



RESEARCH ARTICLE

Influence of growth regulators on herb yield and andrographolide content in kalmegh (*Andrographis paniculata* Wall.Ex.Nees.) var. CIM Megha

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Abstract

The experiment was to study the effect of growth regulators on herb yield and andrographolide content in kalmegh. Kalmegh is referred to as Maha tita in Sanskrit, Rice bitters in the West Indies and King of bitters in England. Its characteristic bitterness is mainly attributed to a secondary metabolite, the diterpenoid lactone andrographolide, which is present at about 3 % in the leaves and 2 % in the stems. In the current investigation, considerable variation in yield and quality traits was recorded across the 13 treatments at College of Horticulture, Rajendranagar, Sri Konda Laxman Telangana Horticultural University. Different concentrations (100, 150 and 200 mg L⁻¹) of plant growth regulators (gibberlic acid (GA₃), naphthalene acetic acid (NAA), cycocel and benzy amino purine) were prepared by dissolving respective growth hormone powders on 2–5 drops of ethyl alcohol and plants of individual treatments were sprayed with the respective plant growth regulators at 30, 60 and 90 days after transplanting (DAT), while plots were treated with distilled water. The yield attributes revealed that treatment T₂ (GA₃ at 150 ppm) produced the highest values for fresh herb yield per plot (5.01 kg), dry herb yield per plot (2.10 kg), fresh herb yield per hectare (97.08 q), dry herb yield per hectare (40.27 q), number of capsules per plant (780.21) and seed yield per plot (8.90 g), with all differences being statistically significant. With respect to quality parameters, T₆ (NAA at 200 ppm) resulted in the maximum andrographolide content (2.69 %). However, the highest andrographolide yield (106.28 kg/ha) and drying percentage (34.68 %) were obtained under T₂ (GA₃ at 150 ppm). The improvement in yield and quality can be attributed to better source-sink relationships, enhanced biomass accumulation and stimulation of secondary metabolite biosynthesis by growth regulators.

Keywords: andrographolide; growth regulators; herb yield; kalmegh

Introduction

Kalmegh (*Andrographis paniculata* Wall. ex Nees), a member of the family Acanthaceae, is native to India and has been an integral component of traditional Indian medicinal systems since ancient times. In Sanskrit it is referred to as Maha tita, while it is commonly known as rice bitters in the West Indies and King of bitters in England (1). The plant is recognised by various vernacular names across different regions, including Create (English), Bhuinimb (Sanskrit), Kirayat (Hindi), Kalmegh (Bengali) and Nela Vemu (Telugu). It is considered one of the bitterest medicinal plants, characterised as an erect or sometimes recumbent annual herb, which may persist as a perennial under managed conditions (2).

The pronounced bitterness of kalmegh is primarily attributed to the presence of a diterpenoid lactone andrographolide, occurring at approximately 3 % in the leaves and 2 % in the stems. In addition to this, the plant contains other bioactive constituents such

as neo-andrographolide, a non-bitter compound of pharmacological importance (3).

Andrographolide, an alkaloid-like secondary metabolite, plays a crucial role in plant defence mechanisms against herbivores and other biotic stresses. Beyond its ecological function, it holds significant value for human use, particularly in the development of medicines, pharmaceuticals, agrochemicals and biopesticides. A large proportion of modern therapeutic agents are derived from or are chemically modified versions of naturally occurring compounds. The expanding commercial relevance of secondary metabolites in recent years has therefore intensified scientific interest in their biosynthesis and regulation reported in kalmegh (4,5).

Growth regulators directly influence growth of the plant. These are chemical substances and when applied in small amounts, they bring rapid changes in the phenotypes of the plant and influence the plant growth, right from seed germination to senescence either

by enhancing or by stimulating the natural growth regulatory system. Plant growth substances are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit, seed development, ultimately enhance the productivity of crops and played an important role in secondary metabolism governed in plants (6). Therefore, the present study aims to evaluate the influence of different plant growth regulators on both the yield and andrographolide accumulation in kalmegh var. CIM Megha, with a view to improving its pharmaceutical and agronomic performance under field conditions.

Materials and Methods

The study was conducted during kharif season of 2021 at PG Research Block, College of Horticulture, Rajendranagar, Sri Konda Laxman Telangana Horticultural University, Telangana, India. The experimental farm is located in a sub-tropical zone at a latitude of 17.32° N, longitude of 78.40° E and an altitude of 536 m above mean sea level. The soil of the site was classified as sandy loam and physical properties of the soil comprised 72.3 % sand, 7.2 % silt and 21.6 % clay, as determined by the Bouyoucos hydrometer method. The crop was raised under irrigated conditions and irrigation was applied at regular intervals based on crop requirements and prevailing weather conditions to maintain optimum soil moisture throughout the growth period. The design was randomized block design (RBD) comprising 13 treatments, each replicated. The plot size is 3 × 2 m, plant spacing 30 × 20 cm and number of plants plot⁻¹ = 100. The treatments included different concentrations of plant growth regulators: T₁: gibberlic acid (GA₃) at 100 ppm, T₂: GA₃ at 150 ppm, T₃: GA₃ at 200 ppm; T₄: naphthalene acetic acid (NAA) at 100 ppm, T₅: NAA at 150 ppm, T₆: NAA at 200 ppm; T₇: cycocel at 100 ppm, T₈: cycocel at 150 ppm, T₉: cycocel at 200 ppm; T₁₀: benzyl amino purine (BAP) at 100 ppm, T₁₁: BAP at 150 ppm, T₁₂: BAP at 200 ppm; and T₁₃: control, which received 100 % recommended dose of fertilisers (RDF: 87 : 75 : 50 kg NPK/ha) along with distilled water spray. Farmyard manure (FYM) at 10 t/ha, was incorporated during the final ploughing before transplanting.

The different concentrations (100, 150 and 200 mg L⁻¹) of plant growth regulator (GA₃, NAA, cycocel and benzy amino purine) were prepared by dissolving respective growth hormone powders on 2–5 drops of ethyl alcohol or alkaline solutions separately and made the final volume of 1000 mL with double distilled water. The plants of individual treatments were sprayed with respective plant growth regulator at 30, 60 and 90 days after transplanting (DAT), while control plots were treated with distilled water.

Yield parameters

The recorded data on yield parameters like fresh herb yield per plot (kg), dry herb yield per plot (kg), fresh herb yield per hectare (q), fresh herb yield per hectare (q), number of capsules per plant, seed yield per plot (g) were statistically analysed.

Statistical analysis

The experimental data collected on various yield components of plant were subjected to Fisher's method of "Analysis of variance" (ANOVA). Wherever *F*-test was significant for comparison amongst the treatments means an appropriate value of critical differences (CD) was worked out. Otherwise against CD values abbreviation NS (Non-significant) was indicated. All the data were

analysed and the results are presented and discussed at a probability level of 0.05 %.

Andrographolide content (%)

Andrographolide content was analysed by using the standard procedure (7).

(a) Sample preparation for estimation of andrographolide (%)

One gram of finely powdered plant sample was extracted with 50 mL of methanol by refluxing on a water bath for 1 hr. The extract was filtered and the residue was subjected to two additional reflux extractions, each lasting 1 hr, using fresh methanol (50 mL). All filtrates were pooled and concentrated to dryness under reduced pressure. The resulting residue was reconstituted in 25 mL of methanol and filtered through a 0.45 µm nylon membrane filter before being transferred into HPLC vials for analysis.

(b) Calibration

A standard stock solution was prepared by dissolving 2 mg of andrographolide standard in methanol in a 5 mL volumetric flask. The stock solution was stored at 4 °C and remained stable for at least 30 days, as confirmed by repeat analysis. Calibration curves were generated using standard concentrations ranging from 2.0–40.0 µg/mL, over which the detector response showed good linearity. Data acquisition and processing were carried out using Millennium 32 software (Milford, MA, USA).

(c) Analytical method

All samples were analysed in triplicate (n = 3) to ensure repeatability of the method. The results are expressed as mean ± standard deviation (SD). Instrumental repeatability was assessed by repeated injection (n = 6) of the same standard solution.

Limit of detection (LOD) and limit of quantification (LOQ):

LOD and LOQ were determined based on the standard deviation of the response (σ) and the slope (S) of the calibration curve using the following equations:

$$\text{LOD} = 3.3 \times (\sigma/S)$$

$$\text{LOQ} = 10 \times (\sigma/S)$$

The LOD and LOQ for andrographolide were found to be approximately 0.2–0.5 µg mL⁻¹ and 0.6–1.5 µg mL⁻¹ respectively (values may be adjusted based on actual data).

Recovery studies were performed by spiking pre-analysed samples with known amounts of andrographolide standard at three levels (80 %, 100 % and 120 %). The percentage recovery ranged from 95–105 %, indicating good accuracy of the method.

Quantitative analysis of andrographolide was performed using a Waters HPLC system equipped with a 2996 photodiode array detector (Waters Pvt. Ltd.). Chromatographic separation was achieved on an XBridge™ C18 column (4.6 × 250 mm, 5.0 µm particle size). The mobile phase consisted of water (A) and 1 : 1 mixture of methanol and reagent alcohol (B), delivered using a gradient program starting from 35A : 65B and progressing to 45A : 55B over 25 min. Each analysis was followed by a 5 min wash with 100 % B and a 10 min equilibration period. The column temperature was maintained at 25 °C and detection was carried out at 225 nm with a constant flow rate. Identification of andrographolide was confirmed by spiking samples with an authentic standard and comparing both retention times and UV spectra.

Andrographolide content (%) =

$$\frac{\text{Calculated conc. of sample in } \mu\text{g/mL} \times \text{dilution factor}}{1000}$$

Andrographolide yield (kg/ha): The andrographolide yield was calculated by using the formula

$$\text{Andrographolide yield (kg/ha)} = \frac{\text{Andrographolide content (\%)} \times \text{Dry herb yield (kg/ha)}}{100}$$

Drying percentage (%): The drying percentage (%) was calculated by using the formula

$$\text{Drying percentage (\%)} = \frac{\text{Weight of shade dried herbs}}{\text{Weight of fresh herbs}} \times 100$$

Results and Discussion

Fresh and dry herb yield per plot

The results presented in Table 1 indicated that maximum fresh herb yield per plot (5.01 kg) was recorded with T₂ (GA₃ at 150 ppm). And it was statistically on par with T₆ (NAA at 200 ppm) (4.66 kg), followed by T₇ (Cycocel at 100 ppm) (4.24 kg). In contrast, minimum fresh herb yield per plot (3.02 kg) was recorded in the control treatment (T₁₃: 100 % RDF + distilled water spray). The similar trend was observed for dry herb yield per plot, where T₂ (GA₃ at 150 ppm) produced the maximum yield (2.10 kg), statistically comparable with T₆ (NAA at 200 ppm) (1.97 kg) and followed by T₇ (Cycocel at 100 ppm) (1.90 kg). The minimum dry herb yield per plot (1.24 kg) was again noted under the control treatment.

The observed enhancement in fresh and dry leaf biomass may be attributed to increased accumulation of biomolecules associated with cell division and cell enlargement, leading to thicker and larger leaves. Gibberellic acid (GA₃) is well documented for its role in stimulating cell division and elongation, which in turn positively influences growth attributes such as leaf number, leaf area and leaf area index. An increase in leaf area enhances photosynthetic efficiency, resulting in greater dry matter accumulation and overall biomass production. Comparable results have been reported earlier in lavender (8) and in kalmegh (9).

Fresh and dry herb yield per hectare

With respect to yield on a hectare basis, T₂ (GA₃ at 150 ppm) recorded the maximum fresh herb yield (97.08 q/ha). And it was statistically at par with T₆ (NAA at 200 ppm) (91.64 q/ha), followed by T₇ (Cycocel at 100 ppm) (83.25 q/ha). The minimum fresh herb yield per hectare (57.07 q/ha) was observed in the control. A similar pattern was evident for dry herb yield per hectare, wherein T₂ (GA₃ at 150 ppm) registered the highest yield (40.27 q/ha), remaining comparable with T₆ (NAA at 200 ppm) (38.51 q/ha) and followed by T₇ (Cycocel at 100 ppm) (36.48 q/ha). The control treatment recorded the minimum dry herb yield (24.90 q/ha).

The increase in fresh and dry biomass of *Andrographis paniculata* (Burm.f.) Nees under plant growth regulator treatments may be due to enhanced synthesis and accumulation of primary metabolites such as carbohydrates, reducing sugars, proteins and starch. These biochemical improvements likely promoted overall vegetative growth through better source-sink relationships. Gibberellic acid is known to stimulate growth by mobilising stored starch, thereby supporting root and shoot elongation. Similar positive effects of growth regulators on growth and productivity have been documented in *Asparagus racemosus* Willd. (10) and in kalmegh by earlier workers (5, 9).

Number of capsules per plant

The data presented in Table 1 revealed that significantly maximum number of capsules per plant (780.21) was observed with T₂ (GA₃ at 150 ppm) and was at par with T₆ (NAA at 200 ppm) (758.14), T₃ (GA₃ at 200 ppm) (730.32) followed by T₁ (GA₃ at 100 ppm) (710.43). The minimum number of capsules per plant (420.09) was recorded with T₁₃ Control (100 % RDF + distilled water spray). Increase in the number of capsules per plant, which resulted from improved development of both primary and secondary branches. Application of plant growth regulators promoted vigorous vegetative growth, leading to enhanced photosynthetic activity and efficient translocation of assimilates from source tissues (leaves) to sink organs (capsules and seeds).

Seed yield per plot (g)

The data presented in Table 1 showed that T₂ (GA₃ at 150 ppm) produced the maximum seed yield per plot (8.90 g). And it was

Table 1. Effect of growth regulators on fresh herb yield/plot (kg) and dry herb yield/plot (kg), fresh herb yield/ha (q), dry herb yield/ha (q), number of capsules per plant and seed yield per plot (g) of kalmegh

Treatments	Fresh herb yield/plot (kg)	Dry herb yield/plot (kg)	Fresh herb yield/ha (q)	Dry herb yield/ha (q)	Number of capsules per plant	Seed yield/plot (g)
T ₁ : GA ₃ at 100 ppm	4.05	1.82	79.57	34.21	710.43	8.04
T ₂ : GA ₃ at 150 ppm	5.01	2.10	97.08	40.27	780.21	8.90
T ₃ : GA ₃ at 200 ppm	4.13	1.88	80.94	36.31	730.32	8.21
T ₄ : NAA at 100 ppm	3.59	1.62	70.65	32.17	618.10	6.96
T ₅ : NAA at 150 ppm	3.75	1.65	72.80	31.53	640.62	7.04
T ₆ : NAA at 200 ppm	4.66	1.97	91.64	38.51	758.14	8.43
T ₇ : Cycocel at 100 ppm	4.24	1.90	83.25	36.48	695.26	7.89
T ₈ : Cycocel at 150 ppm	3.87	1.68	75.48	32.33	656.71	7.23
T ₉ : Cycocel at 200 ppm	3.42	1.55	68.83	29.72	528.85	6.64
T ₁₀ : Benzylaminopurine at 100ppm	3.53	1.58	69.49	30.34	580.19	6.80
T ₁₁ : Benzylaminopurine at 150ppm	3.91	1.73	76.97	33.35	686.15	7.65
T ₁₂ : Benzylaminopurine at 200ppm	3.36	1.51	66.86	29.25	502.02	6.38
T ₁₃ : Control (100 % RDF kg NPK/ha + Distilled water spray)	3.02	1.24	57.07	24.90	420.09	5.10
S.Em±	0.15	0.06	2.17	0.91	18.60	0.21
C.D.at 5 %	0.44	0.18	6.34	2.65	54.28	0.62

GA₃: gibberellic acid; NAA: naphthalene acetic acid; RDF: recommended dose of fertilisers; ppm: parts per million; NPK: nitrogen, phosphorus and potassium; S.Em±: Standard error of mean; C.D. ($p = 0.05$): critical difference at the 5 % level of significance.

statistically on par with T₆ (NAA at 200 ppm) (8.43 g), followed by T₃ (GA₃ at 200 ppm) (8.21 g). The minimum seed yield per plot (5.10 g) was recorded in the control treatment (T₁₃: 100 % RDF + distilled water spray). The enhanced seed yield under GA₃ application may be attributed to an increase in the number of capsules per plant, which resulted from improved development of both primary and secondary branches. Application of plant growth regulators promoted vigorous vegetative growth, leading to enhanced photosynthetic activity and efficient translocation of assimilates from source tissues (leaves) to sink organs (capsules and seeds). This improved source-sink relationship likely facilitated greater accumulation of photoassimilates in reproductive structures, thereby increasing seed yield. Similar trends have been reported earlier in kasturbhendi (11) and coriander (12). Beyond the optimal concentration, increases in parameters (like biomass, seed yield, or secondary metabolites) often plateau or decline, indicating toxicity or inefficiency.

Quality analysis

The data pertaining to the quality parameters viz. andrographolide content (%) andrographolide yield (kg/ha) and drying percentage (%) were recorded as influenced growth regulators are presented in the Tables 2 and Fig. 1. that significantly maximum andrographolide content (2.69 %) was observed with T₆ (NAA at 200 ppm) and was at par with T₂ (GA₃ at 150 ppm) (2.64 %), T₇ (Cycocel at 100 ppm) (2.59 %) followed by T₃ (GA₃ at 200 ppm) (2.51 %). The minimum andrographolide content (1.73 %) was recorded with T₁₃

control (100 % RDF + distilled water spray) (Fig. 2).

PGRs play an important role in mitigating the stress, releasing and increasing the secondary metabolites which were blocked in the vacuole of the plant.

Effect of NAA and GA₃ on andrographolide content may be due to enhanced biosynthetic pathway of andrographolide. It was noted that an increase in growth, biomass and chlorophyll and leaf area may contribute to produce the secondary metabolites. These all are much correlated to our findings and conformity which support that the improvement or enhancing the quality and quantity can be achieved with the applications of growth regulators. These results were very similar with early results in ajwain (13) and in kalmegh (4, 5).

Foliar spray of PGRs on ajwain, showed a progressive increase in the content of thymol in the essential oil as compared to the control (13). Significant increase was reported in morphine yield of opium per plant (104 %) using foliar application of GA₃ and triacontanol in combination in opium poppy (14). These results were very similar with the early findings in kalmegh (4, 5, 7).

Andrographolide yield (kg/ha)

The data presented in Table 2 revealed that significantly maximum andrographolide yield (106.28 kg/ha) was observed with T₂ (GA₃ at 200 ppm) followed by T₆ (NAA at 200 ppm) (103.08 kg) and T₇ (Cycocel at 100 ppm) (94.40 kg/ha). The minimum andrographolide yield (42.10 kg/ha) was recorded with T₁₃ control (100 % RDF + distilled water spray).

Table 2. Effect of growth regulators on andrographolide percentage (%) andrographolide yield/ha (kg) and drying percentage (%) of kalmegh

Treatments	Andrographolide percentage (%)	Andrographolide yield/ha (kg)	Drying percentage (%)
T ₁ : GA ₃ at 100 ppm	2.46	88.83	33.12
T ₂ : GA ₃ at 150 ppm	2.64	106.28	34.68
T ₃ : GA ₃ at 200 ppm	2.51	93.62	32.16
T ₄ : NAA at 100 ppm	2.08	66.85	33.82
T ₅ : NAA at 150 ppm	2.11	69.06	32.35
T ₆ : NAA at 200 ppm	2.69	103.08	33.94
T ₇ : Cycocel at 100 ppm	2.59	94.40	34.22
T ₈ : Cycocel at 150 ppm	2.32	77.32	33.31
T ₉ : Cycocel at 200 ppm	2.01	61.80	32.01
T ₁₀ : Benzylaminopurine at 100 ppm	2.06	64.56	33.67
T ₁₁ : Benzylaminopurine at 150 ppm	2.44	83.74	33.15
T ₁₂ : Benzylaminopurine at 200 ppm	2.01	61.00	32.14
T ₁₃ : Control (100 % RDF kg NPK/ha + Distilled water spray)	1.73	42.10	31.76
S.Em±	0.04	1.15	0.46
C.D.at 5%	0.11	3.36	1.35

GA₃: gibberellic acid; NAA: naphthalene acetic acid; RDF: recommended dose of fertilisers; ppm: parts per million; NPK: nitrogen, phosphorus and potassium; S.Em±: Standard Error of Mean; C.D. ($p = 0.05$): critical difference at the 5 % level of significance.

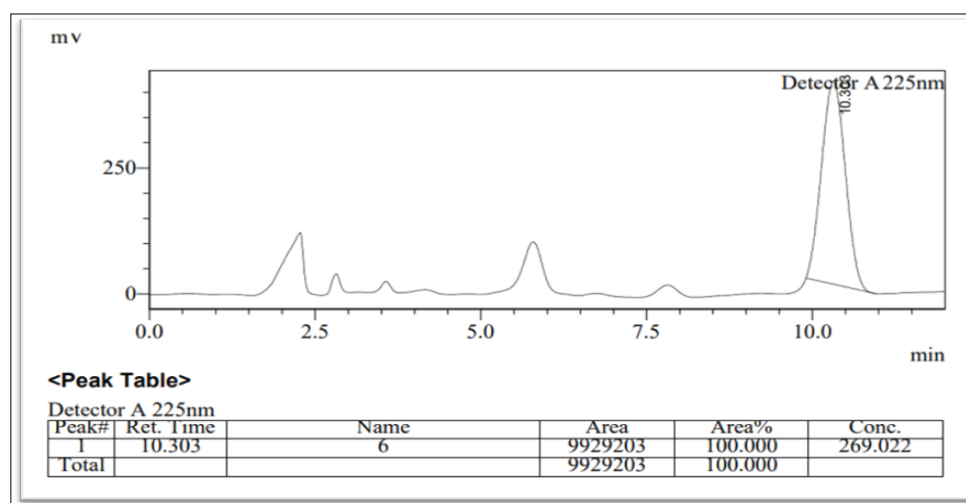


Fig. 1. High-performance liquid chromatography chromatogram for methanolic extract of T₆.

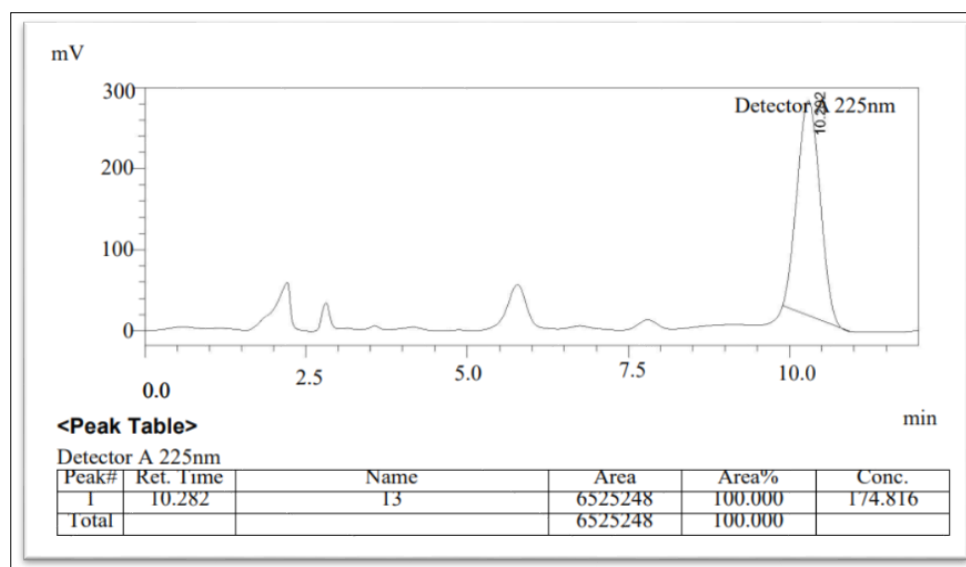


Fig. 2. High-performance liquid chromatography chromatogram for methanolic extract of T₁₃.

The maximum andrographolide yield can be ascribed to the maximum dry herb yield/ha (4027 kg/ha) coupled with fairly higher andrographolide content (2.64 %) due to application of GA₃ at 150 ppm. This result was in accordance with previous findings in fennel (15).

Drying percentage (%)

The data presented in Table 2 revealed that significantly maximum drying percentage (34.68 %) was observed with T₂ (GA₃ at 150 ppm) and was at par with, T₇ (Cycocel at 100 ppm) (34.22 %), T₆ (NAA at 200 ppm) (33.94 %), T₄ (NAA at 100 ppm) (33.82 %), T₁₀ (Benzylaminopurine at 100 ppm) (33.67 %) followed by T₈ (Cycocel at 150 ppm) (33.31 %). The minimum drying percentage (31.76 %) was recorded with T₁₃ Control (100 % RDF + distilled water spray).

The maximum drying percentage obtained by the application of growth regulators may be due to the better sink efficacy. The involvement of growth regulating substances with sink efficiency in influencing the drying percentage and yield potential was reported in kalmegh (9). It is evident from the data that control has registered significantly lower yields and drying percentage than growth regulator sprays which indirectly manipulate the morphological, physiological and growth parameters in fodder maize (16).

Conclusion

Results from the present investigation clearly demonstrated that foliar application of plant growth regulators were significantly influenced growth, yield and quality attributes of kalmegh. The treatment gibberellic acid (GA₃) at 150 ppm (T₂) was showed most significant results with respect to yield of both fresh and dry herb, seed yield andrographolide yield and drying percentage, while naphthalene acetic acid (NAA) at 200 ppm recorded highest andrographolide content. Overall, the study concluded that judicious application of both GA₃ and NAA emerged as one of viable agronomic strategy for improving both productivity and medicinal quality of *Andrographis paniculata* under field conditions.

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Authors' contributions

AKK and GS conceptualised the research and designed the experiments. AKK and BNK contributed the experimental materials. DA carried out the field and laboratory experiments. DA and GS performed the statistical analysis of the data. DA, PG and GS prepared the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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