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Impact of plant growth hormones on growth, physiology and alkaloid content of kalmegh (*Andrographis peniculata* Burn F. Ex)

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Abstract

This study was conducted at A field experiment was carried out in the research farm of RAK College of Agriculture, Sehore (Madhya Pradesh) during *Kharif* (2015-16). A field experiment consisting of Plant Growth Hormones Cycocel (100, 150 and 200 ppm), GA₃ (100, 150 and 200 ppm) and NAA (100 and 150 ppm) and water spray as control to study the impact of plant growth Hormones on plant height, No. of branches, Fresh weight of leaf/plant, Dry weight of leaf/plant. Chlorophyll content, Photosynthesis rate, and leaf andrographolide content of Kalmegh (*Andrographis peniculata* Burn F. Ex). Significantly higher Photosynthesis rate was observed by treatment GA₃ 100 ppm (23.15) at 70 days of crop as compare to treatment GA₃ 150 ppm (22.43) respectively and Cycocel 100 ppm (57.72) at 70 days maximum Chlorophyll content recorded. The applied with NAA at 100 ppm (3.05% w/w) and GA₃ 150 ppm (2.93% w/w) was very effective and recorded maximum percentage of leaf andrographolide respectively. The foliar application of NAA 100 ppm at 90 days dry weight of leaf per plant maximum recorded (2.73 g/plant).

Keywords: Andrographolide, medicinal plant, cycocel, GA₃ spray, ppm, chlorophyll content, photosynthesis rate, HPLC

1. Introduction

Kalmegh (*Andrographis paniculata* Burn F. Ex) is a large genus of herbs and shrubs. In the family of Acanthaceae. Kalmegh is an annual erect or recumbent plant. Distribution mostly in the tropical and moist region. It comprises of 19 species the plant is found in India and Shrilanka. It is indigenous to India. In India it is grown in Assam, Bihar, Karnataka, Kerala Madhya Pradesh, Andhra Pradesh and West Bengal. Kalmegh also known as "King bitter" is one among the prioritized medicinal plant in India and this herbs is being used mainly useful in treating fever, treating liver related diseases, jaundice, diabetes. Snake bites. The leaf and the whole herb contain the medicinal important. The fresh and dried leaves of Kalmegh are used as drugs in India. Growth of a plant is greatly affected by much environmental condition which affected the physiology of plant. The leaves of Kalmegh contain maximum active principle like Andrographolide, homo-Andrographolide andrographosterol and andrographone. Andrographolide the major constituent in leaves which is bitter sub-stance (Gorter, 1911) [1]. The average Andrographolide contain varied from 12.44 to 33.52 mg/g in dried leaves (Prathanturug, 2007) [8] found maximum at 90-120 (Maheshwari *et al.*, 2000) [4]. It has been already used for treating cancer as it promotes cell differentiation in tumour cell (Mutsuda *et al.* 1994) [3]. The recognition of such growth control mechanism has introduced the possibility of modifying growth and development of plant by manipulating hormone level in different organs and various stages in the life cycle. One of the way to achieve this is by exogenous application of chemical growth regulators (Thams, 1985). The term plant growth regulators however applied in phyto hormone as well as synthetic compound (Nickell, 1978). Plant growth regulators are organic compound, other than nutrients, that modify plant physiology processes. PGRs called bio stimulants act side plant cell to stimulate or inhibit specific enzyme or enzyme system and help regulate plant metabolism. To specific PGRs are used to modify crop growth rate and growth pattern during the various stages of development, from germination through harvest and post-harvest preservation. Growth hormone chemicals that have positive influences on major medicinal plant can be of value. The final test, however is that harvested yields must be increase or medicinal quality enhanced in order for growth hormones to be profitable. The synthetic growth regulators chemicals are being extremely important and valuable for manipulating the growth and yield of medicinal plants.

2. Materials and Methods

This study was conducted at A field experiment was carried out in the research farm of RAK College of Agriculture, Sehore (Madhya Pradesh) during *Kharif* 2016). There were eight treatments Cycocyl (100, 150 and 200 ppm), GA₃ (100, 150 and 200 ppm) and GA₃ (100 and 150 ppm) and the water being the control. This study was done on the base of randomized complete block design. The treatment was replicated 3 times. The plant growth regulators were sprayed in three stages viz. seedling stage, vegetative stages and reproductive stage. The local kalmegh variety seeds were sown in main field. The whole plot was divided into 3 blocks each representing the replication. Each block was then divided into unit plot of 2x3m size. Seedlings were transplanted at 30x15cm spacing. The experiment plot fertilized with urea, single super phosphate and murate of potash at the rate of NPK 75 kg, 75 kg and 50 kg ha⁻¹ respectively. Nitrogen was applied at two equal splits, one at the time of transplanting and another as top dressing at 30 day after sowing. Nitrogen at 20 kg ha⁻¹ was used for top dressing. All the operations done regularly during growing season. Different plant growth parameter and physiological observations were recorded on five randomly selected plants from every treatment on 50 days, 70 days and 90 days after transplanting of seeding. The collected data includes plant height, No. of branches, Fresh weight of leaf/plant, Dry weight of leaf/plant. Chlorophyll content was determined by using Minoltas SPAD 502 plus Chlorophyll meter. The instruments measuring the relative amount of chlorophyll present in plant leaves in unit of SPAD (Soil Plant Analysis Development). It is work on the principal of value of measured chlorophyll correspondent to base on light transmitted by the leaf in two wave length reason in which the absorbance of chlorophyll is different. The

photosynthesis rate was measured in micro mole CO₂ m⁻² sec⁻¹ at flowering stage by using automatic Li-cor 6400 photosynthesis system, USA as per method suggested by (Kannan *et al*, 2007). The data on yield attributed and grain yield were collected at the time of harvested. The andrographolide content was estimated by HPLC analysis in leaf samples separately using replicated pooled samples of crop. Finally mean data of the all characters were computed for statistical analysis as per standard procedure given by (Panse and Sukhtme 1989) [7].

3. Results and Discussions

3.1 Plant height (cm): The plant height per plant at 50, 70 and 90 days of the crop is presented in (Table- 1). The effect of different concentration of plant growth hormones was significant for all growth stages except 50 days of crop on plant height. The plant height increases with the advancement in growth stage in Kalmegh. The maximum plant height was recorded with treated GA₃ @100ppm (27.84) at 70 days. Closely followed by NAA @ 100 ppm (26.59) and NAA @150 ppm (23.93) which were at par and plant height per plant was lowest in control (23.18) as compare to treated with plant growth hormones Cycocel@ 100 ppm (22.51), Cycocel 150 ppm (22.13) and Cycocel @ 200 ppm (22.62). At 90 day maximum plant height observed with treated GA₃ @ 150 ppm (47.33). Closely followed by NAA @ 100 ppm (44.68), NAA @ 200 ppm (44.63) which were at par, and plant height per plant was lowest in control (34.74) as compare to treated with plant growth hormones Cycocel@ 100 ppm (33.30), Cycocel @150 ppm (33.42) and Cycocel @ 200 ppm (32.92). Similar results reported on the medicinal plant (*Gloriosa Superba* L.) by K. Kannabiran and Padmanaban (2016) [2].

Table 1: The Impact of Plant Growth Hormones on Growth of kalmegh (*Andrographis paniculata* Nees.)

Symbol	Treatment	Plant height (cm)			No. of Branches		
		50 Days	70 Day	90 Day	50 Day	70 Day	90 Day
T ₁	Cycocel 100 ppm	11.74	22.51	33.30	13.44	23.55	28.22
T ₂	Cycocel 150 ppm	10.66	22.13	33.42	13.55	23.88	22.66
T ₃	Cycocel 200 ppm	10.79	22.62	32.92	10.55	21.66	23.77
T ₄	GA ₃ 100 ppm	14.49	27.84	43.93	10.88	22.44	26.88
T ₅	GA ₃ 150 ppm	13.78	23.93	47.33	13.00	23.77	24.33
T ₆	GA ₃ 200 ppm	15.76	26.59	44.63	12.11	26.11	23.88
T ₇	NAA 100 ppm	12.84	25.08	44.68	12.22	23.88	24.00
T ₈	NAA 150 ppm	13.06	24.86	41.20	11.55	25.33	22.88
T ₉	Control	11.97	23.18	34.74	9.88	20.22	20.66
	SE (m) ±	0.38	0.95	2.33	0.83	1.27	1.88
	CD at 5%	NS	2.93	7.18	NS	3.91	5.81

3.2 Number of branches/plant: The number of branches per plant at 50 days, 70 days and 90 days of the medicinal plant Kalmegh is presented (Table-1). The impact of different concentration of plant growth hormones was significant except 50 days of crop on number of branches per plant. The number of branches per plant maximum found was in the GA₃ @ 200 ppm (26.11) as compare to NAA @150 ppm (25.33) at 70 days of crop. Closely followed by NAA @ 100 ppm (23.88), Cycocel 150 ppm (23.55), Cycocel 150 ppm (23.88), and NAA 100 ppm (23.88) which were at par and lowest no. of branches found was control (20.22). The favorable effect of GA₃ and NAA in The higher number of branches in the plants applied with GA₃ can be related to enhance physiological activities such as cell division, cell elongation, photosynthesis and translocation of nutrients and photosynthates (Saxena, 1989) [9]. At 90 day maximum no. of

branches observed with treated Cycocel 100 ppm @ 150 ppm (28.22) as compare to GA₃ 100 ppm (26.88). Closely followed by NAA @ 100 ppm (24.00) and GA₃ 150 ppm (24.33) which were at par, and no. of branches per plant was lowest in control (20.66) as compare to treated with plant growth hormones.

3.3 Fresh weight of leaf (g): The data on fresh weight of leaf as influenced by various plant growth hormones at different concentration have been presented in (table -2). The impact of different concentration of plant growth hormones was significant except 50 days of crop on fresh weight of leaf per plant. At 70 days fresh weight of leaf per plant maximum found was treated with Cycocel 150 ppm (7.19) as compare to Cycocel 200 ppm (7.10) followed by Cycocel at 100 ppm (6.82g/plant), NAA at 100 ppm (6.65g/plant). While, fresh

leaf weight was minimum (6.10g/plant) with GA₃ at 100 ppm which was at par with other treatments (control (6.05). At 90 days fresh weight of leaf per plant maximum found was plant spray with GA₃ 100 ppm (14.85 g/plant) as compare to NAA 100 ppm (14.17 g/plant). While, fresh leaf weight was minimum (11.72 g/plant) with GA₃ at 200 ppm which was at par with other treatments control (9.76).

3.4 Dry weight of leaf (g/plant): The data on dry weight of leaf per plant influenced by various plant growth hormones at different concentration have been presented in (table -2). The data clearly indicated that, significant influence of plant growth hormones on dry weight of leaf in all stages. At 50 days dry weight of leaf per plant maximum found was treated with GA₃ 100 ppm (1.20 g/plant) as compare to Cycocel 100 ppm (1.11) followed by NAA at 150 ppm (1.09g/plant), GA₃ at 200 ppm (1.05g/plant). While, dry weight of leaf was minimum (0.97g/plant) with Cycocel at 200 ppm and GA₃ at 150 ppm which was at par with other treatments (control (0.55 g/plant). At 70 days and 90 days dry weight of leaf per plant maximum found was treated with NAA 100 ppm (2.30 g/plant and 2.73 g/plant) as compare to GA₃ 100 & 150 ppm (2.00 and 2.67 g/plant) followed by Cycocel at 100 ppm (1.66g/plant), at 70 days and GA₃ at 200 ppm (2.46g/plant) at

90 days. While, dry weight of leaf was minimum (1.56 and 2.11 g/plant) with Cycocel at 150 ppm 90 days. Which was at par with other treatments control?

3.5 Chlorophyll content (SPAD): The Chlorophyll content (Soil Plant Analysis Development) of the crop is presented in (Table-2). The impact of different concentration of plant growth hormones was significant except 50 days of crop. It was observed that there was a continuous increase in the Chlorophyll content at all the stages of crop growth. Significantly higher Chlorophyll content was observed by treatment Cycocel 100 ppm (57.72) at 70 days of crop as compare to treatment Cycocel 150 ppm (56.30) respectively. Closely followed by Cycocel 200 ppm (55.36), GA₃ 200 ppm (54.80) which were at par, Chlorophyll content was lowest in control (45.74) as compare to treated with plant growth hormones GA₃ 150 ppm (54.46), GA₃ 100 ppm (53.87) respectively. Similarly at 90 days of crop maximum chlorophyll content found was treated with plant hormones Cycocel 100 ppm (57.78) as compare to treated with Cycocel 200 ppm (55.59) respectively. Closely followed by Cycocel 150 ppm (54.26), NAA 100ppm (53.72) which were at par, Chlorophyll content was lowest in control (41.78).

Table 2: The Impact of Plant Growth Hormones on Chlorophyll Content, Photosynthesis rate and Alkaloid Content of kalmegh (*Andrographis paniculata* Nees.)

Symbol	Treatment	Fresh wt. of leaf (g)			Dry wt. of leaf (g)			Chlorophyll content (SPAD)			Photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)			* Leaf Alkaloid content (% w/w)
		50 Days	70 Day	90 Day	50 Days	70 Day	90 Day	50 Days	70 Day	90 Day	50 Days	70 Day	90 Day	
T ₁	Cycocel 100 ppm	5.81	6.82	11.74	1.11	1.66	2.37	46.17	57.72	57.78	18.17	20.83	23.40	1.78
T ₂	Cycocel 150 ppm	5.38	7.19	13.78	1.01	1.56	2.11	51.25	56.30	54.26	18.71	20.56	22.78	1.53
T ₃	Cycocel 200 ppm	5.90	7.10	12.50	0.97	1.90	2.35	44.36	55.36	55.59	16.75	20.0	21.55	1.31
T ₄	GA ₃ 100 ppm	5.72	6.10	14.85	1.20	2.00	2.23	49.11	53.87	50.80	21.98	23.15	24.78	2.70
T ₅	GA ₃ 150 ppm	5.80	6.37	12.84	0.97	1.89	2.67	49.13	54.46	52.45	21.11	22.43	24.21	2.93
T ₆	GA ₃ 200 ppm	5.57	6.59	11.72	1.05	1.84	2.46	50.96	54.80	47.11	19.26	21.78	22.22	2.85
T ₇	NAA 100 ppm	5.86	6.65	14.17	1.03	2.30	2.73	53.70	54.77	53.72	18.90	22.02	23.12	3.05
T ₈	NAA 150 ppm	5.26	6.39	13.43	1.09	1.59	2.33	51.47	54.91	45.62	18.54	20.06	22.83	2.11
T ₉	Control	5.06	6.05	9.76	0.55	1.33	1.71	42.13	45.74	41.78	16.44	18.12	19.90	1.28
	SE (m) \pm	1.62	2.36	4.07	0.25	0.41	0.58	1.24	1.83	0.98	0.52	0.59	0.38	-
	C D at 5%	NS	7.26	12.54	0.76	1.26	1.79	NS	5.64	NS	NS	1.83	1.16	-

*The replicated pooled samples of leaf were used for estimation of alkaloid and hence, could not be analyzed statistically and only mean values have been presented.

3.6 Photosynthesis rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$): The Photosynthesis rate of the crop is presented in (Table-2). The impact of different concentration of plant growth hormones was significant except 50 days of crop. It was observed that there was a continuous increase in the Photosynthesis rate at all the stages of crop growth. Significantly higher Photosynthesis rate was observed by treatment GA₃ 100 ppm (23.15) at 70 days of crop as compare to treatment GA₃ 150 ppm (22.43) respectively. Closely followed by NAA 100 ppm (22.02), GA₃ 200ppm (21.78) which was at par, Photosynthesis rate was lowest in control (18.12) respectively. Similarly at 90 days of crop maximum Photosynthesis rate found was applied with GA₃ 100 ppm (24.78) as compare to treated with GA₃ 150 ppm (24.21) respectively. Followed by Cycocel 100 ppm (23.40), NAA 100ppm (23.12) which were at par, Photosynthesis rate was lowest in control (19.90) respectively.

3.7 Alkaloid content of leaf (% w/w)

Plant Growth hormones treatments caused for higher

andrographolide content in leaf presented in (Table- 2). Plant Growth hormones applied with NAA 100 ppm (3.05% w/w) and GA₃ 150 ppm (2.93% w/w) registered maximum leaf andrographolide as compare to treated with GA₃ 200 ppm (2.85% w/w), GA₃ 100 ppm (2.70% w/w), NAA 150 ppm (2.11 w/w) and Cycocel 100 ppm (1.78 w/w) respectively. Leaf andrographolide was lowest in control (1.28). Similar results have been registered by *Menaria* and *Maliwal* in *fennel*.

4. Conclusion

Kalmegh also known as "King bitter" is one among the prioritized medicinal plant in India and this herbs is being used mainly useful in treating fever, treating liver related diseases, jaundice, diabetes and Snake bites. The leaf and the whole herb contain the medicinal important. The fresh and dried leaves of Kalmegh are used as drugs in India. The leaves of the herb were found to contain the highest amount of Andrographolide. To specific PGRs are used to modify crop growth rate and growth pattern during the various stages

of development, Growth hormone chemicals that have positive influences on major medicinal plant can be of value. The final test, however is that harvested yields must be increase or medicinal quality enhanced in order for growth hormones to be profitable.

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