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Biochemical Constituents in Kalmegh (*Andrographis paniculata* Nees.) Under Various Row Spacing's and Nitrogen Levels

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Abstract: *Andrographis paniculata* (King of bitters), commonly known as Kalmegh is used both in Ayurvedic and Unani System of medicines for possess immunological, antibacterial, antiinflammatory, antithrombotic and hepatoprotective properties. Andrographolide is an interesting pharmacophore with anticancer and immunomodulatory activities and hence has the potential to be developed as an anticancer chemotherapeutic agent as well. The study was carried out at the Research Farm of Dusty Acre Area, under the Department of Crop and Herbal Physiology, JNKVV, Jabalpur during kharif 2008-09 to investigate the influence of three row-spacings viz; 15, 30 & 45 cm and five nitrogen levels viz; 0, 40, 60, 80 and 100 kgha^{-1} on physiological traits and productivity in Kalmegh along with magnitudes of biochemical constituents. The experiment was laid out using split plot design with three replications. 30 cm row-spacing, 80kg Nha^{-1} and their combinations were associated with the highest magnitudes of biochemical constituents viz; nitrogen, protein, fat, fibre, ash, phosphorus, potassium and andrographolide in Kalmegh.

Key words: *Andrographis paniculata* · Andrographolide · Nitrogen levels · Row-spacing

INTRODUCTION

Kalmegh, (*Andrographis paniculata* Nees) is a genus of herbs and shrubs, distribution mostly in the tropical and moist regions. It comprises of about 19 plant species found in India and Sri Lanka, certain parts of Thailand and Bangladesh. In India it is grown in Assam, Bihar, Karnataka, Karla, Madhya Pradesh andhra Pradesh and West Bengal. Kalmegh also known as "King of bitter" is one among the prioritized medicinal plants in India and this herb is being used mainly for treating fever, liver disease, diabetes, snake bite etc. The leaf and the whole herb contain the medicinal properties. Growth of a plant is greatly affected by environmental conditions which mainly affects the physiology of plants. Light, which predominantly originates as radiant flux, is unique among the environmental factors acting as a variable and individual organism may be affected by any one of its aspect such as, intensity, color, duration and direction [1]. The *Andrographis paniculata* methanol extract significantly lowered MDA levels and raised the total antioxidant status in urine samples 24 hours after oral administration [2]. Oral administration of the *Andrographis paniculata* aqueous extract significantly enhanced CAT, SOD and GST activities in the liver of lymphoma bearing mice [3]. Moreover, the *Andrographis paniculata* aqueous extract exhibited more antioxidant action than its ethanol extract in terms of free radical scavenging, xanthine oxidase inhibition and anti-lipid peroxidation [4].

The plant contains andrographolide, neo-andrographolide, deoxy-andrographolide and andrographiside. The leaves contain active principle like andrographolide, homo-andrographolide andrographesterol and andrographone. Andrographolide is the major constituent in leaves which is bitter substance [5]. The leaf of the herb was found to contain the highest amount of andrographolide and the seeds contain the lowest [6]. The average andrographolide content varied from 12.44 to 33.52 mg/g in dried leaves [7] and found maximum at 90-120 days after sowing [8]. Raina *et al.* [9] conducted a study and also reported variation of andrographolide content in dry leaves from 1.14% to 2.60% amongst their collections. Sharma *et al.* [10] have also studied variability at morphological, molecular and biochemical level of *Andrographis paniculata*.

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The four flavonoids from *A. paniculata*, namely 7-*O*-methylwogonin, apigenin, onysilin and 3, 4-dicaffeoylquinic acid are anti-atherosclerotic [11].

Andrographolide is colorless, bitter crystalline compound with analgestic, antithrombotic, thrombolytic, hypoglycemic and antipyretic properties. Andrographolide is also attributed with such other activities like liver protection under various experimental conditions. It is mandatory to standardize the cultivation practices for medicinal plants by considering the overexploitation from their reserved forest areas, because of the virtue of its therapeutic values. The plant geometry and nitrogen fertilization have been found to influence various plant physiological processes particularly photosynthates production which provides energy for synthesis of various biochemical constituents. Hence an attempt was made to optimize the effective plant geometry with nitrogen fertilization for production of biochemical constituent's resultant of various metabolic reactions.

MATERIALS AND METHODS

Experiment: The experiment was carried out at the Research Farm, Dusty Acre area, Department of Crop and Herbal Physiology, JNKVV, Jabalpur (M.P.) during the kharif season of 2008-09 to investigate the effect of row spacings and nitrogen levels on biochemical constituents in Kalmegh. The experiment was laid out in a split plot design with three replications. Three row spacings viz; 15 cm (S_1), 30cm (S_2) and 45 cm (S_3) were taken as main treatments, while five nitrogen levels viz; 0 (N_0), 40 kg ha^{-1} (N_1), 60 kg ha^{-1} (N_2), 80 kg ha^{-1} (N_3) and 100 kg ha^{-1} (N_4) as subtreatments. Seeds of kalmegh were sown in the field adopting recommended cultural practices.

Biochemical Estimation: Nitrogen and Protein contents were estimated by AOAC. [12], while Phosphorus by Koing and Johnson [13], Potassium by Black [14], Moisture, Fat, Fiber and Ash by AOAC, [15] and Andrographolide by Rajpal [16] respectively.

RESULTS AND DISCUSSION

Investigations revealed significant differences among various main treatments, subtreatments and their interactions for various biochemical constituents (Table 1). The results revealed that various treatment

Table 1: Biochemical constituents in kalmegh

Main Treatments	Nitrogen (%)	Protein (%)	Phosphorus (%)	Potassium (%)	Fat (%)	Fiber (%)	Ash (%)	Andrographolide (%)
S_1 (15 cm)	0.47	2.99	0.41	1.69	1.80	0.13	0.03	0.054
S_2 (30 cm)	1.23	6.99	0.51	2.14	2.41	0.31	0.17	0.148
S_3 (45 cm)	0.90	6.68	0.48	2.06	2.36	0.24	0.05	0.134
Sem ±	0.04	0.07	0.002	0.015	0.01	0.01	NS	0.0002
C.D. 5%	0.15	0.26	0.007	0.060	0.05	0.03	NS	0.0010
Sub Treatments								
N_0 (Control)	0.22	2.46	0.13	0.82	1.40	0.07	0.02	0.004
N_1 (40 kg ha^{-1})	0.31	3.37	0.16	1.17	2.05	0.18	0.03	0.087
N_2 (60 kg ha^{-1})	0.35	5.42	0.17	1.2	2.34	0.23	0.05	0.131
N_3 (80 kg ha^{-1})	0.90	7.84	0.19	1.38	2.58	0.32	0.17	0.178
N_4 (100 kg ha^{-1})	0.80	8.67	0.19	1.32	2.58	0.34	0.14	0.160
Sem ±	0.04	0.09	0.005	0.027	0.04	0.01	0.04	0.0004
C.D. 5%	0.12	0.26	0.014	0.078	0.13	0.02	0.11	0.0011
Interactions								
S_1N_0	0.34	1.98	0.19	1.31	1.38	0.06	0.02	0.003
S_1N_1	0.47	3.06	0.25	1.67	2.00	0.11	0.03	0.041
S_1N_2	0.49	2.99	0.24	1.52	1.50	0.10	0.03	0.033
S_1N_3	0.54	3.70	0.27	2.08	2.08	0.17	0.04	0.127
S_1N_4	0.50	3.22	0.26	1.86	2.05	0.20	0.04	0.068
S_2N_0	0.42	2.56	0.23	1.43	1.42	0.09	0.03	0.004
S_2N_1	0.61	3.95	0.28	2.18	2.15	0.27	0.04	0.135
S_2N_2	0.61	4.03	0.3	2.2	2.75	0.27	0.05	0.179
S_2N_3	2.45	11.60	0.35	2.48	2.89	0.49	0.39	0.214
S_2N_4	2.05	12.81	0.36	2.39	2.87	0.44	0.33	0.210
S_3N_0	0.35	2.84	0.21	1.36	1.41	0.07	0.03	0.004
S_3N_1	0.50	3.09	0.27	1.97	2.02	0.16	0.03	0.086
S_3N_2	0.66	9.26	0.31	2.27	2.79	0.31	0.08	0.182
S_3N_3	1.53	8.23	0.32	2.32	2.76	0.30	0.06	0.193
S_3N_4	1.47	9.97	0.33	2.36	2.82	0.38	0.05	0.202
Sem ±	0.073	0.15	0.008	0.046	0.076	0.012	NS	0.0007
C.D. 5%	0.21	0.45	0.024	0.134	0.22	0.04	NS	0.0019

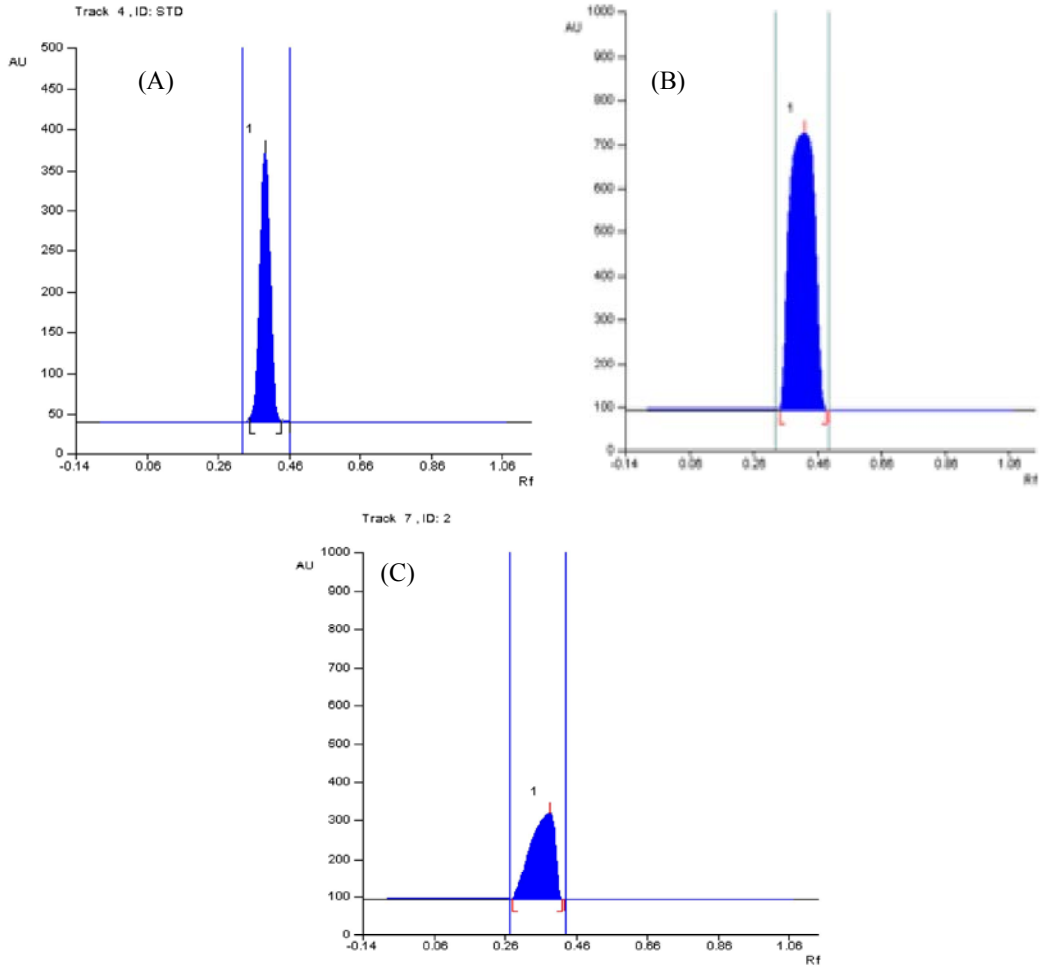

Fig. 1: Chromatogram of (A) Andrographolide standard (B) Interactions of maximum andrographolide content in S_2 (30 cm) row-spacing with N_3 (80 kg ha^{-1}) nitrogen level (C) Interactions of minimum andrographolide content in S_2 (30 cm) and N_0 (Control).

combinations of nitrogen and row spacings affected the nitrogen and protein contents. Significantly maximum nitrogen and protein were recorded in the 30cm row spacing (1.23 and 6.99%) and minimum in 15cm row spacing (0.47 and 2.99%) respectively. Among sub treatments maximum was estimated in 80 kg ha^{-1} (0.90 and 8.67%) and minimum in control (0.22 and 2.46%). In interactions S_2N_3 (2.45 and 12.81%) reported highest and S_1N_0 (0.34 and 1.98%) lowest magnitudes. The rate and duration of protein deposition were essentially independent events, controlled and influenced by different factors [17].

The investigations pertaining to phosphorus and potassium revealed that among main treatments S_2 (0.51 and 2.14%) recorded the significant maximum phosphorus and potassium and S_1 (0.41 and 1.69%) minimum, respectively. Among sub treatments maximum was estimated in N_3 (0.19 and 1.38%) and minimum in N_0 (0.13 and 0.82%). In interactions S_2N_3 (0.36 and 2.48%) reported significantly highest and S_1N_0 (0.19 and 1.31%) was associated with the lowest magnitude.

Nitrogen, protein, phosphorus and potassium contents varied significantly. In main treatments maximum nitrogen, protein, phosphorus and potassium contents was estimated in S_2 (30 cm row spacing) and minimum in S_1 (15 cm row spacing). Among Sub treatments maximum nitrogen and phosphorus contents was obtained in N_3 (80 kg ha^{-1}) and maximum potassium and protein contents was estimated in N_4 (100 kg ha^{-1}). Minimum was noted in N_0 (control) which suggests the direct involvement of nitrogen in increasing protein contents in kalmegh. Among interactions S_2N_3 reported the significant maximum nitrogen & potassium contents and minimum was found in S_1N_0. Treatment combination S_2N_4 had the significant

highest phosphorus and protein contents. Sanjutha, [18] reported the nutrient and andrographolide contents in Kalmegh as (N- 2.88, P- 0.32, K- 3.12% and 1.13%) in addition to the organic and inorganic nutrients.

Fat and fibre analysis indicated that among main treatments S_2 (2.41 and 0.31%) showed the significant maximum fat and fibre contents and S_1 (1.80 and 0.13%) minimum. Among sub treatments N_4 (2.58 and 0.34%) had the significant more fat and fibre over rest of the sub treatments. N_0 (1.40 and 0.07%) was associated with significant lowest value. In interactions S_2N_4 (2.89 and 0.49) reported significantly highest and S_1N_0 (1.38 and 0.06%) was associated with the lowest magnitude. S_2 (0.17 and 0.148%) recorded the maximum ash and andrographolide contents and S_1 (0.03 and 0.054%) showed the minimum. Among sub treatments N_3 (0.17 and 0.178%) possessed the significant more ash and andrographolide and N_0 (0.02 and 0.004%) was associated with significant lowest value. In interactions S_2N_3 (0.39 and 0.214%) had the highest while treatment combination S_1N_0 (0.02 and 0.003%) was associated with the lowest value.

Fibre, fat, ash and andrographolide percent varied significantly in all treatments and interactions. In main treatments maximum content was noted in S_2 (30 cm row spacing) and minimum in main treatment S_1 (15 cm row spacing). In Sub treatments N_4 (100 kg ha^{-1}) noted the significant maximum fibre and fat percent while ash and andrographolide content were maximum in N_3 (80 kg kg ha^{-1}) over rest of the sub treatments. Minimum content was recorded in N_0 (control). In interactions S_2N_4 had the significant highest fibre and fat percent and minimum was found in S_1N_0. Treatment combination S_2N_3 had the significant highest ash and andrographolide contents, respectively (Figure 1).

Andrographolide is an important biochemical constituent used to protect liver [5] and also used as an antipyretic [19]. The absolute recovery was found to be in the range of 97.4 - 99.4 %. The interday CV was 0.4 to 1.4 and intraday 1.05 to 2.8 % [20]. The Andrographolide continued to accumulate in plant leaves and the alkaloid contents varied at various growth stages significantly. A pattern of progressive increase in andrographollide contents in fresh as well as dry leaves was noted with increase in crop age. Minimum content of andrographollide was estimated at 30 DAS and maximum at 120 DAS. Planting of Kalmegh in second fortnight of July and harvesting after 120 DAS was found ideal for obtaining higher herbage yield as well as andrographolide content [8]. Growing of kalmegh strain CIM-AP-3 and planting at a closer spacing of 30 X 15 cm accommodating 2,22,222 plant ha^{-1} is suggested for obtaining maximum dry biomass and diterpenoid lactones (andrographolide and neoandrographolide) yield the subtropical climate of North India [21]

CONCLUSION

Thus the investigations revealed that 30 cm row-spacing, 80 kg ha^{-1} N level and their combinations possessed the highest magnitudes of biochemical constituents viz; nitrogen, protein, fat, fibre, ash, phosphorus, potassium and andrographolide percentage in kalmegh.

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