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SHORT COMMUNICATION

Optimization of plant population and nitrogen requirement for commercial cultivation of Kalmegh (*Andrographis paniculata* Nees) under Indo-Gangetic plains of north India

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ABSTRACT

To optimize the plant population density and nitrogen requirement of Andrographics paniculata under indo-Gangetic plains, a field experiment was conducted in a sandy loam soil during rainyseason (July-October) at the research farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow. Sixteen treatments comprising of four plant population densities (66,666, 111,111, 222,222 and 444,444 plants ha^{-1} by planting at 50 x 30cm, 30 x 30cm, 30 x 15cm and 15 x 15 cm spacing, respectively) and four nitrogen levels (0, 40, 80 and 120 kg ha^{-1}) were tested in a factorial randomized block design with three replications. Yield contributing traits such as plant height, number of branches per plant, leaf area index (LAI), dry biomass and content of diterpenoid lactones (Andrographolide + neo-andrographolide) registered a significant increased at a plant population of 222,222 plants ha^{-1} and application of 80 kg ha^{1} nitrogen. The optimum dose of N was worked out to be 90.7 kg ha^{1} that gave a maximum dry biomass yield of 3833.7 kg ha^{1}.

INTRODUCTION

Kalmegh (*Andrographis paniculata* Nees.), is an annual medicinal herb of the Family Acanthaceae. The plant is widely used in Indian systems of medicine like Ayurveda, Yunani, Shiddha and Homeopathy (AYUSH) for hepato-protection. The plant is also known to possess antipyretic, antihistamic, analgesic, antibacterial, anti-inflammatory, antifertility and immuno-suppressive properties owing to its bitter diterpenoid lactones commonly known as andrographolides [5, 7, 8]. Kalmegh is also an important herbal remedy for dysentery, diarrhea, enteritis, fever, cough, sore throat, tonsillitis, bronchitis, arthralgia, menstrual and postpartum haematometra, hypertension and snake bite [1, 2, 3, 4]. Commercial cultivation of kalmegh has been intiated in india to meet the growing market demands of raw herb and purified andrographolids. Amongst the various agronomic factors that need to be standardized for realizing the maximum yield potential of a crop under a given agro-climate,

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optimization of plant population density and fertilizer applications treatments are of primary consideration. In an earlier report [7] we have shown that a plant density of 222, 222 plants/ ha favored the high biomass production in kalmegh under northern Indian agro-climate. We now report here the interactive influence of plant density and nitrogen dosages on biomass and andrographolides yield in *A. paniculata* under Indo-Gangetic plains of north India.

MATERIALS AND METHODS

Experimental site and soil

The present field experiment was conducted during rainy season (July-October) at the research farm of the Central Institute of Medicinal and Aromatic Plants, Lucknow located at $26^0 5'$N, $80^0 5'$E and an altitude of 120 m above mean sea level. The soil (pH 8.0) of the experimental field was a sandy loam (typic ustifluvent), having 0.3% organic carbon, low available nitrogen (alkaline $KMnO_4$ extractable N was 46 mg kg^{-1}) and medium phosphorus (Olsen P 7.0 mg kg^{-1}) and exchangeable potassium (74 mg kg^{-1}) contents.

Treatments and experimental design

Sixteen treatments comprising of four plant densities (66,666, 111,111, 222,222 and 444,444 plants ha^{-1} by planting at 50 x 30cm, 30 x 30cm, 30 x 15cm and 15 x 15 cm, spacing, respectively) and four nitrogen levels (0, 40, 80 and 120 kg ha^{-1}) were tested in a factorial randomized block design with three replications. Individual gross and net plot size was 3.5 m x 3.0 m and 3.0 m x 2.1 m, respectively.

Cultural operations

Thirty-five days old seedlings of *A. paniculata* were transplanted at the specified spacing according to the plant density treatments in the first fortnight of July, 2009 and 2010. The crop received a basal fertilizer dressing of 50 kg ha^{-1} each of P_2O_5 and K_2O before planting. Nitrogen doses as per treatment were applied in three equal splits, one third as basal and rest top dressed at 30 and 60 days after transplanting (DAT). Two manual weedings were carried out at 30 and 60 DAT to minimize the weed competition. The crop was harvested at 105 DAT in the last week of October, 2009 and 2010.

Measurement of growth and metabolite production

Data on growth and yield parameters such as plant height, leaf area index and leaf: stem ratio were recorded in ten randomly selected plants from each plot before harvest at the onset of seed setting stage. Diterpenoid lactones (andrographolide + neo-andrographolide) contents in the dried shoot biomass were estimated in the harvested samples through HPLC analysis [6]. The total diterpenoid lactones yield under respective treatment was calculated by multiplying their content with herb yield. For HPLC analysis, 1.0 g air dried shoot samples of *A. paniculata* were powered and extracted with methanol (3 x 10 ml, 12 hr at room temp), filtered and concentrated under vacuum till dryness. To this extract 5 ml methanol was added and filtered through Millipore filter (0.45µm) before a known amount was subjected to HPLC analysis. The HPLC analysis was carried out using 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 20µl sample loop, a PDA detector (SPD-M10A) and a LC-10 workstation. Separation was carried using a C_{18} Water make µBondpak column (300mmx3.9mm, I.D. 10µm); a mobile phase consisting of acetonitrile: water (30:70) at a flow rate of 1.0 ml min^{-1} and a UV detection at 230 nm. 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 for factorial RBD and treatment differences were separated using CD at 5% level of probability.

RESULTS AND DISCUSSION

Plant growth and biomass yield

The data presented in Table 1 suggested that the plants were significantly taller under higher plant densities as compared to lower density and reverse was true for leaf : stem ratio and number of branches per plant. Leaf area index (LAI) and dry biomass yield increased significantly with increase in plant population up to 222,222 plants ha^{-1}, beyond which there was no significant gain. The higher plant height but lower leaf: stem ratio and number of branches per plant under higher plant population were probably due to higher inter and intra row plant competition resulting in induced apical growth and less lateral branching. Higher LAI and dry biomass production under higher plant population were due to higher number of plants per unit area. Similarly observations were also made by our group in an earlier study [7] where less number of branches per plant and higher biomass production was obtained at a plant population density of 222,222 plants ha^{-1} in Kalmegh. Improved plant height, number of branches per plant, LAI and dry biomass accumulation was also observed with increase in nitrogen (N) levels up to 80 kg ha^{1}. Further increase in N levels to 120 kg ha^{1} did not bring significant change in growth and biomass yield. Lower leaf: stem ratio under higher N levels was also noted due to excessive senescence of lower leaves on account of mutual shading due to higher plant growth. There was a quadratic ($Y=1903.2+38.875X – 0.205X^2$) response to nitrogen levels (Figure 1). The optimum dose of N was worked out to be 90.7 kg ha^{1} and dry biomass yield at this optimum dose of N was worked out to be 3833.7 kg ha^{1}.

Diterpenoid lactones content and yield

Diterpenoid lactones (andrographolide + neo-andrographolide) content in shoots of kalmegh ranged from 1.78 - 1.94% and was not significantly influenced by plant population (spacing) and

Table 1. Biomass and diterpenoid lactones yield of kalmegh as affected by plant densities and nitrogen application

Treatments	Plant height (cm)	No. of branches $plant^{-1}$	Leaf area index	Leaf : stem ratio	Dry biomass yield ($kg\ ha^{-1}$)	Andrographolide + neoandrographolide content (% dry wt.)	Andrographolide + neoandrographolide yield ($kg\ ha^{-1}$)
Plant density ha^{-1} 66,666	60.8*	28	2.02	2.08	2,690	1.94	51.1
111,111	64.8	25	2.24	1.90	3,051	1.87	57.1
222,222	67.3	22	2.64	1.85	3,401	1.83	62.2
444,444	70.4	18	2.73	1.83	3,446	1.78	61.3
SEm ±	0.8	0.7	0.04	0.02	88	0.1	1.5
LSD (P=0.05)	2.4	2	0.12	0.06	254	NS	4.4
Nitrogen levels ($kg\ ha^{-1}$)							
0	62.3	16	1.67	2.02	1,846	1.85	34.2
40	66.3	22	2.45	1.95	3,343	1.90	63.5
80	68.1	27	2.70	1.86	3,607	1.92	69.3
120	66.6	28	2.81	1.83	3,792	1.85	70.1
SEm ±	0.8	0.7	0.04	0.02	88	0.1	1.5
LSD (P=0.05)	2.4	2	0.12	0.06	254	NS	4.4

*- mean values, of 2 year data; NS-Non significant

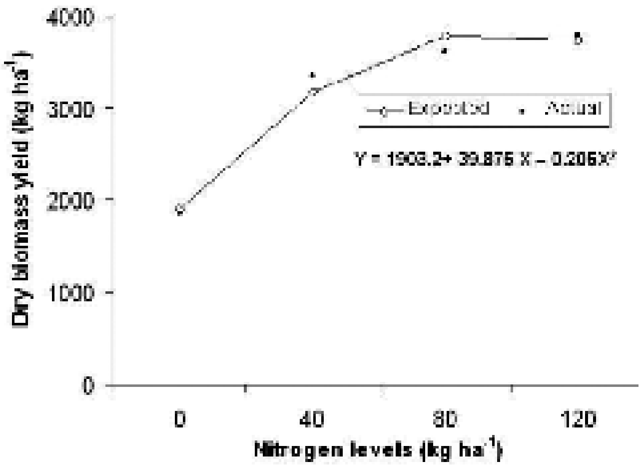

Figure 1. Response of kalmegh to nitrogen levels

nitrogen treatments (Table 1). However, the net yield of these bioactive molecules was significantly improved under closer spacing or increase in population density mainly due to enhanced dry biomass yield.

CONCLUSION

On the basis of results obtained in the present study it can be concluded that maintaining a plant population of 222,222 plants ha^{-1} by planting at a spacing of 30x15 cm and application of 90.7 kg N ha^{-1} are optimal agronomic treatments for obtaining maximum net returns from the commercial cultivation of Kalmegh under Indo-Gangetic plains of north India.

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