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# Nutrient impact: Unveiling the growth and biochemical dynamics of Kalmegh (Andrographis paniculata Nees.) through zinc and iron nutrition

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#### Abstract

A field experiment at Kitturu Rani Channamma College of Horticulture, Arabhavi, assessed the impact of Zinc and Iron on Kalmegh (*Andrographis paniculata* Nees.) growth and biochemical attributes in the northern dry zone of Karnataka during 2019-2020. With ten treatments replicated thrice in an RCBD, observations at 30, 60, 90 days after planting, and harvest showed significant increases in plant height (19.72 cm to 40.88 cm), branches (17.43 to 28.15), north-south spread (8.79 cm to 29.00 cm), and eastwest spread (11.32 cm to 32.86 cm) with Zinc (0.25%) and Iron (0.25%) foliar application alongside RDF. Chlorophyll-a, chlorophyll-b, and total chlorophyll content (1.37, 1.71, 3.08 mg g<sup>-1</sup> of tissue) peaked with Zinc (0.25%) and Feso4 (0.25%) foliar application alongside RDF (T<sub>10</sub>). These findings highlight the positive influence of Zinc and Iron nutrition on Kalmegh, offering valuable insights for agricultural practices in similar agro-ecological contexts and enriching our understanding of nutrient management in horticulture.

Keywords: Kalmegh, RDF, FeSO4, ZnSO4 and Chlorophyll

# Introduction

Kalmegh, scientifically known as *Andrographis paniculata* Nees, is part of the Andrographis genus, comprising twenty-six species, primarily found in South India. Commonly referred to as 'kalmegh,' this herbaceous plant belongs to the Acanthaceae family and is indigenous to India and Sri Lanka (Kirtikar and Basu, 1975)<sup>[9]</sup>. The plant's fresh and dried leaves, as well as the extracted juice, are recognized as official drugs in the Indian pharmacopoeia, employed in traditional medicine across China, India, and Southeast Asia.

Characterized by its erect herbaceous structure, Kalmegh grows to a height of 30–90 cm, featuring petiolated lanceolate leaves with whitish flowers borne on spreading racemes. The fruit, known as a capsule, measures 2 cm in length, contains several brownish-yellow seeds, and holds significance as the 17<sup>th</sup> prioritized crop among 32 medicinal plants in India (Kala *et al.*, 2007; Anon., 2016; Sharma *et al.*, 2009) <sup>[7, 2, 15]</sup>. Traditionally regarded as a "cold property" herb, Kalmegh is utilized to alleviate body heat during fevers and eliminate toxins. Its therapeutic properties stem from an enzyme induction mechanism, offering anti-inflammatory, antibiotic, anti-malarial, anti-hepatitic, and anti-pyretic benefits. Notably, its immune-stimulating properties aid in treating various ailments, including dysentery, diarrhea, cholera, fever, diabetes, bronchitis, hypertension, piles, and gonorrhea. Research has documented the plant's anti-HIV properties attributed to the presence of the active constituent Andrographolide (Sajwan, 2008) <sup>[13]</sup>. Key constituents like andrographolide-A, andrographolide-B, and related diterpenoids contribute to immune stimulation, anti-inflammatory effects, fertility enhancement, liver protection, and bile secretion stimulation (Kataky and Handique, 2010) <sup>[8]</sup>.

In the realm of nutrient management, the importance of micronutrients cannot be overstated. Zinc, a vital element, plays a pivotal role in photosynthesis, auxin biosynthesis, protein synthesis, enzyme system activation, cell division, cell elongation, and chlorophyll formation in plants (Deepak, 2010)<sup>[3]</sup>.

Additionally, Iron is indispensable for photosynthesis, participating in in chlorophyll synthesis and ensuring the maintenance of chloroplast structure and function (Gyana and Sunit, 2015)<sup>[6]</sup>. Understanding and optimizing nutrient management are critical factors influencing both the quantity and quality of harvested produce.

# **Materials and Methods**

A field experiment titled "Standardization of Nutrient Requirement in Kalmegh (*Andrographis paniculata* Nees.)" was conducted at the Department of Plantation, Spices, Medicinal, and Aromatic Crops, Kittur Rani Channamma College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalkot, Karnataka. The experimental site's soil composition consisted of sandy loam. The study, carried out during the rabi season of 2019 and 2020, adopted a Randomized Complete Block Design with three replications. The chosen variety for experimentation was kalmegh var. CIM-Megha, developed by the Central Institute of Medicinal and Aromatic Plants, Lucknow. This particular variety is distinguished by its early maturation, tolerance to iron deficiency, and superior performance in terms of dry biomass yield and andrographolide content.

# **Treatment Details**



Healthy and uniformly sized seedlings, aged forty-five days, were meticulously chosen and transplanted into the main field. The transplantation process involved placing the seedlings at a specific spacing of 30 cm between rows and 15 cm between individual plants. This careful arrangement aims to optimize the utilization of available space and promote optimal growth and development of the transplanted seedlings in the main field.

#### **Observation recorded Plant height (cm)**

Plant height measurements were conducted by measuring from the ground level to the tip of each plant, utilizing a meter scale. The average height value was then computed based on measurements taken from five randomly selected plants. The recorded data was expressed in centimeters (cm), providing a representative and averaged assessment of the plants' vertical growth. This systematic approach ensures accuracy and reliability in capturing the overall height characteristics of the observed plant specimens.

# Number of branches

The total count of primary branches per plant was determined by observing and recording the number from the specifically tagged plants. Subsequently, the average number of primary branches per plant was calculated, providing a representative value that reflects the typical branching pattern of the observed plants. This methodical approach ensures a comprehensive understanding of the plants' branching characteristics, contributing to a more accurate and standardized assessment.

# Plant spread (E-W, N-S) (cm)

The East-West and North-South spread of the plants were meticulously measured and recorded. Subsequently, the average spread values were calculated, providing a representative measure of the plants' overall lateral growth. These averages, expressed in centimeters, offer valuable insights into the spatial development and coverage of the observed plants in both the East-West and North-South orientations. This systematic recording and averaging process enhance the precision of the assessment of the plants' horizontal expansion.

# **Biochemical parameters**

# Chlorophyll content (mg g<sup>-1</sup> of tissue)

The determination of total chlorophyll, chlorophyll "a," and chlorophyll "b" content was carried out using the DMSO method. The procedure involved harvesting the third fully expanded leaf from the top, which was then cut into small pieces. A known weight of these leaf pieces was placed in a test tube containing 7.0 ml of dimethyl sulphoxide (DMSO). The test tube was incubated at 65 °C for 30 minutes. Subsequently, the leaf residue was separated by decanting the solution, and the final volume was adjusted to 10 ml with DMSO. The absorbance of the extract was measured at 645, 663, and 440 nm using a UV-vis Spectrophotometer (Elico, SL - 159), and a blank was run using DMSO. The total chlorophyll, chlorophyll "a," and chlorophyll "b" contents were then calculated employing the formulas provided by Sadasivam and Manicham (1996)<sup>[12]</sup>. The results were expressed in milligrams per gram of fresh weight, providing a quantitative assessment of the chlorophyll concentration in the plant tissue extract.

mg chlorophyll a per g tissue = 
$$12.7 (A663) - 2.69 (A645) \times \frac{v}{1000 \times w \times a}$$
  
mg chlorophyll b per g tissue =  $22.9 (A645) - 4.68 (A663) \times \frac{v}{1000 \times w \times a}$   
mg total chlorophyll per g tissue =  $20.2 (A645) + 8.02 (A663) \frac{v}{1000 \times w \times a}$ 

Where, A = absorbance at specific wavelengths, v = final volume of chlorophyll extracted (ml)

w = fresh weight of the sample (g) and a = path length of light (1 cm)

### **Results and Discussion**

The combined data analysis of plant height, influenced by the application of RDF, Iron, and Zinc on Kalmegh during the years 2019 and 2020, revealed noteworthy outcomes. The most significant plant height, at 30, 60, 90 days after planting, and at harvest (19.72 cm, 27.71 cm, 36.23 cm, and 40.88 cm, respectively), was achieved through the foliar application of Iron and Zinc (0.25%) along with RDF ( $T_{10}$ ). Following closely was  $T_7$ , involving soil application of Iron and Zinc (20 & 25 kg ha<sup>-1</sup>) with RDF, recording plant heights of 18.72 cm, 27.47 cm, 35.75 cm, and 39.30 cm at the respective stages. Conversely, the minimum plant height was observed in  $T_1$  (RDF) at all stages of crop growth (15.28 cm, 24.60 cm, 31.02 cm, and 35.33 cm at 30, 60, 90 days after planting, and at harvest, respectively) (Table 1).

Regarding the number of branches,  $T_{10}$ , involving foliar application of Zinc (0.25%) and Feso<sub>4</sub> (0.25%) with RDF, exhibited the maximum number of branches at all stages (17.43, 23.00, 26.22, and 28.15 at 30, 60, 90 days after planting, and at harvest, respectively). Following closely was  $T_7$ , with soil application of Iron and Zinc (20 & 25 kg ha<sup>-1</sup>) along with RDF, recording substantial branch numbers. The minimum number of branches occurred in  $T_1$  (RDF) at all growth stages (13.29, 20.27, 23.67, and 25.08 at 30, 60, 90 days after planting, and at harvest, respectively) (Table 2).

Furthermore,  $T_{10}$ 's foliar application of Iron and Zinc (0.25%) with RDF recorded the maximum north-south plant spread (8.79, 17.44, 24.65, and 29.00 cm at 30, 60, 90 days after planting, and at harvest, respectively).

In contrast, the minimum north-south spread was observed with

 $T_1$  (RDF) at all stages of crop growth (7.41 cm, 15.04 cm, 22.71 cm, and 27.03 cm at 30, 60, 90 days after planting, and at harvest, respectively) (Table 3).

Similarly,  $T_{10}$  displayed the significantly maximum east-west plant spread (11.32, 18.32, 26.43, and 32.86 cm at 30, 60, 90 days after planting, and at harvest, respectively), while,  $T_1$  (RDF) demonstrated the minimum spread at all plant growth stages (9.28 cm, 16.20 cm, 24.15 cm, and 31.42 cm at 30, 60, 90 days after planting, and at harvest, respectively) (Table 4).

The enhanced growth attributes, possibly due to sufficient zinc availability, may have stimulated physiological and metabolic processes in the plant. Zinc's role in regulating auxin concentration and increasing cation exchange capacity of roots might contribute to this effect. Additionally, iron, a key element in photosynthesis, plays a vital role in chlorophyll synthesis and is part of enzymes associated with energy transfer and various metabolic processes (Abhijit Y.C, 2017)<sup>[1]</sup>.

The synergistic effects of Iron and Zinc, along with their prolonged availability, contribute to improved crop growth and higher yields (Meena *et al.*, 2003; Gaffar *et al.*, 2011) <sup>[10, 4]</sup>. This aligns with findings in chickpea, coriander, fenugreek, and fennel reported by Singh and Gupta (1996), Meena (2003) <sup>[10]</sup>, Sammauria (2007) <sup>[14]</sup>, and Gupta (2012) <sup>[5]</sup>, respectively.

Moreover,  $T_{10}$ 's foliar application of Iron and Zinc (0.25%) with RDF recorded the highest chlorophyll-a, chlorophyll-b, and total chlorophyll content (1.37, 1.71, and 3.08 mg g<sup>-1</sup> of tissue, respectively). This was followed by  $T_7$ , involving soil application of Iron and Zinc (20 & 25 kg ha<sup>-1</sup>) with RDF. Conversely, the application of RDF alone ( $T_1$ ) demonstrated the least chlorophyll-a, chlorophyll-b, and total chlorophyll content (1.07, 1.34, and 2.41 mg g<sup>-1</sup> of tissue, respectively). This further underscores the crucial role of iron and zinc in metabolic processes, DNA synthesis, respiration, and photosynthesis, contributing to chlorophyll production and overall plant health (Sammauria, 2007; Gupta, 2012) <sup>[14, 5]</sup> (Table 5).

Treatments		Rab	i -2019			Rab	<i>i-</i> 2020			Pool	led data	
Treatments	<b>30 DAP</b>	60 DAP	<b>90 DAP</b>	At harvest	<b>30 DAP</b>	60 DAP	<b>90 DAP</b>	At harvest	<b>30 DAP</b>	60 DAP	<b>90 DAP</b>	At harvest
$T_1$	14.80	23.23	29.07	34.37	15.77	25.97	32.97	36.30	15.28	24.60	31.02	35.33
$T_2$	15.67	23.45	30.23	35.77	16.00	26.63	34.63	38.63	15.83	25.04	32.43	37.20
<b>T</b> <sub>3</sub>	16.09	23.51	30.40	36.27	16.99	26.67	34.67	39.33	16.54	25.09	32.53	37.80
$T_4$	17.08	24.21	30.47	37.07	18.58	27.53	35.87	39.87	17.83	25.87	33.17	38.47
<b>T</b> <sub>5</sub>	17.02	24.02	30.60	35.90	17.77	27.20	35.20	39.20	17.39	25.61	32.90	37.55
$T_6$	17.27	25.19	33.83	36.87	18.16	28.40	36.40	40.07	17.72	26.80	35.12	38.47
<b>T</b> <sub>7</sub>	18.17	25.71	34.27	38.37	19.27	29.23	37.23	40.23	18.72	27.47	35.75	39.30
$T_8$	17.63	23.30	30.97	36.37	18.21	27.67	35.67	38.67	17.92	25.48	33.32	37.52
<b>T</b> 9	17.87	24.72	31.33	36.43	18.71	27.43	35.10	39.10	18.29	26.08	33.22	37.77
T10	19.06	26.16	35.20	40.50	20.38	29.27	37.27	41.27	19.72	27.71	36.23	40.88
S.E m ±	0.77	0.60	0.64	0.58	0.76	0.92	0.99	0.81	0.70	0.73	0.64	0.58
CD (5%)	2.29	1.77	1.90	1.72	2.26	2.73	2.94	2.40	2.08	2.18	1.91	1.73
CV (%)	7.84	4.24	3.50	2.73	7.34	5.76	4.83	3.56	6.94	4.89	3.32	2.65

Table 1: Effect of Iron and Zinc on plant height (cm) in kalmegh (Andrographis paniculata Nees.)

$\begin{array}{c} T_1 - RDF \ (75:\ 75:\ 50 \ kg \ NPK + FYM \ 25 \ t \ ha^{-1}) \\ T_2 - T_1 + ZnSO4 \ 10 \ kg \ ha^{-1} \\ T_3 - T_1 + ZnSO4 \ 20 \ kg \ ha^{-1} \\ T_4 - T_1 + FeSO4 \ 25 \ kg \ ha^{-1} \\ T_5 - T_1 + FeSO4 \ 25 \ kg \ ha^{-1} \\ T_6 - T_1 + ZnSO4 \ 10 \ kg \ + \ 15 \ kg \ FeSO4 \ ha^{-1} \end{array}$	Soil application
T <sub>7</sub> - T <sub>1</sub> + ZnSO <sub>4</sub> 20 kg + 25 kg FeSO <sub>4</sub> ha <sup>-1</sup>	
T <sub>8</sub> - T <sub>1</sub> + ZnSO <sub>4</sub> 0.25 %	Foliar
T9 - T1+ FeSO4 0.25%	rona
$T_{10} - T_1 + ZnSO_4 0.25\% + FeSO_4 0.25\%$	application

Table 2: Effect of Iron and Zinc on number of branches of kalmegh (Andrographis paniculata Nees.)

Treatmonta		Rabi -	Rabi- 2020				Pooled data					
Treatments	30 DAP	60 DAP	<b>90 DAP</b>	At harvest	30 DAP	60 DAP	<b>90 DAP</b>	At harvest	<b>30 DAP</b>	60 DAP	90 DAP	At harvest
$T_1$	10.81	19.95	21.95	23.87	11.33	25.40	25.40	26.30	13.29	20.27	23.67	25.08
$T_2$	10.94	20.18	22.85	24.80	11.38	25.80	25.80	26.47	13.47	20.53	24.33	25.63
$T_3$	11.80	21.63	23.63	25.57	12.49	26.87	26.87	27.40	14.40	21.92	25.25	26.48
$T_4$	11.63	21.61	23.61	25.33	12.22	26.40	26.40	27.20	15.11	21.87	25.01	26.27
T5	12.25	21.88	23.88	25.73	12.84	26.43	26.43	27.53	15.01	22.14	25.16	26.63
T <sub>6</sub>	13.35	22.14	24.14	27.00	14.61	27.43	27.43	28.33	15.76	22.44	25.79	27.67
<b>T</b> <sub>7</sub>	13.78	22.53	24.53	26.77	14.63	27.70	27.70	28.60	16.53	22.83	26.12	27.68
$T_8$	13.16	21.97	23.28	26.33	13.81	27.07	27.07	28.00	15.69	22.30	25.18	27.17
T9	12.92	21.89	23.89	25.77	13.51	27.05	27.05	27.67	15.81	22.18	25.47	26.72
T10	14.47	22.74	24.74	27.20	14.93	27.70	27.70	29.10	17.43	23.00	26.22	28.15
S.E m ±	0.64	0.69	0.65	0.65	0.66	0.91	0.91	0.69	0.42	0.68	0.70	0.66
CD (5%)	1.90	2.06	1.93	1.94	1.96	2.70	2.70	2.06	1.26	2.03	2.08	1.95
CV (%)	8.87	5.54	4.75	4.38	8.65	5.88	5.88	4.33	4.81	5.38	4.82	4.25



Table 3: Effect of Iron and Zinc on north south (cm) plant spread in Kalmegh (Andrographis paniculata Nees.)

T		Rabi -	2019		Rabi- 2020				Pooled data			
1 reatments	30 DAP	60 DAP	<b>90 DAP</b>	At harvest	30 DAP	60 DAP	<b>90 DAP</b>	At harvest	30 DAP	60 DAP	90 DAP	At harvest
$T_1$	6.73	14.37	21.95	26.25	8.08	15.70	23.48	27.81	7.41	15.04	22.71	27.03
$T_2$	6.98	14.40	22.03	26.62	8.11	15.73	23.93	28.22	7.55	15.07	22.98	27.42
T3	7.31	14.50	22.81	27.95	8.48	16.00	24.47	29.55	7.89	15.25	23.64	28.75
$T_4$	7.23	14.97	22.83	27.85	8.36	15.97	24.46	29.38	7.79	15.47	23.64	28.61
T5	7.48	14.99	22.92	26.70	8.75	16.42	24.59	28.60	8.11	15.70	23.75	27.65
T <sub>6</sub>	7.92	14.90	23.36	28.21	9.03	16.80	24.93	29.25	8.48	15.85	24.14	28.73
$T_7$	7.95	14.97	23.61	27.69	9.15	16.90	25.28	29.36	8.55	15.94	24.45	28.53
$T_8$	8.00	15.40	23.21	27.67	8.97	16.73	24.87	29.01	8.48	16.07	24.04	28.34
<b>T</b> 9	7.80	15.33	23.64	27.21	8.83	16.66	24.64	28.91	8.32	15.99	24.14	28.06
T10	8.21	16.61	23.73	28.45	9.37	18.27	25.57	29.55	8.79	17.44	24.65	29.00
S.E m ±	0.51	0.49	0.75	0.68	0.46	0.50	0.65	0.57	0.48	0.48	0.68	0.61
CD (5%)	1.53	1.45	2.22	2.02	1.37	1.47	1.92	1.70	1.43	1.41	2.03	1.82
CV (%)	11.76	5.63	5.62	4.28	9.15	5.20	4.54	3.42	10.23	5.22	4.97	3.77



Table 4: Effect of Iron and Zinc on East west (cm) plant spread in kalmegh (Andrographis paniculata Nees.)

Truesday and a		Rabi -	Rabi- 2020				Pooled data					
Treatments	30 DAP	60 DAP	<b>90 DAP</b>	At harvest	30 DAP	60 DAP	<b>90 DAP</b>	Harvest	<b>30 DAP</b>	Poiled data   0 DAP 60 DAP 90 DA   9.28 16.20 24.15   9.60 16.28 24.32   9.84 16.62 24.35   9.60 16.46 24.31	90 DAP	At harvest
$T_1$	8.73	15.37	23.40	30.93	9.83	17.03	24.90	32.10	9.28	16.20	24.15	31.42
$T_2$	9.18	15.45	23.64	31.03	10.03	17.11	25.01	32.29	9.60	16.28	24.32	31.66
<b>T</b> <sub>3</sub>	9.26	15.85	23.47	31.02	10.43	17.39	25.23	32.55	9.84	16.62	24.35	31.79
$T_4$	8.99	15.66	23.59	30.84	10.21	17.26	25.03	32.43	9.60	16.46	24.31	31.64
T5	9.62	16.24	24.24	31.36	10.65	18.21	25.84	32.69	10.14	17.22	25.04	32.03

$T_6$	9.62	17.23	24.98	31.91	11.10	18.86	26.43	33.66	10.36	18.04	25.71	32.79
$T_7$	10.01	17.04	25.44	31.70	11.75	18.97	27.04	33.70	10.88	18.01	26.24	32.70
$T_8$	9.35	16.22	24.56	31.63	11.03	18.49	26.22	33.50	10.19	17.35	25.39	32.57
T9	9.59	16.51	24.74	31.39	10.54	18.41	26.21	33.17	10.06	17.46	25.48	32.28
$T_{10}$	10.56	17.42	25.72	31.96	12.08	19.22	27.13	33.76	11.32	18.32	26.43	32.86
S.E m ±	0.66	0.99	1.03	0.90	0.90	1.12	1.08	3.44	0.77	1.05	1.05	0.90
CD (5%)	1.96	2.95	3.06	2.67	2.66	3.33	3.20	10.24	2.29	3.12	3.12	2.68
CV (%)	12.06	10.54	7.31	4.96	14.41	10.72	7.20	18.08	13.20	10.57	7.24	4.85



Table 5: Effect of Iron and Zinc on chlorophyll content (mg g<sup>-1</sup> of tissue) in Kalmegh (Andrographis paniculata Nees.)

T		Rabi -2019	1		Rabi- 2020			Pooled data	a
1 reatment	Chlorophyll a	Chlorophyll b	<b>Total Chlorophyll</b>	Chlorophyll a	Chlorophyll b	<b>Total Chlorophyll</b>	Chlorophyll a	Chlorophyll b	<b>Total Chlorophyll</b>
$T_1$	1.01	1.34	2.35	1.13	1.35	2.48	1.07	1.34	2.41
$T_2$	1.07	1.55	2.62	1.17	1.36	2.52	1.12	1.45	2.57
T <sub>3</sub>	1.09	1.41	2.50	1.24	1.45	2.69	1.17	1.43	2.60
$T_4$	1.07	1.38	2.46	1.25	1.39	2.64	1.16	1.39	2.55
T <sub>5</sub>	1.08	1.52	2.60	1.30	1.56	2.86	1.19	1.54	2.73
T <sub>6</sub>	1.21	1.58	2.80	1.28	1.65	2.93	1.25	1.62	2.86
T <sub>7</sub>	1.24	1.64	2.89	1.34	1.70	3.03	1.29	1.67	2.96
T <sub>8</sub>	1.13	1.59	2.72	1.21	1.61	2.82	1.17	1.60	2.77
T <sub>9</sub>	1.18	1.55	2.72	1.31	1.60	2.91	1.24	1.57	2.82
T <sub>10</sub>	1.34	1.70	3.04	1.40	1.72	3.12	1.37	1.71	3.08
S.E m ±	0.18	0.11	0.20	0.15	0.09	0.15	0.15	0.10	0.15
CD (5%)	0.54	0.34	0.58	0.45	0.28	0.45	0.44	0.29	0.46
CV (%)	27.47	12.94	12.71	20.82	10.48	9.31	21.45	10.97	9.72

#### Conclusion

The findings from the current study highlight that, the foliar application of Zinc and Iron at a concentration of 0.25%, coupled with the application of Recommended Dose of Fertilizer (RDF), led to the attainment of maximum growth attributes and chlorophyll content in Kalmegh. This outcome holds significance for cultivating Kalmegh in the northern dry zone of Karnataka, emphasizing the positive impact of this specific nutrient management approach on the plant's overall development and physiological processes.

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