dry biomass production of kalmegh at different growth stages (mean of 2004 and 2005)

	Dry biomass yield (ton ha^{-1})		
Treatments	Pre-flowering (80 DAT)	Flowering (92 DAT)	Seed setting (105 DAT)
Strains			
AP-3	2.77	3.93	4.00
AP-10	1.77	2.33	2.50
AH-89	1.83	2.07	2.23
Local	2.13	3.03	2.87
LSD (p = 0.05)	0.60	0.68	0.56
Spacing (cm)			
45×30	1.52	2.25	2.55
30×30	1.98	2.95	2.92
30×15	2.88	3.33	3.22
LSD (p = 0.05)	0.52	0.58	0.49

Table 3: Andrographolide, neoandrographolide and total diterpenoid lactones yield (kg ha^{-1}) of kalmegh at different growth stages (mean of 2004 and 2005)

	Andrographolide yield			Neoandrographolide yield			Total diterpenoid lactones yield		
Strains	Pre-flowering	Flowering	Seed setting	Pre-flowering	Flowering	Seed setting	Pre-flowering	Flowering	Seed setting
AP-3	67.80	64.20	76.20	7.70	11.50	6.60	75.50	75.70	82.80
AP-10	36.90	32.90	45.90	6.20	7.20	6.80	43.10	40.10	52.70
AH-89	37.80	27.70	43.20	5.30	6.80	7.10	43.10	34.50	50.30
Local	52.60	49.70	54.70	6.50	9.00	8.20	59.10	58.70	62.90
LSD (p = 0.05)	2.70	2.72	4.37	0.36	0.34	0.62	2.88	2.72	4.37
Spacing (cm)									
45×30	30.00	34.90	46.40	4.20	7.60	6.90	34.20	42.50	53.30
30×30	46.30	44.60	54.90	6.10	9.40	7.40	52.40	54.00	62.30
30×15	70.50	48.40	62.50	9.50	9.30	8.20	80.00	57.70	70.70
LSD (p = 0.05)	2.03	2.03	3.20	0.27	0.25	0.46	2.18	2.03	3.20

and main branches emergence height from ground were also noted. While in AP-3, the branches maintained an angle of 60° from main shoot it was 45° for others. The emergence of primary branches in case of AP-3 was from very close to the ground level whereas in case of other strains it was 4-5 cm above from the ground level. Strain AH-89 had the lowest values of above parameters. Spacing had no specific effect on various growth characteristics, except number of branches and plant spread that decreased with decrease in plant spacing.

Biomass production: Dry shoot biomass of all the strains increased up to flowering stage, beyond which there was very little gain in dry biomass, particularly under wider spacing of 45×30 cm whereas in closer spacing of 30×30 cm and 30×15 cm dry biomass reduced. It might be due to fall of lower leaves which became yellow and dried due to mutual shading (Table 2).

The strains varied significantly with respect to biomass production. AP-3 produced significantly higher biomass than remaining strains. At harvest, dry biomass production by AP-3 was 33.4-79.4% higher than remaining strains (Table 2). It was possibly due to open plant canopy, resulting in better utilization of solar energy than remaining strains (Table 1).

Spacing had significant influence on dry biomass production. Closer spacing of 30×15 cm produced higher dry biomass by 89.5 and 45.5% at pre flowering, 48 and 12.9% at flowering and 26.3 and 10.3% at seed setting stage as compared to 45×30 and 30×30 cm spacing, respectively. It was due to better utilization of natural and applied growth resources by dense plant population.

Diterpenoid lactones content and yield: There was no significant difference in andrographolide, neoandrographolide and total diterpenoid lactones content of different strains planted on different spacings at different growing stages. Andrographolide and total diterpenoid lactones contents in dry herb were maximum 2.06-2.47 and 2.36-2.73% at pre-flowering stage and the lowest 1.34-1.64 and 1.67-1.94%, respectively at flowering stage. Significant variations in andrographolide, neoandrographolide and total diterpenoid lactones yield were noted among the strains. AP-3 yielded the maximum total diterpenoid lactones (82.8 kg ha^{-1}) where as AH-89 produced the minimum (50.3 kg ha^{-1}). Overall, the advantage in diterpenoid lactones yield with AP-3 was 31.6-70.7% over remaining strains (Table 3). Andrographolide, neoandrographolide and total diterpenoid lactones yield varied with plant spacing. Decrease in plant spacing or increase in population density led to increase in diterpenoid lactones production. The total diterpenoid lactones yield at harvest with 45×30, 30×30 and 30×15 cm was 53.3, 62.3 and 70.7 kg ha^{-1}, respectively. These variations were mainly due to variations in dry biomass yield.

CONCLUSION

Growing of kalmegh strain CIM- AP-3 and planting at a closer spacing of 30×15 cm accomodating 2,22,222 plants ha^{-1} is suggested for obtaining maximum dry biomass and diterpenoid lactones (andrographolide and neoandrographolide) yield under the subtropical climate of North India.

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