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Effect of different levels of nutrient on productivity, and diterpene lactone content of Kalmegh [*Andrographis paniculata* (Burm. f.) Wall. ex Nees]

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Abstract

An investigation was conducted at the research farm in Doddaballapura, India, during the years 2023–24 to examine the impact of varying nutrient levels on the productivity and diterpene lactone content of Kalmegh [*Andrographis paniculata* (Burm. f.) Wall. ex Nees]. The findings indicated that significant increases in plant height (51.41 cm), branches per plant (24.43), fresh herb yield per hectare (9212.93 kg), and dry herb yield per hectare (2982.70 kg) were achieved with the application of 100% Poultry manure + 2% AMC (T₄), comparable to (T₈) 50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya at 125 DAS. Moreover, the highest levels of total andrographolide (2.89%) and total diterpene lactone content (3.01%) were observed in treatment (T₇), which involved the application of 50% Vermicompost, 50% Neem cake, 2% AMC, and 3% Panchagavya, at 85 days after sowing, similar to treatment (T₆) 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya, when compared to the control group.

Keywords: Phytochemical, growth, yield, poultry manure, FYM, neem cake

Introduction

Kalmegh [*Andrographis paniculata* (Burm. f.) Wall. ex Nees] belongs to the family ' Acanthaceae. It is an annual herb that can grow from 30-100 cm tall. The stem is distinctly four angular, leaves are opposite, simple, and narrowly egg-shaped to lance shaped. Flowers is auxiliary, and terminal racemes or panicles combined into a pyramidal inflorescence. The crop is distributed in tropical region of India, Hong Kong, Thailand, Brunel, Singapore and Bangladesh. It can be found in a variety of habitats, such as plains, hill sides, coastlines and cultivated in farms and waste lands. The climatic requirement for the plant is hot and humid conditions with ample sunshine. Depending upon area of cultivation, harvesting is done in October to November. The flowering and fruiting are done throughout the year, especially from May to August (Katakya and Handique, 2010) [12].

It is also known as “Kalmegha,” is one among the 32 prioritized medicinal plants of India by National Medicinal Plants Board (Verma *et al.* 2019) [33]. Its estimated consumption in India is over 250 tons per year (Sharma *et al.* 2008) [27]. It is one of the most widely used medicinal plants in *Ayurvedic* formulations, (Katta *et al.* 2007) [13], conventionally used as blood purifier, tonic, febrifuge, etc., In addition, it is also used in noncodified traditional practices to treat jaundice, malaria, tuberculosis, etc. (Parrotta, 2001) [23]. It is reported that most of the activities of *A. paniculata* are attributed to its diterpenoid contents namely andrographolide (A1), neoandrographolide (A2), and 14-deoxy-11,12-didehydroandrographolide (A3) (Chao and Lin, 2010; Jarukamjorn and Nemoto, 2008; Mishra *et al.* 2007 and Niranjan *et al.* 2010) [5, 10, 18, 21]. A1 has been shown to possess anti-inflammatory, anticancer and cardioprotective properties (Kumar *et al.* 2004; Chao *et al.* 2011 and Woo *et al.* 2008) [14, 4, 34]. A2 has been reported for anti-malarial and hepatoprotective activities (Kapil *et al.* 1993 and Misra *et al.* 1992) [11, 19]. The A3 has been reported as a hypotensive agent and for antiplatelet activity (Thisoda *et al.* 2006 and Yoopan *et al.* 2007) [32, 35].

The whole herb is the source of several diterpenoids of which the bitter water-soluble lactone andrographolide is important and is distributed all over the plant body in different proportions. The leaves contain the maximum (2.5%) andrographolide content while the stem contains lesser amount (2.0%) of this active principle. In addition, the plant is an important source of flavonoids, sesquiterpenes, phenylpropanoids. The roots contain the flavonoids andrographin, panicotin, aplgenin-4', 7-dimethyl ether, mono-*o*-methyl within and hydroxy- 7,8,2',3'-trimethoxy flavone and β -sitosterol. In Japan several flavonoids named as andrographidin A, B, C, D, E, F have been extracted from the roots and whose content varies from 0.015 to 0.15 percent. Sesquiterpenes like paniculide A, B and C have been reported to be obtained from the callus tissues. Phenyl propanoid eugenol has also been recorded in the aerial parts of the plant. The diterpenoid and sesquiterpenoid compounds occurring in *A. paniculata* have been referred to as paniculides and andrographolides. Besides flavonoids, caffeic acid chlorogenic acid, caffeoylquinic acids are also present in this plant (Farooqui and Sreeramu, 2004) [8].

Studies have demonstrated that prolonged utilization of inadequate doses of nutrients in imbalanced proportions has led to significant depletion of nutrient reserves in Indian soils, resulting in multiple nutrient deficiencies and a decrease in crop productivity. The utilization of inorganic fertilizers has been attributed to more than a 50 percent increase in crop yield, but it reduces the crop quality attributes, which lead to poor market price to the grower. There is widespread recognition that solely employing organic manure application can represent a comprehensive approach to achieving sustainable agriculture, as it is nature-based, environmentally friendly, and ensures resource conservation for the future. Embracing the use of organic manures to fulfill crop nutrient requirements is anticipated to become increasingly necessary in the years ahead, as these manures not only enhance yield but also sustain the physical, chemical, and biological properties of soil. However, organic sources suitable for soil incorporation are becoming scarce. The essential elements encapsulated in organic manures undergo gradual mineralization, thus becoming available to crops, thereby promoting growth, enhancing nutrient content, and improving soil fertility. Organic manures such as Farmyard manure, Vermicompost, Panchagavya, Neem cake, and Poultry manure are regarded as repositories of various essential nutrients crucial for plant growth. Although organic manures alone may not fully meet crop nutrient requirements, they play a vital role. Farmyard manure comprises a decomposed mixture of dung, urine from farm animals, along with litter, and residual material from roughages or fodder fed to cattle. Poultry manure, also known as chicken manure, constitutes organic waste primarily derived from the feces and urine of chickens. Poultry bird excreta ferment rapidly. Research indicates that poultry manure can reduce soil bulk density, enhance water retention capacity, increase organic matter content, oxygen diffusion rate, and promote soil aggregate stability. The combined application of plant nutrients from chemical fertilizers and organic manures has been shown to foster greater growth and nutrient levels compared to their individual application. Consequently, this study was undertaken to investigate the impact of varying nutrient levels on the productivity and diterpene lactone content of Kalmegh [*Andrographis paniculata* (Burm. f.) Wall. ex Nees].

Material and Methods

An experiment was conducted during rabi, 2023 at the research

farm in Doddaballapura, India, which is geographically situated at 13.29°N latitude and 77.53°E longitude at an altitude of 900 m above mean sea level, under the eastern dry zone of Karnataka. The soil of the experimental site was sandy loamy in texture, neutral in soil pH (7.20), with an optimum EC level of 0.08 dS/m, low in organic carbon, available nitrogen, and medium in available phosphorous and potassium. The experiment was laid out in a Randomized Block Design with three replications and nine treatments. *viz.*, T₁-100% NPK RDF (75:75:50 kg/ha) + 100% Micronutrient (Super six 25kg/acre), T₂-100% FYM (25 MT/ha)+ 2% AMC, T₃-100% Vermicompost (15 MT/ha) + 2% AMC, T₄-100% Poultry manure (10 MT/ha) + 2% AMC, T₅-50% NPK RDF(37.5:37.5:25 kg/ha) + 50% Neem cake (300 kg/ha) + 2% AMC, T₆-50% FYM (12.5 MT/ha) + 50% Neem cake (300 kg/ha) + 2% AMC + 3% Panchagavya (foliar and drenching application), T₇-50%Vermicompost (7.5 MT/ha) + 50% Neem cake (300 kg/ha) + 2% AMC + 3% Panchagavya (foliar and drenching application), T₈-50% Poultry manure (5 MT/ha) + 50% FYM (12.5 MT/ha) + 50% Neem cake (300 kg/ha) + 2% AMC + 3% Panchagavya (foliar and drenching application) and T₉-Control. The quantity of conventional and organic manures was applied as per the design of the research experiment. Kalmegh seeds, variety CIM-Megha, were procured from the CIMAP Regional Station, Bengaluru, and seeds were sown in a research experiment plot with a net plot size of 8 m² and a spacing of 30 x 20 cm. Light irrigation was given at the time of sowing; subsequent irrigation was given twice a week based on the dry spell. Plant samples of total andrographolide were collected to record dry matter production at 85, 105, and 125 days after sowing. Necessary plant protection measures were taken to safeguard the crop from pests and diseases. Five plants from each plot were randomly chosen and tagged in order to record observations. The crop was weeded at regular intervals to keep the plot weed-free at 20, 50, and 80 days after sowing. Fresh herbage yield was estimated by harvesting the crop with a sickle 15 cm above the ground level at 85, 105, and 125 days after sowing and immediately weighted for obtaining plot yield. After recording the fresh herbage yield, the plants were placed under shade for 5–6 days and obtained a dry herbage yield.

Statistical Analysis

The data generated from the experiment were analyzed using OPSTATS of the statistical package (Sheoran *et al.*, 1998) [28]. Analysis of variance (ANOVA) was performed using OPSTATS ANOVA procedure. Means were separated using Fisher's protected least significant difference (LSD) test at a probability level of $p < 0.05$.

Detail steps of preparation of Panchagavya

1. **Day 1-3:**
 - Step 1:** Mix Cow Dung (7 kg) and Cow Ghee (1 kg) thoroughly.
 - Step 2:** Keep the mixture for 3 days, mixing both morning and evening.
2. **Day 4-18:**
 - Step 3:** After 3 days, add Cow Urine (10 liters) and Water (10 liters) to the mixture.
 - Step 4:** Keep this mixture for 15 days, mixing both morning and evening.
3. **Day 19-30:**
 - Step 5:** After 15 days, add the following ingredients to the mixture:
Cow Milk (3 liters)

Cow Curd (2 liters)
Tender Coconut Water (3 liters)
Jaggery (3 kg)
Well ripened Bananas (12 nos.)

Step 6: Keep this final mixture for an additional 12 days, ensuring regular mixing both morning and evening.

4. Day 31:

Step 7: Panchagavya is ready for use.

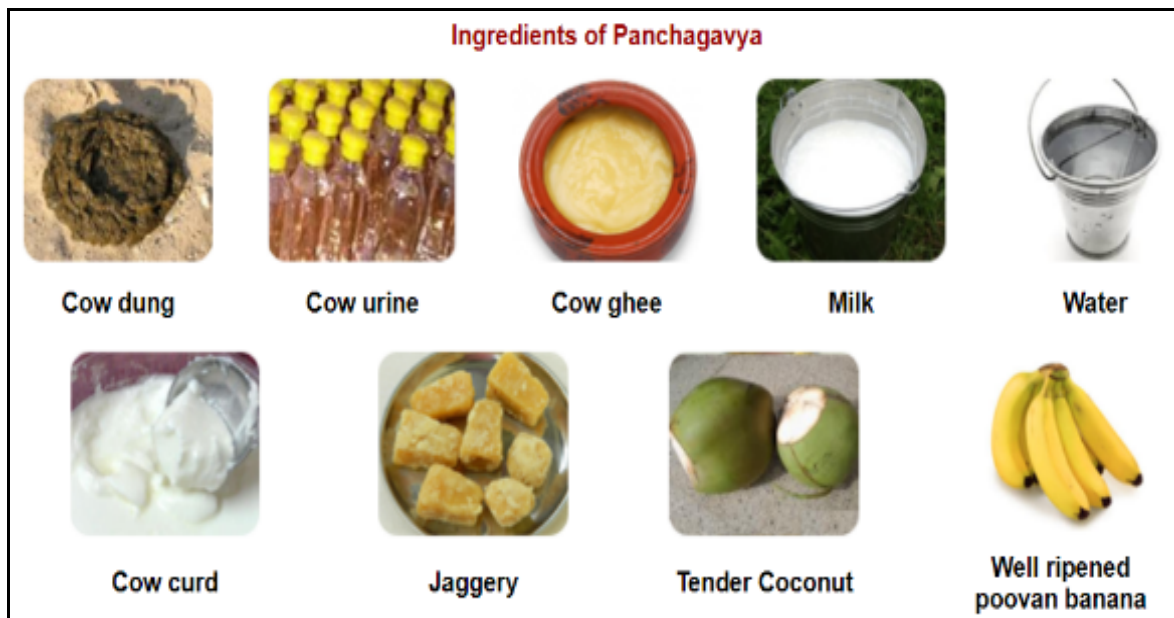


Plate 1: Ingredients of Panchagavya

HPLC Instrumentation and conditions

Preparation of Standard and Sample Solutions

Stock solutions of Andrographolide and the sample were prepared in methanol at approximate concentrations of 1.0 mg/mL and 40.0 mg/mL, respectively. Standard and sample solutions for analysis were then prepared by diluting these stock solutions with acetonitrile to obtain approximate concentrations of 0.5 mg/mL for Andrographolide and 4.0 mg/mL for the sample.

Instrumentation

The HPLC method development and validation was done by using Waters HPLC e2695 Separation Module with 2998 PDA Detector, empower software and Reliant C-18 4.6-mm x 25 cm;5- μ m packing L1 column.

Chromatographic conditions

UV detection was employed at 223 nm, gradient elution (solvent A & B) was used at a flow rate of 1.5 ml/min. and injection volume was set to 20 μ l. The total run time of standard was 45 min. Where Solvent A is Potassium dihydrogen phosphate solution and B is degassed Acetonitrile. The method follows the guidelines established by the USP. The retention times of peaks are compared to the retention time of Andrographolide to identify different diterpene lactones. Andrographolide has a relative retention time of 1.00, which serves as a reference for calculating the relative retention times of other diterpene lactones provided in Table 1.

Table 1: The relative retention times of the different diterpene lactones

Analyte	Relative Retention time
Andrographolide	1.00
Neoandrographolide	1.16
4-Deoxy-11,12-didehydroandrographolide	1.31
Andrograpanin	1.50

Results and Discussion

Morphological attributes

Plant height (cm)

The combined application of conventional, organic manures, and bio-stimulants in various combinations was found to have significant effects on the plant height of the Kalmegh at different stages of growth period. Among the different treatments experimented with, the application of 100% Poultry manure + 2% AMC (T₄) registered a maximum plant height of 25.73 cm, 47.13 cm, and 51.41 cm at 85, 105, and 125 DAS, respectively. However, the treatments T₅ (22.40 cm); T₈ (43.80 cm), T₇ (43.63 cm), T₅ (43.20 cm) and T₇ (48.50 cm), T₂ (48.28 cm), T₁ (48.11 cm), were on par with each other respectively. The lowest plant height of 17.80 cm, 38.87 cm, and 42.79 cm at 85, 105, and 125 DAS, respectively, were recorded in control (T₉). The increased plant height might also be due to the application of poultry manure, which contains high nitrogen content and was made available to plants through mineralization to stimulate plant growth, increase uptake of primary nutrients, and fast movements of photosynthates within the plant system (Sathya and Maheshwari, 2017) [26].

Number of branches per plant

The evaluation of the data revealed that the application of organic manures and bio-stimulant significantly differed in the number of branches per plant at all stages of crop growth (Table 2). Among the various combinations experimented, the maximum number of branches per plant recorded was 12.73 and 19.13 at 85, 105 DAS respectively in the treatment T₈ (50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya), which is on par with treatments T₄, T₅, T₆ and T₄, T₆, T₇ (12.67, 12.40, 12.00 and 18.53, 18.20, 18.20) at 85 and 105 DAS, respectively). However, at 125 DAS, the maximum number of branches per plant (24.43) was noticed in T₄ (100% Poultry manure + 2% AMC) which is on par with

treatment T₇, T₂, T₃. The lowest number of branches in the plant was recorded in treatment T₉ (10.27, 16.33, and 18.51 at 85, 105, and 125 DAS, respectively). The poultry manure applied contains most macro and micro-nutrients which has a significant effect on the number of branches per plant. This finding is in conformity with the work of Alabi (2006) [1]. Poultry manure and biofertilizers had a significant effect on the number of branches per plant as it activated vegetative growth. These results showed that the proper dose of nitrogen and phosphorus was influential on the number of branches per plant at the final harvesting stage. A similar result was reported by Hemalatha and Suresh (2012) [9]. Moreover, AMC and Panchagavya were applied in solution form to the plant as foliar spray is more readily available and established a greater number of branches. This phenomenon was also reported by Singh *et al.*, (2011) [30] and Sunil Kumar *et al.*, (2011) [31].

Number of leaves per plant

The effect of application of conventional, organic manures, and bio-stimulants in different combinations on the number of leaves of Kalmegh at different crop growths was significant, as shown in Table 2. However, treatment T₄ (100% Poultry manure + 2% AMC) registered the maximum number of leaves per plant (37.93) at 85 DAS, which is on par with T₈, T₅, and T₆. The maximum number of leaves per plant was 49.27 and 62.49 at 105 and 125 DAS, respectively, in T₈ (50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya), which was on par with T₄, T₅ and followed by T₄, T₅ at 105 and 125 DAS. The lowest number of leaves (23, 34.93, and 43.41) at 85, 105, and 125 DAS, respectively, were recorded in control (T₉). An important factor in a plant's ability to create more biomass through photosynthetic efficiency is the number of leaves and branches on the plant. Particularly organic manures are a highly efficient organic plant growth input that stimulates the growth of numerous plant species (Kumaresan *et al.*, 2023) [16].

Stem girth (mm)

The data shown in Table 2 indicates that the stem girth of the Kalmegh at various crop growth stages was significantly impacted by the integrated application of conventional, organic, and bio-stimulants in various combinations. However, treatment T₄ (100% Poultry manure + 2% AMC) registered the maximum girth of stem (3.51 mm) at 85 DAS, which is followed by T₂, T₈, T₆, T₇, T₃ and T₅ compared to control, and the maximum stem girth 4.80 mm and 5.79 mm at 105 and 125 DAS was registered in T₈ (50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya) which was on par with T₄, T₂ and T₄, T₆ respectively. The lowest stem girth was found in the T₉ control at stages of growth (2.30 mm, 3.23 mm, and 4.44 mm at 85, 105, and 125 DAS, respectively). The percentage of growth was increased for plant height and number of branches at different growth stages, but number of leaves and stem girth increased in initial growth stage and further declined till harvest due to maturation and leaf senescence. This might be due to balanced application of organic manures in mixture with inorganic fertilizers could be ascribed to enhancement in soil health and raised availability of both macro and micro nutrients to the crop or it might be because of fact that nutrient released from both organic and inorganic fertilizers would have resulted in the extended nutrient availability which in turn increased the translocation of photosynthates and optimum vegetative growth, according to Naleena *et al.* (2019) [20]; Cheena *et al.* (2020) [6].

Yield Traits

Fresh and dry yield per plant (g)

The data on fresh and dry yield per plant are presented in Table 3, it was found that the use of conventional fertilizers, organic manures, and biofertilizers in various combinations had a significant effect on the fresh and dry yield per plant of Kalmegh. In comparison to the control at 85, 105, and 125 DAS, the maximum fresh weight per plant (14.89, 26.50, and 55.28 g) and dry weight per plant (4.49, 15.33, and 17.90 g) were recorded in treatment T₄ (100% Poultry manure + 2% AMC), which is on par to T₈ (50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya).

Fresh and dry yield per plot (kg)

According to findings, it was determined that the integrated application of conventional fertilizers, organic manures, and biofertilizers in different combinations had significant effects on the fresh and dry yield per plot of Kalmegh. The maximum fresh weight per plot (1.98, 3.52 and 7.35 kg) and dry weight per plot (0.60, 2.04 and 2.38 kg) were recorded in treatment T₄-100% Poultry manure + 2% AMC as compared to control at 85, 105 and 125 DAS respectively which is on par with T₈ (50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya).

Fresh and dry yield per hectare (kg)

The data pertaining to fresh and dry yield per hectare represented in Table 3 and Fig. 1. It is found that the fresh and dry yield per hectare of Kalmegh was significantly influenced by the integrated application of conventional fertilizers, organic manures and biofertilizers in different combinations. The maximum fresh weight per hectare (2481.47, 4416.65 and 9212.93 kg/ha) and dry weight per hectare (747.59, 2555.55 and 2982.70 kg/ha) were recorded in treatment T₄-100% Poultry manure + 2% AMC as compared to control at 85, 105 and 125 DAS respectively which is on par with T₈ (50% Poultry manure + 50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya). This is attributed to increases in plant growth and yields brought about by the accumulation of secondary metabolites in plants and the continuous availability of all plant nutrients and enzymes needed for crop growth. These results are consistent with the Kalmegh research studied by Arpana and Bagyaraj (2007) [2]. The higher yield attributable characteristics by higher supply of nutrients, a suitable physical and biological environment, with raised organic carbon in the soil leading to greater root activity and nutrient uptake could account for the superior yield achieved in Kalmegh because of the integration of conventional fertilizers and organic manures. Application of organic manures along with conventional are enhanced of soil physical environment might be helped in better development of root growth, reported by Bhargavi *et al.*, 2017 [3]. Similar results were observed by Kumar *et al.* (2018) [15] and Nishchitha *et al.* (2018) [22]. Therefore, the highest herbage yield noted in the present experiment with the applying of organic fertilizers and bio-stimulants can be attributed to mineralization and mobilization of nutrients which increase plant nutrient absorption. This assists in the development of more plant tissues, resulting in abundant vegetative growth, these results are in conformity with the findings of Sanjutra *et al.* (2008) [25], Ramesh *et al.* (2011) [24], (Mishra and Jain., 2013) [17] and Shwetha *et al.* (2021) [29].

Phytoconstituent Traits**Total andrographolide and diterpene lactone (%)**

The effect of integrated application of conventional, organic manures, and bio-stimulants in different combinations on the total diterpene andrographolide lactone of Kalmegh at different harvesting stages was significant, as shown in Table 4 and Fig. 2 and 4. However, treatment T₇ (50% Vermicompost, 50% Neem cake, 2% AMC, and 3% Panchagavya) registered the highest total andrographolide and total diterpene lactone content (2.89% and 3.01%) and (2.35% and 2.57%) at 85 and 105 DAS, which is followed by T₆ (50% FYM + 50% Neem cake + 2%

AMC+ 3% Panchagavya) and highest total andrographolide and total diterpene lactone content (2.35% and 2.39%) at 125 DAS was registered in T₅ (50% NPK RDF + 50% Neem cake + 2% AMC followed by T₆ (50% FYM + 50% Neem cake + 2% AMC+ 3% Panchagavya) compared to control. It was observed that the phytoconstituent levels in *Andrographis paniculata* significantly increased between 85 to 100 days after sowing, followed by a decline after 120 days. Therefore, harvesting Kalmegh at 85 days after sowing will result in peak phytoconstituent levels. The Similar results were also noticed by Deshmukh *et al.* (2018)^[7] in Kalmegh.

Table 2: Effect of different combination of nutrient on growth parameter of Kalmegh

Treatments	85 DAS				105 DAS				125 DAS			
	PH (cm)	BPP	NLP	SG (mm)	PH (cm)	BPP	NLP	SG (mm)	PH (cm)	BPP	NLP	SG (mm)
T ₁ 100% RDF + 100% Micronutrient	19.67	11.60	30.00	2.64	42.60	17.40	38.40	3.48	48.11	18.81	45.43	5.07
T ₂ 100% FYM + 2% AMC	19.00	11.07	31.40	3.42	39.56	17.80	39.07	4.26	48.28	21.43	46.45	5.41
T ₃ 100% Vermicompost + 2% AMC	21.17	11.47	32.47	2.80	43.60	17.20	39.33	4.06	47.23	20.68	44.42	4.69
T ₄ 100% Poultry Manure + 2% AMC	25.73	12.67	37.93	3.51	47.13	18.53	40.60	4.64	51.41	24.43	57.56	5.61
T ₅ 50% NPK RDF + 50% Neem cake + 2% AMC	22.40	12.40	34.27	2.79	43.20	18.00	42.67	3.55	46.28	22.60	53.51	5.10
T ₆ 50% FYM + 50% Neem cake + 2% AMC+ 3% PNG	21.50	12.00	33.47	3.13	41.40	18.20	39.33	4.07	46.69	23.07	46.43	5.53
T ₇ 50% Vermicompost + 50% Neem cake + 2% AMC+ 3% PNG	20.61	11.60	31.87	2.93	43.63	18.20	39.60	3.86	48.50	23.65	49.50	4.68
T ₈ 50% Poultry manure + 50% FYM + 50% Neem cake +2% AMC+ 3% PNG	20.84	12.73	36.27	3.40	43.80	19.13	49.27	4.80	46.88	21.68	62.49	5.79
T ₉ Control	17.80	10.27	23.00	2.30	38.87	16.33	34.93	3.23	42.79	18.51	43.41	4.44
Mean	20.97	11.76	32.30	2.99	42.64	17.87	40.36	3.99	47.35	21.65	49.91	5.15
S. Em±	1.40	0.47	3.71	0.45	1.52	0.83	2.91	0.41	1.17	0.97	0.12	0.27
CD at 5%	4.18	1.42	7.26	1.34	4.56	2.51	8.72	1.22	3.51	2.90	0.36	0.81

PNG- Panchagavya; AMC- Arka microbial consortium; PH: Plant height (cm); BBP-Branches per plant; NLP- Number leaves per plant; SG- Stem girth (mm)

Table 3: Effect of different combination of nutrient on yield parameter of Kalmegh

85 Days after sowing						
Treatments	FWPP (g)	DWPP (g)	FW (kg/plot)	DW (kg/plot)	FW (kg/ha)	DW (kg/ha)
T ₁ 100% RDF + 100% Micronutrient	7.28	3.07	0.97	0.41	1212.59	512.22
T ₂ 100% FYM + 2% AMC	12.22	3.26	1.63	0.43	2037.03	543.89
T ₃ 100% Vermicompost + 2% AMC	8.67	3.37	1.15	0.45	1444.44	562.41
T ₄ 100% Poultry Manure + 2% AMC	14.89	4.49	1.98	0.60	2481.47	747.59
T ₅ 50% NPK RDF + 50% Neem cake + 2% AMC	12.67	3.93	1.68	0.52	2111.10	655.00
T ₆ 50% FYM + 50% Neem cake + 2% AMC+ 3% PNG	9.11	3.60	1.21	0.48	1518.51	599.44
T ₇ 50% Vermicompost + 50% Neem cake + 2% AMC+ 3% PNG	10.89	3.60	1.45	0.48	1814.81	599.44
T ₈ 50% Poultry manure + 50% FYM + 50% Neem cake +2% AMC+ 3% PNG	13.00	4.15	1.73	0.55	2166.66	692.03
T ₉ Control	7.19	2.93	0.96	0.39	1197.96	488.33
Mean	10.66	3.6	1.42	0.48	1776.06	600.04
S. Em±	1.32	0.35	0.20	0.05	219.82	57.93
CD at 5%	3.95	1.04	0.59	0.14	659.04	173.67
105 Days after sowing						
Treatments	FWPP (g)	DWPP (g)	FW (kg/plot)	DW (kg/plot)	FW (kg/ha)	DW (kg/ha)
T ₁ 100% RDF + 100% Micronutrient	15.67	6.61	2.08	0.88	2611.10	1101.85
T ₂ 100% FYM + 2% AMC	21.17	7.83	2.82	1.04	3527.76	1305.55
T ₃ 100% Vermicompost + 2% AMC	16.67	7.50	2.22	1.00	2777.77	1250.00
T ₄ 100% Poultry Manure + 2% AMC	26.50	15.33	3.52	2.04	4416.65	2555.55
T ₅ 50% NPK RDF + 50% Neem cake + 2% AMC	19.17	7.39	2.55	0.98	3194.43	1231.48
T ₆ 50% FYM + 50% Neem cake + 2% AMC+ 3% PNG	17.67	7.67	2.35	1.02	2944.43	1277.77
T ₇ 50% Vermicompost + 50% Neem cake + 2% AMC+ 3% PNG	14.67	7.67	1.95	1.02	2444.43	1277.77
T ₈ 50% Poultry manure + 50% FYM + 50% Neem cake +2% AMC+ 3% PNG	25.17	14.83	3.35	1.97	4194.43	2472.21
T ₉ Control	12.56	5.00	1.67	0.67	2093.32	833.33
Mean	18.80	8.87	2.50	1.18	3133.81	1478.39
S. Em±	2.65	1.45	0.37	0.21	690.30	242.25
CD at 5%	7.96	4.36	1.11	0.63	2069.61	726.29
125 Days after sowing						
Treatments	FWPP (g)	DWPP (g)	FW (kg/plot)	DW (kg/plot)	FW (kg/ha)	DW (kg/ha)

T ₁	100% RDF + 100% Micronutrient	40.22	13.15	5.35	1.75	6703.68	2191.35
T ₂	100% FYM + 2% AMC	38.11	11.50	5.07	1.53	6351.83	1916.66
T ₃	100% Vermicompost + 2% AMC	38.56	10.41	5.13	1.38	6425.90	1734.50
T ₄	100% Poultry Manure + 2% AMC	55.28	17.90	7.35	2.38	9212.93	2982.70
T ₅	50% NPK RDF + 50% Neem cake + 2% AMC	33.11	11.66	4.40	1.55	5518.50	1944.07
T ₆	50% FYM + 50% Neem cake + 2% AMC+ 3% PNG	40.78	12.67	5.42	1.68	6796.27	2111.10
T ₇	50% Vermicompost + 50% Neem cake + 2% AMC+ 3% PNG	38.22	12.51	5.08	1.66	6370.34	2084.56
T ₈	50% Poultry manure + 50% FYM + 50% Neem cake +2% AMC+ 3% PNG	52.00	15.65	6.92	2.08	8666.63	2608.69
T ₉	Control	30.89	9.82	4.11	1.31	5148.13	1636.10
	Mean	40.80	12.81	5.43	1.70	6799.36	2134.42
	S. Em±	4.14	1.44	0.63	0.20	690.30	239.45
	CD at 5%	12.42	4.31	1.90	0.60	2069.61	717.92

PNG- Panchagavya; AMC- Arka microbial consortium FWPP-Fresh weight per plant; DWPP; Dry weight per plant

Table 4: Effect of different combination of nutrient and harvesting stages of total diterpene lactone content in Kalmegh

Treatments		85 DAS		105 DAS		125 DAS	
		TA (%)	TD (%)	TA (%)	TD (%)	TA (%)	TD (%)
T ₁	100% RDF + 100% Micronutrient	2.61	2.74	1.40	1.77	1.74	1.97
T ₂	100% FYM + 2% AMC	2.69	2.87	2.02	2.32	2.04	2.23
T ₃	100% Vermicompost + 2% AMC	2.34	2.49	1.95	2.00	1.80	2.02
T ₄	100% Poultry Manure + 2% AMC	1.96	2.66	2.15	2.35	1.95	2.20
T ₅	50% NPK RDF + 50% Neem cake + 2% AMC	2.88	2.74	1.96	2.26	2.35	2.39
T ₆	50% FYM + 50% Neem cake + 2% AMC+ 3% PNG	2.85	3.00	2.09	2.37	2.13	2.17
T ₇	50% Vermicompost + 50% Neem cake + 2% AMC+ 3% PNG	2.89	3.01	2.25	2.57	1.78	1.83
T ₈	50% Poultry manure + 50% FYM + 50% Neem cake +2% AMC+ 3% PNG	2.52	2.66	1.86	2.16	1.80	1.84
T ₉	Control	1.96	2.04	1.36	1.60	1.76	1.79
	Mean	2.52	2.69	1.89	2.15	1.93	2.05
	S. Em±	0.01	0.01	0.02	0.01	0.00	0.00
	CD at 5%	0.02	0.02	0.05	0.02	0.01	0.01

PNG- Panchagavya; AMC- Arka microbial consortium; TA- Total andrographolide; TD- Total diterpene lactone

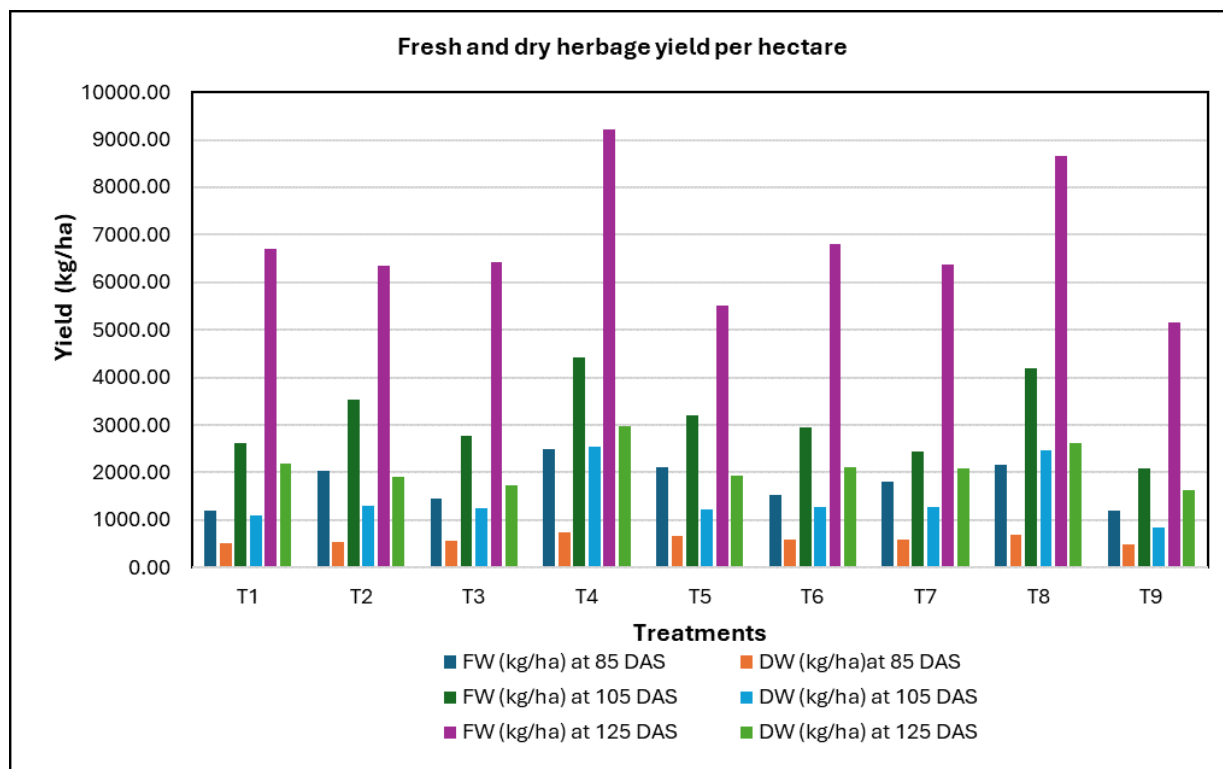


Fig 1: Fresh and dry herbage yield per hectare at different harvesting stages of Kalmegh

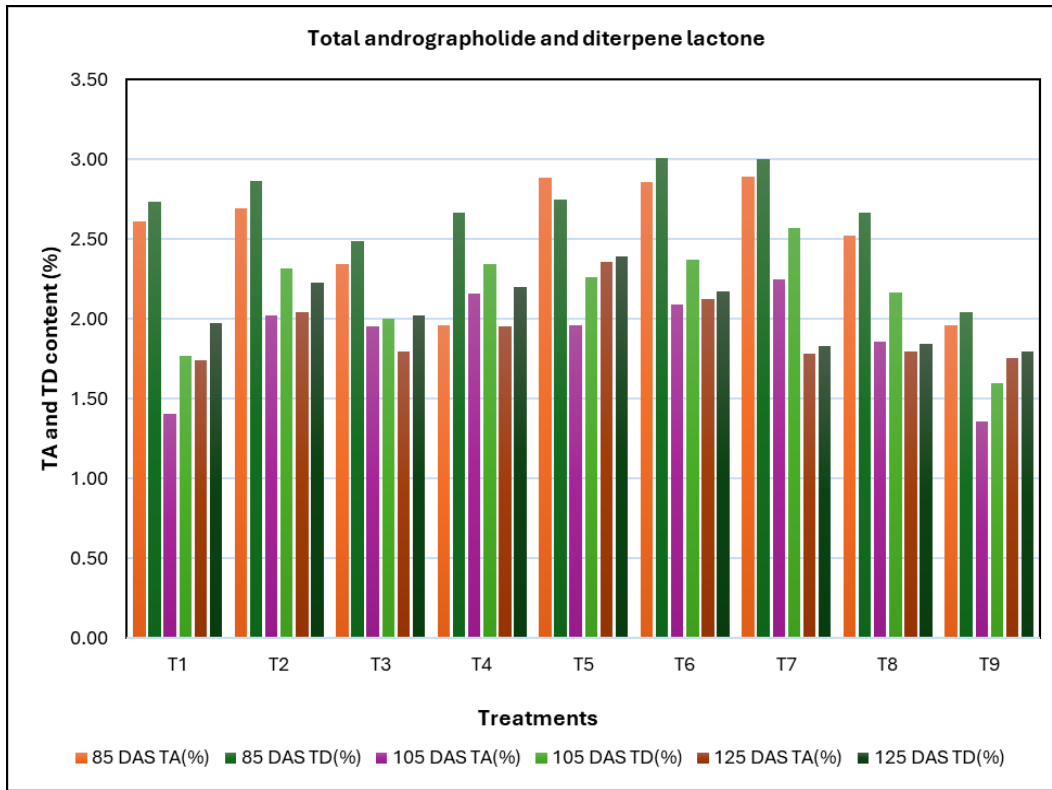


Fig 2: Total andrographolide and diterpene lactone at different harvesting stages of Kalmegh

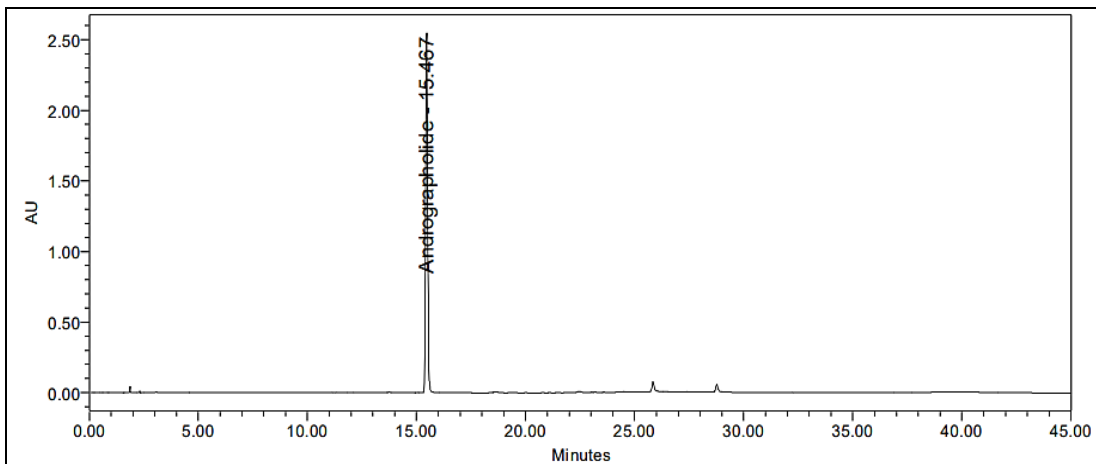


Fig 3: Chromatographic representation of Andrographolide standard

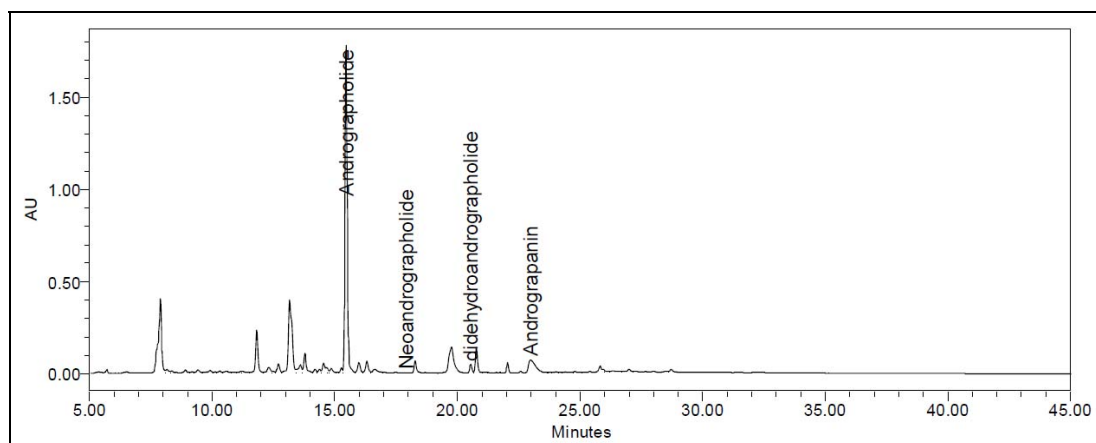


Fig 4: Chromatographic representation of Total Diterpenoid content

Conclusion

From this investigation, the highest growth and herbage yield was obtained in the treatment combination T₄ and T₈ at different harvesting stages, and application of T₆ (50% FYM + 50% Neem cake + 2% AMC + 3% Panchagavya) showed the highest total diterpene lactone and total andrographolide content at 85 days after sowing. Maximum productivity and total diterpene content were significant noticed in treatment combination of T₆ (50% FYM (12.5 MT/ha) + 50% Neem cake (300 kg/ha) + 2% AMC + 3% Panchagavya) and T₅-(50% NPK RDF (37.5:37.5:25 kg/ha) + 50% Neem cake (300 kg/ha) + 2% AMC). From the above results, it may be stated that the cultivation of organic compost and the use of bio-stimulant along with neem cake in an integrated manner is beneficial in improving the productivity and phytoconstituent level of Kalmegh.

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