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Spatial and harvesting influence on growth, yield, quality, and economic potential of Kalmegh (*Andrographis paniculata* Wall Ex. Nees)

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Abstract

Andrographis paniculata, commonly known as Kalmegh, is used both in *Ayurvedic* and *Unani* system of medicines because of its immunological, antibacterial and hepatoprotective properties. This study was carried out to investigate the influence of four harvesting times (120, 135, 150 days after planting and at seed maturity) and four planting distances (30×15, 30×10, 20×15 and 20×10 cm) on growth, dry herbage biomass, seed yield and quality traits of *Andrographis paniculata* at CCS Haryana Agricultural University, Hisar, India in the two years 2005 and 2006. The treatments were laid out in a split plot design with three replications. The maximum values for dry herbage biomass yield (5.14 t ha^{-1}), net returns (760.00 EUR ha^{-1}), B:C ratio (2.59), andrographolide content (2.63 %) and total yield (135.00 kg ha^{-1}) were detected 135 days after planting with an optimum planting distance of 30×15 cm. However, the maximum iron content was estimated 120 days after planting. The highest dry herbage (4.58 t ha^{-1}) and maximum seed yield (19.7 kg ha^{-1}) were registered at plants that were lined out with a distance of 20×10 cm.

Keywords: Kalmegh, *Andrographis paniculata*, capsule, herbage yield, andrographolide content, iron content, net returns

1 Introduction

Kalmegh (*Andrographis paniculata*), an annual herb, belongs to the family Acanthaceae and constitutes one of the nineteen species of the genus *Andrographis*. In India, Kalmegh is well known under different vernacular names such as Kirta, Kiryata, Kalpnath, Create, Green Chirata and King of Bitters. It grows abundantly in Southeast Asian countries such as Sri Lanka, Pakistan, Malaysia and Indonesia and has been cultivated extensively for medicinal purpose in India, China, Thailand, East and West Indies and Mauritius (Mishra *et al.*, 2007; Kanokwan & Nobuo, 2008; Niranjan *et al.*, 2010; Kataky & Handique, 2010). The fresh and dried leaves of Kalmegh and extracted juice are the official drugs of Indian pharmacopoeia.

Four lactones, *viz.* deoxyandrographolide, andrographolide, neoandrographolide and deoxydidehydroandrographolide were found in *Andrographis paniculata* (Sangalungkarn *et al.*, 1990) and are common forms used in clinics (Tipakorn *et al.*, 2003). The leaves and roots are stomachic, tonic, antipyretic, febrifuge and cholagogue (Kanniappan *et al.*, 1991) and can provide a rich source of iron used by anaemic patients. The production volume of Kalmegh achieved by commercial agriculture is not exactly available in the literature. However, the total production volume achieved from wild growing plants is about 5,000 tonnes per year, mainly from the states of Madhya Pradesh, Uttar

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Pradesh, West Bengal and Bihar (Kumar, 2006). The estimated demand of Kalmegh for the year 2005 was about 2,200 tonnes (Kumar, 2006). Due to its pharmacological properties, the Kalmegh herb is collected indiscriminately from the wild sources causing a sharp decline in the availability of this herb to the industry.

Very few studies have been carried out in the development of agrotechniques for its commercial cultivation (Maheshwari *et al.*, 2002; Singh & Singh, 2005; Singh *et al.*, 2011). The crop geometry or plant spatial arrangement and appropriate harvesting time are considered to be very important for crop quality. The active principle of a plant varies with time interval, different environmental conditions (National Research Centre for Medicinal and Aromatic Plants, 2001; Kumar *et al.*, 2002) and time of harvesting had a major influence on productivity and quality of Kalmegh (Nemade *et al.*, 2001). As per the earlier reports, the quality of Kalmegh decreases with the delay in harvesting (National Research Centre for Medicinal and Aromatic Plants, 2001). Therefore, a proper planting distance and an optimum harvest time need to be standardized for this crop. The present study was undertaken with the objective to maximize biomass production of the medicinal Kalmegh plant as well as the content of andrographolide and iron due to the determination of optimum plant density and harvesting stage.

2 Materials and methods

2.1 Study area

The experiments were carried out on fields situated in the Hisar district that constitute one of the 21 districts of Haryana state, India (elevation: 215.2 m a.s.l., lat. 29°10'N, long. 75 46'E). Hisar has a typical semi-arid subtropical climate with hot and dry winds during summer. The maximum temperature of around 48°C prevails during summer months of May and June and minimum temperature around freezing point accompanied by frost occurs in winter months of December and January. The total rainfall as well as its distribution in this region is subjected to large variations. About 80 to 90 percent of the total rainfall (about 400 mm) is received from South West monsoon during July to September. A few downpours of cyclonic rains occur during December and January or late spring. The common crops that are cultivated in this region are wheat, cotton, cluster bean, pearl millet, green gram and mustard. The forest area is less than 6 % of the total state area. The soil of the experimental field was sandy loam in texture (sand 64.43 %, silt 17.3 % and clay 19.27 %), low in available nitrogen (191 kg/ha), medium in available phosphorus (16 kg/ha) and rich in available potash (744 kg/ha) with pH 8.5.

2.2 Treatments and experimental design

There were two main factors (i) four harvesting times: 120, 135, 150 and 170 days after transplanting (DAP) and (ii) four planting distances: 30 cm × 15 cm, 30 cm × 10 cm, 20 cm × 15 cm and 20 cm × 10 cm. These were factorially combined to give 16 treatments, which were laid out in a split plot design, with harvesting time as the main plots and planting distance as sub plots with three replications each. The field experiments were conducted from July to October in both years 2005 and 2006 with a plot size of 4.0 m × 3.6 m (Fig.1).

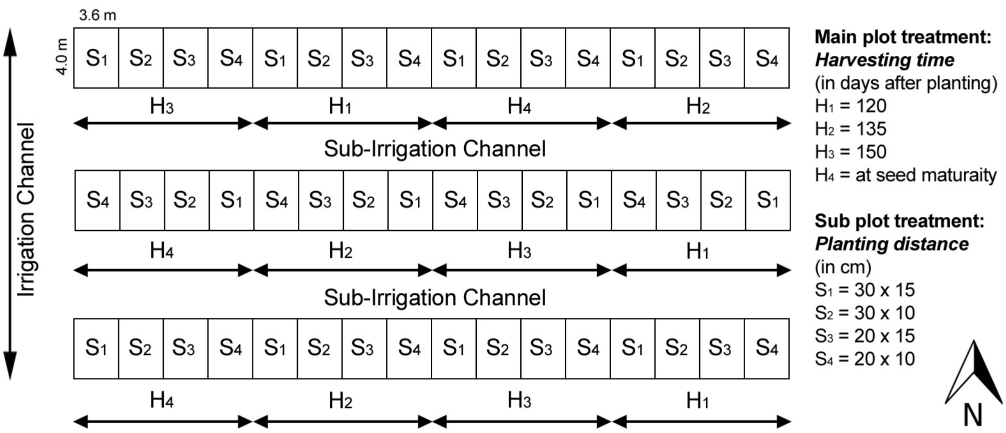

Fig. 1: *Experimental design to study the impact of harvesting time and planting distance on Kalmegh growth characteristics in the Hisar district, India in 2005 and 2006.*

2.3 Field preparation and cultural operation

The field was prepared to a fine tilth after thorough incorporation of 10 t well rotten farmyard manure. For raising crop, 35 days old seedlings were transplanted on 5th July 2005 and 15th July 2006, respectively. At the time of transplanting, nitrogen was applied with 25 kg/ha and additional 25 kg/ha were scattered on day 30 after transplanting. Manual weeding took place at week three and four, and irrigation was done at week two, four and seven during the crop season.

2.4 Plant growth and biomass production

The growth and yield attributes were recorded from five randomly selected plants taken from each treatment plot. Fresh, dry herbage and seed yield were recorded as per the time of harvesting. Dry herbage samples were first sun dried and afterwards in the oven at around 65°C until a constant dry weight was obtained.

2.5 Andrographolide content and yield

The andrographolide content was estimated by using the method of Srivastava *et al.* (1959). The dry herb (whole plant part) weighing 10 g was transferred in a Soxhlet extraction apparatus and extracted with 200 ml of ethyl alcohol (95 %) for 6 hours. The extract was continuously boiled until a volume of 50 ml has been reached, and thereafter, 30 ml of distilled water was added. This solution was partitioned with 2×25 ml portions of benzene for 2 minutes and the benzene layer was rejected. Distilled water (20 ml) was added to the aqueous layer, and the combined aqueous solution was partitioned for 2 minutes with 3×25 ml ethyl acetate, which was sufficient to extract all the bitter principles. The combined ethyl acetate extract was treated with anhydrous sodium sulphate (about 40–50 g) and filtered through filter paper. Washing was done with 10–15 ml ethyl acetate and mixed with the original filtrate. The solvent was then removed from the water bath, and the residue was kept in oven at 100°C for 2 to 3 hours until the dry weight became constant. The weight of dry residue was found as andrographolide content (in g). The andrographolide yield (kg/ha) was calculated on the basis of dry herb yield.

2.6 Iron content

The iron content in acid digested plant sample was determined with the help of atomic absorption spectrometer (AAS), which is based on the principle that the atoms of metallic element (Fe), which normally remain in ground state under flame conditions, absorb energy when subjected to tradition of specific wavelength. The absorption of radiation is proportional to the concentration of atoms of that element. The absorption of radiation by the atoms is independent of the wavelength of absorption and temperature.

2.7 Calculations

Weight of plant material taken = 1 g

Volume made after digestion = 100 ml

Dilution factor = 100/1 = 100 times

Content of Fe in 1 ml plant sample = $M\,\mu$g

Content of Fe in 100 ml plant sample = $M \times 100\,\mu$g

Content of Fe in 1 g plant sample = $M \times 100\,\mu$g

Content of Fe in 100 g plant sample = $M \times 100 \times 100\,\mu$g

Content of Fe in 100 g plant sample = $M \times 10$ mg

Where, M stands for Fe concentration in μg per 1 ml aliquot as read from standard curve against the sample readings. Iron yield was calculated on the basis of dry herbage yield.

2.8 Statistical analysis

In order to evaluate the comparative performance of the various treatments, data were analyzed by using the technique of analysis of variance described by Fisher (1958). All tests of significance were made at 5 % level. The statistical method as suggested by Panse & Sukhatme (1961) was followed for the analysis and interpretation of experimental results.

3 Results

3.1 Growth and seed yield attributes

Both treatments harvesting time and planting distance had a significant impact on all plant growth and yield parameters (Table 1). With regard to the impact of harvesting time, plant height increased significantly up to 150 DAP (84.2 cm) in both years, while the rate of increase was observed maximum between 120 DAP and 135 DAP (76.4 cm to 81.5 cm) and minimum between 150 DAP and harvesting stage (84.2 to 85.0 cm). The number of branches was highest at harvesting stage. The mean performance of leaf to stem ratio among all treatments showed a gradual decrease from 135 DAP (0.22) to seed maturity (0.16). The capsule length increased significantly up to 135 DAP (18.8 cm) but no significant differences among treatments of 135 DAP, 150 DAP and at seed maturity could be observed. The number of capsules per plant increased significantly up to seed maturity (335.5) and maximum rate of capsule formation was noticed between 135 (254.5) and 150 (318.6) DAP, and the lesser capsules were recorded when the crop was harvested on day 120 after transplanting.

Table 1: *Growth and yield attributes of Kalmegh as affected by different planting distance and harvesting time. Values constitute the mean of five measurements on five randomly selected plants per treatment. Seed maturity was reached at harvesting time 170 DAP.*

Treatment	*Plant height (cm)*			*Number of branches per plant*			*Leaf/Stem ratio*			*Capsule length (cm)*			*Number of capsules per plant*		
	2005	*2006*	*Mean*	*2005*	*2006*	*Mean*	*2005*	*2006*	*Mean*	*2005*	*2006*	*Mean*	*2005*	*2006*	*Mean*
Harvesting time															
120 DAP	78.0	74.8	76.4	32.5	30.1	31.3	0.16	0.20	0.18	16.7	17.1	16.9	229.3	201.4	215.4
135 DAP	83.8	79.2	81.5	32.9	31.2	32.1	0.21	0.23	0.22	18.1	19.4	18.8	272.6	236.3	254.5
150 DAP	85.9	82.4	84.2	33.0	31.8	32.4	0.15	0.19	0.17	18.5	20.6	19.6	334.5	302.6	318.6
170 DAP	87.0	83.0	85.0	34.6	32.4	33.5	0.14	0.17	0.16	18.6	21.0	19.8	349.6	321.4	335.5
CD at 5 %	1.4	1.6	–	NS	2.0	–	0.02	0.02	–	0.4	0.7	–	6.5	9.3	–
Planting distance															
30 × 15 cm	85.1	78.9	82.0	35.4	33.4	34.4	0.18	0.22	0.20	18.0	19.0	19.5	344.5	297.1	320.8
30 × 10 cm	86.5	79.0	82.8	33.2	31.0	32.1	0.18	0.20	0.19	18.0	19.4	18.7	310.3	278.9	294.6
20 × 15 cm	86.3	80.2	83.3	33.1	31.2	32.1	0.17	0.18	0.18	17.9	19.1	18.5	271.6	248.0	259.8
20 × 10 cm	87.8	81.0	84.4	31.3	29.6	30.5	0.16	0.19	0.18	17.8	19.2	18.5	261.0	236.4	248.7
CD at 5 %	NS	NS	–	2.3	2.9	–	NS	0.03	–	NS	NS	–	8.2	10.2	–

DAP = days after transplanting, CD = critical difference

The mean performance of two years exhibited a maximum plant height at a planting distance of 20 × 10 cm (84.4 cm), whereas, the growth parameters, number of branches per plant (34.4) and leaf to stem ratio (0.20) were recorded maximum at a plant spacing of 30 × 15 cm (Table 1). Capsule length was not significantly affected by planting distance during the two years and mean values varied from 18.5 to 19.5 cm. The number of capsules per plant was highest at a planting distance of 30 × 15 cm (320.8). Dry herbage biomass increased significantly until 135 days DAP (5.14 t/ha) and decreased to 4.16 t/ha (after 150 DAP) and 3.47 t/ha (at seed maturity) (Table 2). The decrease in dry herbage yield was 20.0, 23.5 and 48.1 per cent when harvesting was done at 120 DAP, 150 DAP and at seed maturity, respectively. The seed yield varied significantly up to seed maturity stage with the maximum of 21.5 kg/ha. The maximum dry biomass yield of 4.6 t/ha and highest seed yield of 19.7 kg/ha was harvested from plants with a planting distance of 20 × 10 cm.

3.2 *Andrographolide and iron content*

The highest content of andrographolide was recorded at a crop age of 135 DAP (2.63 %) and 120 DAP (2.44 %) (Table 3). Thereafter, the andrographolide content decreased due to the senescence of the lower leaves with minimum content at seed maturity stage (1.73 %). Also, the androgapholide yield reduced within older plants with maximum realization after 135 days of transplanting (134.9 kg/ha). Iron content was found highest (24.42 mg per 100 g of dry matter) in plants harvested at 120 DAP and total iron uptake at 135 DAP. Concerning the impact of plant spacing on andrographolide content, analyses revealed a peak content at a planting distance of 30 × 15 cm, whereas andrographolide yield (96.75 kg/ha) was highest in plants that were planted in a distance of 20 × 10 cm. The application of different planting distances failed to exert any significant effect on iron content. However, maximum iron yield of 0.93 kg/ha was recorded at a planting distance of 20 × 10 cm and 20 × 15 cm.

3.3 *Impact of harvesting time and planting distance on gross return, net return and B/C ratio*

The maximum gross returns of €1,235/ha, highest net returns of €759/ha and better Benefit-Cost ratio (2.59) was obtained from plants treated with a harvesting time of 135 DAP (Table 4). At seed maturity, net returns and B/C ratio decreased to €513/ha and 2.08, respectively. The smallest planting distance of 20 × 10 cm resulted in maximum gross returns of €1,178/ha, net returns of €699/ha and a B/C ratio of 2.46.

Table 2: *Impact of harvesting time and planting distance on fresh, dry herbage yield and seed yield of Kalmegh. Values constitute the mean of five measurements on five randomly selected plants per treatment. Seed maturity was reached at harvesting time 170 DAP.*

Treatment	*Dry herbage yield (t/ha)*			*Seed yield (kg/ha)*		
	2005	*2006*	*Mean*	*2005*	*2006*	*Mean*
Harvesting time						
120 DAP	4.51	4.04	4.28	14.2	10.8	11.3
135 DAP	5.45	4.82	5.14	17.3	15.9	16.6
150 DAP	4.11	4.21	4.16	19.4	17.4	18.4
170 DAP	3.44	3.5	3.47	22.6	20.3	21.5
CD at 5 %	0.25	0.19	–	1.3	1.2	–
Planting distance (cm)						
30 × 15 cm	4.09	3.69	3.89	15.9	13.3	14.6
30 × 10 cm	4.26	3.98	4.12	18.3	14.9	16.6
20 × 15 cm	4.52	4.24	4.38	19.1	17.1	18.1
20 × 10 cm	4.65	4.5	4.58	20.4	18.9	19.7
CD at 5 %	0.21	0.17	–	1.5	1.3	–

DAP = days after transplanting, CD = critical difference

Table 3: *Impact of harvesting time and planting distance on andrographolide and iron content and their uptake in Kalmegh plants. Values constitute the mean of five measurements on five randomly selected plants per treatment. Seed maturity was reached at harvesting time 170 DAP.*

Treatment	*Andrographolide content (%)*			*Iron content (mg/100g dry matter)*			*Andrographolide yield (kg/ha)*			*Iron yield (kg/ha)*		
	2005	*2006*	*Mean*	*2005*	*2006*	*Mean*	*2005*	*2006*	*Mean*	*2005*	*2006*	*Mean*
Harvesting time												
120 DAP	2.41	2.46	2.44	23.93	24.91	24.42	108.8	99.4	104.1	1.08	1.01	1.05
135 DAP	2.66	2.59	2.63	20.70	23.62	22.16	145.0	124.8	134.9	1.13	1.14	1.14
150 DAP	2.05	2.30	2.18	17.59	21.23	19.41	84.2	96.8	90.5	0.72	0.89	0.81
170 DAP	1.66	1.80	1.73	16.01	20.48	18.22	57.2	63.0	60.1	0.55	0.71	0.63
CD at 5 %	0.03	0.09	–	0.21	0.70	–	3.8	6.2	–	0.06	0.11	–
Planting distance												
30 × 15 cm	2.31	2.40	2.36	19.20	23.24	21.22	94.4	88.6	91.50	0.80	0.86	0.83
30 × 10 cm	2.26	2.31	2.29	19.67	22.81	21.24	96.2	91.94	94.07	0.84	0.91	0.88
20 × 15 cm	2.13	2.24	2.19	19.58	22.79	21.19	96.3	94.98	95.64	0.90	0.96	0.93
20 × 10 cm	2.07	2.16	2.12	19.78	22.22	21.00	96.3	97.20	96.75	0.93	0.93	0.93
CD at 5 %	0.07	0.06	–	NS	NS	–	NS	5.21	–	0.07	0.05	–

DAP = days after transplanting, CD = critical difference

Table 4: *Impact of harvesting time and planting distance on the economic parameters: gross returns, net returns and B/C ratio of Kalmegh plants. Seed maturity was reached at harvesting time 170 DAP.*

Treatment	*Gross returns (€/ha)*			*Net returns (€/ha)*			*B/C ratio*		
	2005	*2006*	*Mean*	*2005*	*2006*	*Mean*	*2005*	*2006*	*Mean*
Harvesting time									
120 DAP	1,018	1,003	1,011	562	502	532	2.23	2.00	2.12
135 DAP	1,232	1,237	1,235	776	741	759	2.70	2.48	2.59
150 DAP	1,024	1,137	1,081	567	636	602	2.24	2.27	2.26
170 DAP	952	1,032	992	495	531	513	2.09	2.06	2.08
Planting distance									
30 × 15 cm	968	968	968	512	466	489	2.12	1.93	2.03
30 × 10 cm	1,034	1,052	1,043	578	550	564	2.27	2.09	2.18
20 × 1 cm	1,093	1,139	1,116	636	637	637	2.39	2.27	2.33
20 × 10 cm	1,135	1,220	1,178	679	718	699	2.49	2.43	2.46

DAP = days after transplanting

4 Discussion

Yield is the most important character for judging the value of the treatment effects on a crop. But a close understanding of morphological and physiological characters, which fundamentally determine the resultant product, is necessary for extrapolation of results in practical application.

4.1 *Growth and seed yield attributes*

The plant height increased up to seed maturity, however, after 120 days of transplanting, the rate of increase in plant height slowed down. The closer spacing of 20 × 10 cm resulted in taller plants as compared to wider spacing, which might be attributed to the competition among plants for natural resources particularly solar radiation. Similar results have also been reported by Ram *et al.* (1998). A delay in harvesting and the reduction in planting distance led to a decrease in leaf to stem ratio of Kalmegh plants, a result that was also reported by Bhan *et al.* (2006). Vijayaraghavan *et al.* (2005) verified a relationship between a reduced leaf to stem ratio and higher plant density, while plant height was enhanced.

4.2 *Biomass production and seed yield*

The economic yield of crop plant depends upon source-sink relationship as well as on the different components of the source (leaf, shoot, number of branches and dry matter) and of the sink (number of capsule, capsule length, seeds per capsule, seed yield per plant). Harvesting Kalmegh plants at day 135 after transplanting resulted in maximum dry herbage yield and it decreased until seed maturity. The reduction in herbage yield was mainly due to lesser dry matter yield per plant and senescence of leaves at later stages of crop growth (Pandey *et al.*, 2003; Maheshwari *et al.*, 2002; Singh *et al.*, 2011). The seed yield at seed maturity was registered maximum due to more number of capsules per plant and capsule length. A short planting distance of 20 × 10 cm produced significantly more herbage and seed yield compared to the other tested plant spacing. Although the growth in terms of dry matter accumulation and yield attributes of individual Kalmegh plants were higher under small plant density, these treatment plots failed to compensate the loss in yield due to lesser number of plants per unit land area. A higher crop yield in Kalmegh at narrow planting distance has also been reported by Rao *et al.* (1990), Kanjilal *et al.* (2002) and Singh & Singh (2005).

4.3 *Andrographolide and iron content*

The decrease in andrographolide content within plants harvested after 135 DAP might be due to the reason that andrographolide is a compound with a possibility to breakdown within aging plants (Pandey *et al.*, 2003; Bhan *et al.*, 2006; National Research Centre for Medicinal and Aromatic Plants, 2001). To our study similar andrographolide contents were also found by Misra *et al.* (2005) within the Kalmegh variety CIM Megha-High yielding.

The lower iron content within plants harvested after 120 DAP might be due to the fact that iron is immobile in plants. Hence, the older leaves contain a higher amount of iron than the stem. After 120 days of planting, leaves in the lower part of the Kalmegh plant fall on the ground therefore iron content is reduced in the entire plant. The iron content was not significantly affected by different planting distance, though the iron uptake was significantly higher at a plant spacing of 20×10 cm due to overall higher herbage yield. A significantly higher andrographolide content within plants was noticed in plants adjusted at a planting distance of 30×15 cm. Patidar *et al.* (2011) also reported a higher andrographolide content within plants grew at wider spacing of 30 cm than at the spacing of 15 cm, whereas, the yield per hectare was higher at 20×10 cm planting distance due to the higher dry herbage yield per hectare.

4.4 Impact of harvesting time and planting distance on gross returns, net returns and B/C ratio

Harvesting of the crop 135 DAP resulted in the highest net return and B/C ratio due to the highest herbage yield at this stage. The maximum gross returns, net returns and B/C ratio at 20×10 cm planting distance was due to higher herbage and seed yield per hectare.

For a profitable yield and successful cultivation of Kalmegh crop under subtropical climatic conditions, a planting distance of 20×10 cm can be suggested. Harvesting on day 135 after transplanting was found to be ideal for obtaining maximum dry herbage biomass yield as well as high contents of andrographolide and iron.

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