

Role of Growth Regulators on Quality and Yield of Kalmegh (*Andrographis paniculata* Nees)

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Abstract

A study was conducted at B.A. College of Agriculture, Anand Agricultural University, Anand during 2019 to find out the influence of foliar treatments on the productivity of Kalmegh. Different treatments which includes T₁ (NAA 50 mg l⁻¹), T₂ (NAA 100 mg l⁻¹), T₃ (NAA 200 mg l⁻¹), T₄ (GA₃ 50 mg l⁻¹), T₅ (GA₃ 100 mg l⁻¹), T₆ (GA₃ 200 mg l⁻¹), T₇ (paclobutrazol 50 mg l⁻¹), T₈ (paclobutrazol 100 mg l⁻¹), T₉ (paclobutrazol 200 mg l⁻¹) and T₁₀ (control) were applied as foliar at 30 days after transplanting. The effect of phytohormones on dry herbage yield at harvest were found significant and highest was (4913.84 kg ha⁻¹) in GA₃ 100 mg l⁻¹ followed by paclobutrazol 100 mg l⁻¹ (4901.68 kg ha⁻¹) and paclobutrazol 200 mg l⁻¹ (4884.48 kg ha⁻¹) as compared to control. Andrographolide content (%) at harvest were recorded significant and highest was (1.453%) observed in GA₃ 200 mg l⁻¹. Higher andrographolide content was recorded in GA₃ 100 mg l⁻¹ (1.403%) and paclobutrazol 100 mg l⁻¹ (1.395%). Results indicated that increased the content of andrographolide by 25.04%, 20.74% and 20.05% in case of GA₃ 200 mg l⁻¹, GA₃ 100 mg l⁻¹ and paclobutrazol 100 mg l⁻¹ respectively as compared to control. Growth parameters leaf dry weight (0.688) and total dry weight (0.654) reported positive significant correlation with quality parameter i.e. andrographolide percentage at harvest. Physiological parameter have positive significant correlation with the quality i.e. andrographolide viz. SLW (0.699), RGR (0.677), CGR (0.692), NAR (0.655) and BMD (0.651).

Keywords: *Andrographis paniculata*, andrographolide, growth regulators, quality, yield

1. Introduction

Andrographis paniculata (Burm.f.) Wall. ex Nees is an herbaceous plant in the family Acanthaceae (Niranjan Reddy et al., 2005) known as kalmegh. It is an annual herb with wide medicinal and pharmacological applications due to the presence of andrographolide, an important bioactive compound. Being a prioritized medicinal plant in India, this medicinal herb has been recommended for cultivation to fulfill the market demands (Basak et al., 2020). Andrographolide, the principal secondary metabolite of *Andrographis paniculata*, displays a wide spectrum of medicinal activities. Andrographolide which is responsible for the curative properties such as antibiotic hepatoprotective (Handa and Sharma, 1990), antifungal (Sule et al., 2012), anti-HIV activity (Carlo et al., 2000, Xu et al., 1996), chikangunia virus (Wintachai et al., 2015), antipyretic and anti-inflammatory (Caceres et al., 1997), and cancerolytic (Matsuda et al., 1994) properties and used as an immunostimulant. The heavy demand for andrographolide in Indian as well as in international markets has motivated Indian farmers to start commercial cultivation of this important medicinal plant (Kanjilal et al., 2002).

Andrographolide, the primary biologically active compound of *Andrographis paniculata*, is produced through

coordinated action of two pathways, the classical cytosolic mevalonate pathway and the alternative plastidial non-mevalonate pathway, i.e. Deoxy-xylulose Phosphate pathway (Das and Bandyopadhyay, 2021).

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, Andrographolide the major constituent in leaves which is bitter substance (Gorter, 1911). Raina et al. (2013) reported wide variability among different accessions of karyatu for andrographolide content which was from 0.72–2.99% on dry weight basis.

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 regulate metabolism. Plant growth regulators promote root and shoot growth (Kim et al., 2018, Steffens et al., 2006), enhance water use



efficiency, promoting flowering and pod setting (Nagel et al., 2001), increase chlorophyll content (Sun et al., 2016), improve photosynthetic rate (Travaglia et al., 2009), enhance the translocation of photoassimilates and increase biomass accumulation (Liu et al., 2019) resulting in enhanced growth and yield. Specific PGRs are used to modify 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. When hormone acts upon plant system, definitely it enters some direct and molecular interactions, which results, eventually in manifestation of measurable physiological and biochemical effects (Kumar and Purohit, 2011). The treatments of various growth regulators might be responsible for increased transport of assimilates from source (leaves) to sink (pods and seeds) and ultimate conversion into final yield (Gudhate et al., 2009). The whole process of manipulation of source-sink relation might have favoured the yield increase. Growth control mechanisms 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 ways to achieve this is by exogenous application of chemical growth regulators. Growth regulators influences on the vegetative growth of plant. Foliar application of the phytohormones directly affect plant and it helps to increase growth and yield of the crop. Use of bioregulators i.e. plant growth regulators composed of growth retardants and promoters, use in right concentration may affect the plant architecture in typical fashion (Hermesz and Ferencz, 2009). Foliar application of plant growth regulators helps to increase androgaholide content (Anuradha et al., 2009). To increase the productivity by way of increasing the herb yield by the use of growth regulators.

2. Materials and Methods

An experiment was carried out at B. A. College of Agriculture, Department of Plant Physiology, Anand Agricultural University, Anand by transplanting during 15th August and harvested at 16th December, 2019. Experiment site was located at 22°35' North Latitude and 72°55' East Longitude and Altitude of 45 m above mean sea level. The crop was fertilized with the application of FYM @ 10 t ha⁻¹ as per recommended dose of basal application. Phytohormones spray solution (NAA, GA₃ and paclobutrazol) of different concentration (50, 100, 200 mg l⁻¹) was prepared from powder form which was dissolved in 2–5 drops of ethyl alcohol or alkaline solution add and make up final volume of 1000 ml with double distilled water. e.g. GA₃ 50 mg l⁻¹ solution prepared by 50 mg GA₃ powder from was dissolved with small amount of alcohol or alkaline solution by stirring and make up final volume of 1000 ml with double distilled water, different concentration was also prepared by the same way for required quality. Foliar sprays of phytohormones were carried out at 30 days after transplanting of a crop and observations were

done time to time.

For the determination androgapholide content in experimental samples are total herbage excluding root, the samples were shade dried, powdered and extracted in methanol. 100 mg powdered sample was extracted in 20 ml methanol by sonication for one minute. The extract centrifuge, filter by 0.45 µm syringe filter and extract used for chemical analysis. A modular UFLC (Shimadzu Corporation, Kyoto, Japan), HPLC system consisting of two LC-20AD pumps, SPD -20A UV-Vis Detector, DGPU-20A3 degasser, SIL-20AC HT auto sampler, a CTO-10ASvp column oven, CBM-20 communications bus module was used for chromatographic separation of analytes on a Merck Rp-18 (250×4.6 mm, 5 µm). The mobile phase was consisted of methanol and water (65:35, v/v) ratio and was delivered at a flow rate of 1.0 ml min⁻¹ and absorbance was set at 229 nm in UV-VIS detector. The column temperature was maintained at 40°C for better resolution and the sample injection volume was 10 µl (Gajbhiye and Khristi, 2010). The compounds were identified based on retention time. The calibration curves prepared by the mixture of standards were diluted serially and the calibration curves were established. The linear equation is,

$$y = mx + c \dots \dots \dots (1)$$

The equation was established between the concentration of the standard injected and the peak area, where y is peak area, x is concentration of standard, m and c are constants. The quantification of plant samples was carried out by using peak area in linear equations and the corresponding concentrations were derived and expressed in % (Gajbhiye and Khristi, 2010). The data collected for the yield were subjected to the statistical analysis by adopting analysis of variance technique as described by Panse and Sukhatme (1984).

3. Results and Discussion

3.1. Effect of phytohormones on dry herbage yield (kg ha⁻¹)

The data regarding dry herbage yield of kalmegh are furnished in Table 1. An appraisal of data indicated that phytohormones treatments exerted their significant consequence on dry herbage yield. At harvest, significantly highest dry herbage yield (4913.84 kg ha⁻¹) was registered under treatment T₅ (GA₃ 100 mg l⁻¹) and it was found statistically at par with treatments T₈ and T₉, while control T₁₀ recorded the lowest (4001.93 kg ha⁻¹) dry herbage yield. Results indicated that herbage yield as influenced by the phytohormones at harvest. The impact of different phytohormones have significant effect on yield. The increase in herbage yield may be due to the growth regulator increase the number of leaves which is responsible for production and accumulation of maximum photosynthates as compared to control. Phytohormones application affect the plant height, leaf number altered the root growth which may contributed to yield increase. Gudhate et al. (2009) results support with our result and indicated that growth regulators improve the biomass in kalmegh while

Table 1: Effect of phytohormones on dry herbage yield and andrographolide content at harvest

Treatments	Dry herbage yield (kg ha ⁻¹) at harvest	Andrographolide content (%) at harvest
T ₁ : NAA 50 mg l ⁻¹	4035.46	1.214
T ₂ : NAA 100 mg l ⁻¹	4170.32	1.258
T ₃ : NAA 200 mg l ⁻¹	4053.14	1.283
T ₄ : GA ₃ 50 mg l ⁻¹	4049.81	1.323
T ₅ : GA ₃ 100 mg l ⁻¹	4913.84	1.403
T ₆ : GA ₃ 200 mg l ⁻¹	4181.93	1.453
T ₇ : Paclobutrazol 50 mg l ⁻¹	4153.43	1.185
T ₈ : Paclobutrazol 100 mg l ⁻¹	4901.68	1.395
T ₉ : Paclobutrazol 200 mg l ⁻¹	4884.48	1.234
T ₁₀ : Control (water spray)	4001.93	1.162
SEm±	251.82	0.061
CD (p=0.05)	748.19	0.182
CV%	10.06	8.20

Maity et al. (2019) reported foliar spray helps to produce higher green leaf yield in Tea.

3.2. Effect of phytohormones on andrographolide content (%) at harvest

The data regarding andrographolide content (%) at harvest of kalmegh are represented in Table 1

An appraisal of data indicated that phytohormones treatments exerted their significant consequence on andrographolide content (%) at harvest. At harvest, significantly maximum andrographolide content (1.453%) was in treatment T₆ (GA₃ 200 mg l⁻¹) and it was statistically at par with phytohormones treatments T₅, T₈, T₄ and T₃, while control T₁₀ recorded the lowest (1.162%) andrographolide content at harvest. The effect of GA₃ on andrographolide content may be due to biosynthesis pathway of andrographolide. The results show that GA₃ 200 mg l⁻¹ showed higher andrographolide followed by GA₃ 100 mg l⁻¹ and Paclobutrazol 100 mg l⁻¹ were highly useful to increase the content of andrographolide by 25.04%, 20.74% and 20.05% respectively as compared to control.

These results were very similar with the result of Gudhate et al. (2009) in kalmegh and explain that the effect of GA₃ on andrographolide content may be due to enhanced biosynthetic pathway of andrographolide. They also noted that increase of growth, biomass, chlorophyll, leaf area may contribute to produce the secondary metabolites. These all are very co-related to our findings and conformity which advocate that the improvement or hastening the quality and

Table 2: Effect of phytohormones on andrographolide content correlation with parameter studied

	Andrographolide %	Dry herbage yield	Plant height	No. of leaves	No. of branches	Leaf dry weight	Shoot dry weight	Root dry weight	Total dry weight
Andrographolide %	1								
Dry herbage yield	0.429	1							
Plant height	0.359	-0.270	1						
No. of leaves	0.510	0.789**	0.096	1					
No. of branches	0.490	0.065	0.932**	0.416	1				
Leaf dry weight	0.688*	0.901**	0.055	0.904**	0.377	1			
Shoot dry weight	0.594	0.844**	0.084	0.952**	0.399	0.923**	1		
Root dry weight	0.554	0.841**	0.224	0.846**	0.521	0.893**	0.916**	1	
Total dry weight.	0.654*	0.891**	0.075	0.945**	0.401	0.982**	0.979**	0.927**	1
R/S ratio	-0.265	-0.246	0.421	-0.372	0.297	-0.326	-0.271	0.083	-0.294
Leaf area	0.631	0.916**	0.001	0.927**	0.323	0.953**	0.961**	0.916**	0.976**
LAI	0.631	0.916**	0.001	0.927**	0.323	0.953**	0.961**	0.916**	0.976**
LAD	0.614	0.911**	0.023	0.930**	0.346	0.944**	0.962**	0.918**	0.972**
LAR	-0.675*	-0.680*	-0.254	-0.836**	-0.536	-0.874**	-0.898**	-0.836**	-0.903**
SLW	0.699*	0.826**	0.077	0.836**	0.387	0.971**	0.836**	0.809**	0.924**
RGR	0.677*	0.735**	0.260	0.887**	0.558	0.893**	0.951**	0.886**	0.940**
CGR	0.692*	0.897**	0.066	0.910**	0.389	0.976**	0.971**	0.933**	0.993**
NAR	0.655*	0.881**	0.160	0.884**	0.484	0.972**	0.932**	0.927**	0.973**
BMD	0.651*	0.890**	0.780	0.947**	0.403	0.982**	0.980**	0.926**	1.000**

Table 2: Continue...



	R/S ratio	Leaf area	LAI	LAD	LAR	SLW	RGR	CGR	NAR	BMD
R/S ratio	1									
Leaf area	-0.269	1								
LAI	-0.269	1.000**	1							
LAD	-0.257	0.998**	0.998**	1						
LAR	0.254	-0.803**	-0.803**	-0.797**	1					
SLW	0.382	0.859**	0.859**	0.845**	-0.880**	1				
RGR	-0.232	0.876**	0.876**	0.879**	-0.975**	0.857**	1			
CGR	-0.26	0.975**	0.975**	0.970**	-0.904**	0.917**	0.943**	1		
NAR	0.22	0.933**	0.933**	0.935**	-0.903**	0.937**	0.938**	0.973**	1	
BMD	-0.295	0.975**	0.975**	0.972**	-0.903**	0.923**	0.940**	0.992**	0.972**	1

* ($p=0.05$) level of significance, ** ($p=0.01$) level of significance

quantity be achieved with the applications of phytohormones.

3.3. Effect of phytohormones on andrographolide content correlation with parameter studied

The results of effect of phytohormones on andrographolide content correlation with parameter studied are presented in Table 2. Growth parameter like plant height, number of leaves, number of primary branches, shoot dry weight, root dry weight, root-shoot ratio were had non-significant correlation with quality parameter i.e. andrographolide % at harvest. Similarly, physiological parameter like leaf area, leaf area index (LAI), leaf area duration (LAD) and yield parameter dry herbage yield had also shown to non-significant correlation with the quality i.e. andrographolide at harvest. Leaf area ratio (LAR) found negatively highly significant correlation with quality parameter i.e. andrographolide percentage at harvest. Growth parameters like leaf dry weight (0.688), total dry weight (0.654) were had positive significant correlation with quality parameter i.e. andrographolide percentage at harvest. Similarly, other physiological parameter had also shown to positive significant correlation with the quality i.e. andrographolide viz. SLW (0.699), RGR (0.677), CGR (0.692), NAR (0.655) and BMD (0.651).

4. Conclusion

The effect of phytohormones on dry herbage yield at harvest were found significant and highest was (4913.84 kg ha⁻¹) in GA₃ 100 mg l⁻¹ followed by paclobutrazol 100 mg l⁻¹ (4901.68 kg ha⁻¹) as compared to control. The effect of phytohormones on andrographolide content were recorded significant and highest was (1.453%) observed in GA₃ 200 mg l⁻¹. Comparatively higher andrographolide content was also recorded in GA₃ 100 mg l⁻¹ (1.403%) and paclobutrazol 100 mg l⁻¹ (1.395%).

5. References

Anuradha, V.E., Jaleel, C.A., Salem, M.E., Gomathinayagam,

M., Panneerselvam, R., 2010. Plant growth regulators induced changes in antioxidant potential and andrographolide content in *Andrographis paniculate* Wall.ex Nees. Pesticide Biochemistry and Physiology 98(2), 312–316.

Basak, B.B., Jat, R.S., Gajbhiye, N.A., Saha, A., Manivel, P., 2020. Organic nutrient management through manures, microbes and biodynamic preparation improves yield and quality of kalmegh (*Andrographis paniculata*) and soil properties. Journal of Plant Nutrition 43(4), 548–562.

Caceres, D., Hancke, J., Burgos, R., Wikman, G., 1997. Prevention of common colds with *Andrographis paniculate* dried extract: A pilot double-blind trial. Phytomedicine 4(2), 101–104.

Carlo, C., Berman, S., John, G., Babish, X., Lynne, S., Melissa, D., Kameron, W., Cynthia, A., Leanna, J., 2000. A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytotherapy Research 14(5), 333–338.

Das, D., Bandyopadhyay, M., 2021. Manipulation of DXP pathway for andrographolide production in callus cultures of *Andrographis paniculata*. Planta 254(2), 23.

Gajbhiye, N.A., Khristi, S., 2010. Distribution pattern of andrographolide and total lactones in different parts of kalmegh plant. Indian Journal of Horticulture 67(4), 591–593.

Gorter, M.K., 1911. The bitter constituent of *Andrographis paniculata* Nees. Recueil des Travaux Chimiques des Pays-Bas 30, 151–160.

Gudhate, P.P., Lokhande, D.P., Dhupal, K.N., 2009. Role of plant growth regulators for improving andrographolide in *Andrographis paniculata*. Pharmacognosy Magazine 5(19), 249–253.

Handa, S., Sharma, A., 1990. Hepatoprotective activity of andrographolide against galactosamine and paracetamol intoxication in rats. The Indian Journal of Medical Research 92(B), 284–292.



- Hermesz, E., Ferencz, A., 2009. Identification of two phospholipid hydroperoxide glutathione peroxidase (gpx4) genes in common carp. Comparative Biochemistry and Physiology (CBP) Part C: Toxicology and Pharmacology 150, 101–101.
- Kanjilal, P.B., Bordoloi, S., Kalita, R., Burman, P., Singh, R., 2002. Cultivation practices for kalmegh (*Andrographis paniculata*) and spiderling (*Boerhaavia diffusa*) in Assam, India. In: Govil, J.N., Pandey, J., Shivkumar, B.G., Singh, V.K. (Eds.), Crop Improvement, Production Technology, Trade and Commerce. Studium Press LLC, USA, 175–180.
- Kim, Y., Seo, C.W., Khan, A.L., Mun, B.G., Shahzad, R., Ko, J.W., Yun, B.W., Park, S.K., Lee, I.J., 2018. Exo-ethylene application mitigates waterlogging stress in soybean (*Glycine max* L.). BMC Plant Biology 18(254), 1–16.
- Kumar, A., Purohit, S.S., 2011. Plant Physiology fundamentals and applications (Student Edn.). Jodhpur, India, 363. ISBN (13): 978-81-88826-56-8.
- Liu, C., Feng, N., Zheng, D., Cui, H., Sun, F., Gong, X., 2019. Uniconazole and diethyl aminoethyl hexonate increase soybean pod setting and yield by regulating sucrose and starch content. Journal of the Science of Food and Agriculture 99(2), 748–758.
- Maity, U., Dutta, P., Laya, B., Chaudhuri, K.M., 2019. Enhancement of green leaf yield in Tea [*Camelia sinensis* (L.) O Kuntze] as a function of pigments by foliar application of Triaccontanol. International Journal of Bio-resource and Stress Management 10(4), 451–455.
- Matsuda, T., Kuroyanagi, M., Sugiyama, S., Umehara, K., Ueno, A., Nishi, K., 1994. Cell differentiation-inducing diterpenes from *Andrographis paniculate* Nees. Chemical and Pharmaceutical Bulletin 42(6), 1216–1225.
- Nagel, L., Brewster, R., Riedell, W., Reese, R., 2001. Cytokinin regulation of flower and pod set in soyabeans (*Glycine max* L Merr.). Annals of Botany 88(1), 27–31.
- Niranjan Reddy, V. L., Malla Reddy, S., Ravikanth, V., Krishnaiah, P., Venkateshwar Goud, T., Rao, T.P., Venkateswarlu, Y., 2005. A new bis-andrographolide ether from *Andrographis paniculate* Nees and evaluation of anti-HIV activity. Natural Product Research 19(3), 223–230.
- Panase, V.G., Sukhatme, P.V., 1984. Statical methods for agriculture workers. ICAR, New Delhi, 97–105.
- Raina, A.P., Gupta, V., Sivaraj, N., Dutta, M., 2013. *Andrographis paniculatea* (Burm. f.) Wall. Ex Nees (kalmegh), a traditional hepatoprotective drug from India. Genetic Resources and Crop Evolution 60(2), 1–9.
- Steffens, B., Wang, J., Sauter, M., 2006. Interaction between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious root in deepwater rice. Planta 223(3), 604–612.
- Sule, A., Ahmed, Q.U., Latip, J., Samah, O.A., Omar, M.N., Umar, A., Dogarai, B.B., 2012. Antifungal activity of *Andrographis paniculata* extracts and active principles against skin pathogenic fungal strains *in vitro*. Pharmaceutical Biology 50, 850–856.
- Sun, F., Feng, N., Zheng, D., Cui, H., He, T., Zhao, J., 2016. Effect of plant growth regulators S3307 and DTA-6 on physiological metabolism and GmAc gene expression in soybean. Scientia Agricultura Sinica 49(7), 1267–1276.
- Travaglia, C., Reinoso, H., Bottini, R., 2009. Application of abscisic acid promotes yield in field-cultured soybean by enhancing production of carbohydrates and their allocation in seed. Crop and Pasture Science 60(12), 1131–1136.
- Wintachai, P., Kaur, P., Lee, R.C., Ramphan, S., Kuadkitkan, A., Wikan, N., Ubol, S., Roytrakul, S., Chu, J.J., Smith, D.R., 2015. Activity of andrographolide against chikungunya virus infection. Scientific Reports 5, 14179.
- Xu, H.X., Wan, M., Loh, B.N., Kon, O.L., Chow, P.W., Sim, K.Y., 1996. Screening of traditional medicines for their inhibitory activity against HIV-1 protease. Phytotherapy Research 10, 207–210.

