

Full Length Research Paper

# Variations in growth of tubers of field grown *Coleus barbatus* as affected by different hormonal treatments

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Accepted 21 October, 2020

The aim of the present study was to determine the effect of Plant Growth Regulators (PGRs) viz. Indole-3-acetic acid (IAA), Gibberellic acid (GA<sub>3</sub>) and Kinetin in their physiological ranges from 10<sup>-7</sup> to 10<sup>-5</sup> M on tuberous roots of *Coleus barbatus* (patharchur) with their respective control. The certified seed material was procured from Forest Research Institute (FRI), Dehradun, Uttarakhand (India). Significant variation was reported in growth of tuberous roots of *Coleus* due to hormonal treatments and out of these three hormones IAA at 10<sup>-5</sup> M was observed highly effective as compared to control, GA<sub>3</sub> and Kinetin. Application of IAA at 10<sup>-5</sup> M, resulted in maximum promotory effect on number of tubers, fresh weight and dry weight, followed by GA<sub>3</sub> at 10<sup>-5</sup> M, Kinetin at 10<sup>-7</sup> M as compared to control. Application of IAA, GA<sub>3</sub> and Kinetin resulted in more number of tubers, which was found increased by c.a. 52, 28 and 16%, respectively as compared to control.

**Key words:** *Coleus barbatus*, indole-3- acetic acid, gibberellin, kinetin.

## INTRODUCTION

Medicinal plants are of great significance to the health of individuals and communities (Hill, 1952). India is well known as the “Emporium of Medicinal Plants”. Due to their great importance, demand of medicinal plants has increased numerous folds. *Coleus barbatus*, belonging to the family of Lamiaceae, is a well known plant throughout the country and is known as patharchur, pashanbhedi in Hindi and Makkadi beru or Mangana beru in Kannada. It is one of the most potential medicinal crops of the future, as its pharmacopieal properties have been discovered only recently. Its tuberous roots are rich source of coleonol which is being developed as a drug for hyper-tension, glaucoma, asthma, congestive heart failures and certain types of cancers. While its foliage is employed in treating intestinal disorders and used as a condiment since long time, it is under cultivation in parts of Rajasthan, Maharashtra, Karnataka and Tamil Nadu. It decreases the blood pressure, cough, heart diseases etc. *C. barbatus* is used medicinally in Africa, Arabia, and

Brazil as well. The root tubers of the plant are prepared and eaten as a condiment in India. Other Indian *Coleus* spp. is used in traditional Ayurvedic healing. Chemical studies of alcoholic extracts of the tubers of *C. barbatus* led to isolation of the coleonol, which has become an important research tool in studying the roles of the enzyme adenylate cyclase and cyclic-AMP in cellular physiology. In addition, coleonol is reported to have been used in the preparation of medicines preventing hair greying and restoring grey hairs to its normal colour. Indiscriminate collection of *C. barbatus* has led to rapid depletion of wild populations resulting in its listing as a plant vulnerable to extinction in India (Gupta, 1988). The genus *Coleus* was first described by De Loureiro (1970). The name *Coleus* is derived from the Greek word *Koleos*, which means sheath around the style (De Loureiro, 1970). *C. barbatus* grows wildly in arid and semiarid regions of India. The pharmacological activities were attributed to coleonol, located in root tubers.

Medicinal plants have curative properties due to the presence of various complex chemical substances of different chemical nature, which are found as secondary plant metabolites in one or more parts of these plants. These plant metabolites, according to their composition,

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**Table 1.** Inputs for land preparation before sowing seeds.

S/No.	Materials	Per acre	Per hectare
1.	Seeds (kg)	20,000	50,000
2.	Farm yard manure (t)	4	10
3.	Fertilizers (kg)	N	10
		P <sub>2</sub> O <sub>5</sub>	24
		K <sub>2</sub> O	20

Half on N, whole of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O may be applied as a basal dose followed by the remaining ½ N at 30 days after planting as top dressing.

are grouped as glycosides, alkaloids, corticosteroids, essential oils etc. Among them, alkaloids form the largest group; including, quinine (*Cinchona*), reserpine (*Rauvolfia*), coleonol (*Coleus*), aconitine (*Aconite*) etc. and glycosides form another important group. Infact, plants provided safe and effective drugs and has no harmful side effects unlike modern synthetic drugs and antibiotics (Aswal and Goel, 1996). Concern for species conservation and a sustained supply of the root material led to consideration of developing *C. barbatus* as a medicinal crop. The focus of the development studies was the increased yield of root tubers and coleonol. As a consequence of this development, *C. barbatus* is now being cultivated as a source of coleonol.

Ironically, the economic cultivation of this miraculous herb has not been realized. Most of its supply still comes from wild resources. There is a need to increase its productivity to cope with the increasing demand. In this context, the use of growth regulators particularly IAA, GA<sub>3</sub> and Kinetin could be useful, as they have been shown to possess potential to enhance crop productivity (De-La-Guardia and Benlloch, 1980; Mousa et al., 2001). The evidence for hormone involvement comes from correlation of hormone concentration with specific development stages, effects of applied hormones and the relationship of hormones to metabolic activities. Plant hormones are signal molecules produced at specific locations and regulate physiological processes in target cells at other locations in very low concentrations. The treatments with growth regulators probably antagonize the effect of growth inhibitory substance and also enhance rate of metabolism during germination (Verma and Tandon, 1988).

Auxin is a generic term for compounds characterized by their capacity to induce elongation in shoot cells. These substances are chemically related to Indole-3-acetic Acid (IAA), which itself appears to be the principal auxin in many plants. They resemble IAA in physiological action. IAA was found as a constituent of human urine. Additionally, it was active in promoting the growth of some plant tissues or organs. IAA was the first phytohormone identified, considering its important role in plant development; main subject of research is its

metabolism. Cooper (1935) was first to examine its effect in stimulating rooting from stem cuttings. Thus, auxin requirement to induce rooting, and subsequent interaction between applied auxin and the tissue at the base of the cutting has been the subject of much research. In case of medicinal plant like Low bush blueberry, Debnath (2007) suggested the role of auxin in serving its various physiological activities.

Gibberellins (GAs) are tetracyclic diterpenoid growth factors that are essential regulators of stem elongation and other plant developmental processes (Hooley, 1994; Swain and Olszewski, 1996). The detailed mechanism of the gibberellins effects on growth is largely unknown. However, it is well documented that this phytohormone affects stem growth, through both cell elongation and cell division (Kende and Zeevaart, 1997). GA<sub>3</sub> is a well known stimulator of cell expansion, cell elongation and elongation of internodes, Huttly and Phillips (1995). Cytokinins are involved in many plant processes, including cell division, shoot and root morphogenesis, cell enlargement, auxiliary bud release and senescence (Kieber, 2002). Therefore, this study was aimed at comparing and characterizing the effects of IAA, GA<sub>3</sub> and Kinetin on growth of tuberous roots of patharchur.

## MATERIALS AND METHODS

A completely randomized block design experiment was carried out at the Department of Botany, R.C.U. Govt. P.G. College campus, Uttarkashi (30° 22' to 31° 25' N and 77° 51' to 79° 27' E), India. The information about habit, habitat, distribution and medicinal uses of *C. barbatus* was collected from various research publications. The seeds of *C. barbatus* were procured from Forest Research Institute (FRI), Dehradun for the study. It thrives better in porous and well drained soils with a pH ranging from 5.5 to 7. However, it does not require very fertile soils and can be economically grown even on the soils with marginal fertility. It prefers humid climate with a RH ranging from 83 to 95% and a temperature range between 10 - 25°C for its successful growth. The annual rainfall recorded ranges from 100 to 160 cm, mainly during June to September months. It is also found to perform well in less humid and warmer regions when grown as an irrigated crop (Table 1).

Crop was planted in third week June with the onset monsoon. Before planting, the field was ploughed deep soon after the receipt of pre-monsoon showers and brought to fine tilts. Further, the land

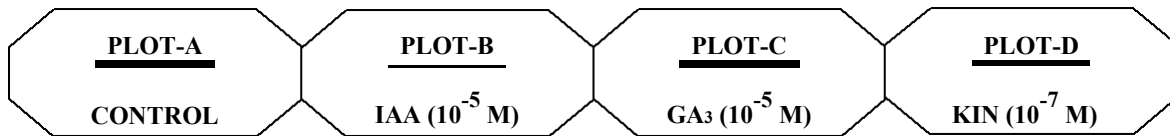


Figure 1. Different concentrations.

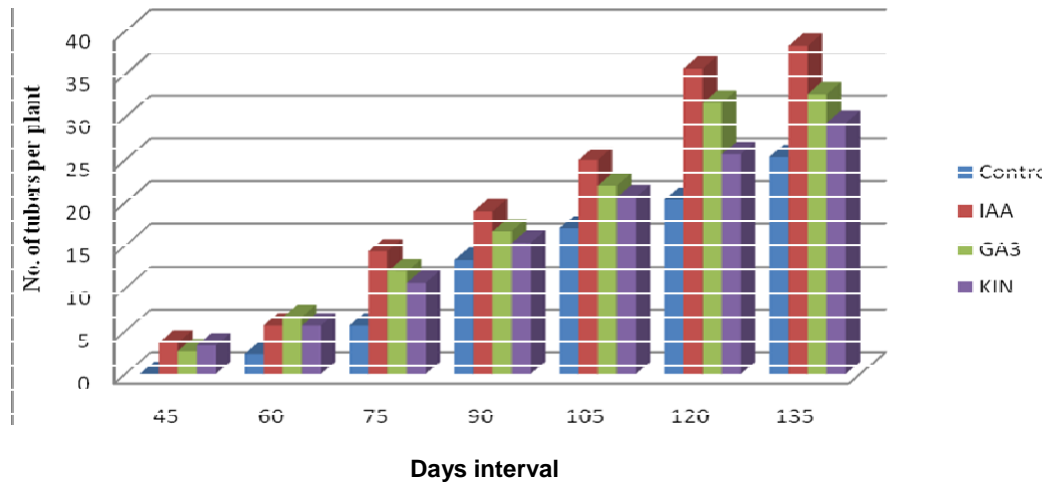


Figure 2. Variation in number of tuber per plant due to hormonal treatment at the interval of 15 days.

was divided into four plots (Plot A, B, C and D) of convenient sizes. The seeds were treated with growth regulators (IAA, GA<sub>3</sub> and Kinetin) to determine their effects on growth of tuberous roots of *C. barbatus*, and then compared with control. Seeds of *C. barbatus* were sown in fields of sandy loam soil, in fixed measurement in 4 plots for the plant. Before sowing, the seeds were pre-soaked and treated with HgCl<sub>2</sub>. Planting was done in-between the ridges of 2 feet and plant to plant distance were kept 1.5 feet. Each plot was fenced by barbed wire to avoid any biotic interference.

In Plot A, no treatment of growth hormones were given. While the rest three plots were treated with different concentrations of IAA (10<sup>-5</sup> M), GA<sub>3</sub> (10<sup>-5</sup> M) and Kinetin (10<sup>-7</sup> M) on alternate days till maturity, as these concentrations of IAA, GA<sub>3</sub> and Kinetin was reported best effective during lab studies, conducted before doing field trial. Plot B, was sprayed with IAA at the concentration of 10<sup>-5</sup> M on alternate days after the seedlings of both the species attained two leaf stages. Plot C, was sprayed with GA<sub>3</sub> at the concentration of 10<sup>-5</sup> M and Plot D was sprayed with Kinetin at the concentration of 10<sup>-7</sup> M, (as this concentration for Kinetin was reported best effective during lab studies) on alternate days after the seedlings of both the species attained two leaf stages. The samples for growth analysis were taken regularly at the interval of 15 days from each plot separately, after the seedling emergence (two leaf stage) till maturity. For each study three phenotypically identical plants from each plot were taken carefully and brought to the laboratory and were washed with running tap water to remove soil particles. The growth measurements in gram per plant were taken on tuberous root for each treatment separately. The mean values of three replicates of each plot were calculated and results were presented in graphical form in Figures 2, 3 and 4 with their statistical analysis shown in Tables 2, 3, and 4, from first sampling to the last one. The mean of each treatment was calculated and for quantitative evaluation of effects of various treatments, the values were used to compare with the control (Figure 1). Analysis of Variance (ANOVA)

was determined by using SPSS package 10.0 version of the software. Data were analyzed and subjected to ANOVA depending upon the experimental design according to Gomez and Gomez (1984).

$$\text{Standard Error Mean (SEM)} = \sqrt{2 \times \text{MSSe} / R}$$

Where, MSSe = Mean Sum of Square of error and R = No. of Replications

$$\text{Critical Difference CD at 5\%} = \text{SEM} \times t \text{ 5\% error degree of freedom}$$

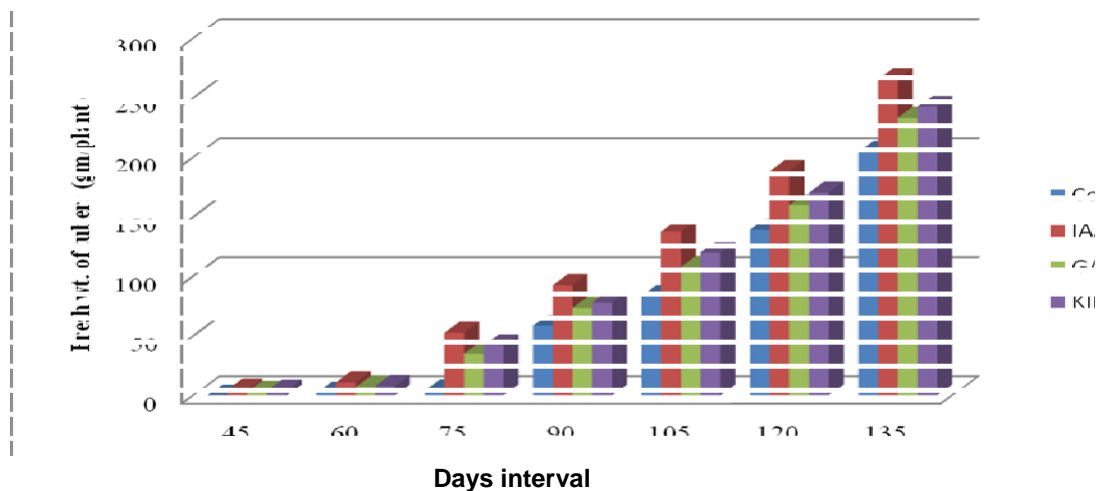
Where, SEM = Standard Error mean and t 5% = Student t value for the degree of freedom.

$$\text{Critical Variance (CV)} = (100 \times \text{MSSe}) / \text{Total of Mean of the treatments.}$$

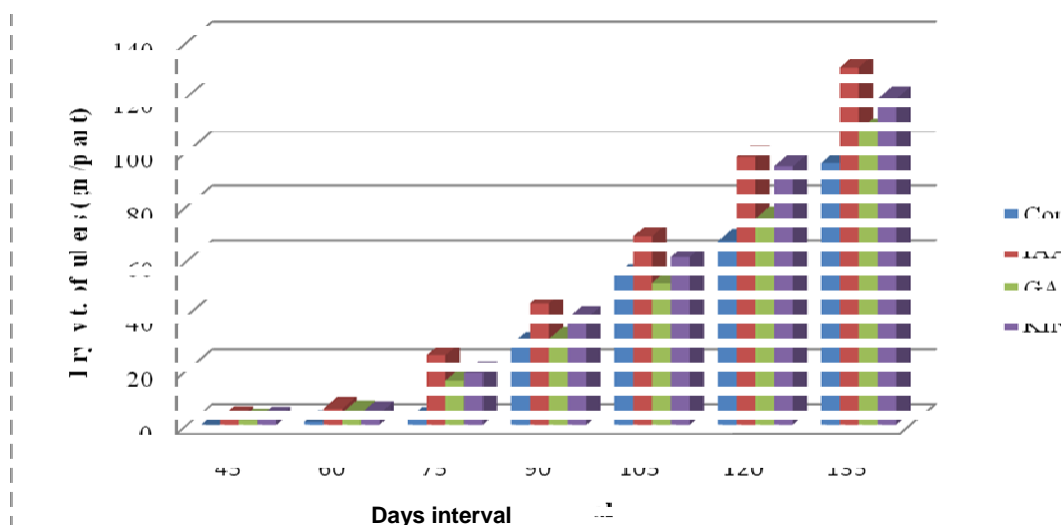
Test for Standard Error mean of mean absorbencies of control and treated plants are made by calculating 't' value (student's t) and by comparing with critical value of 't' at 5% confidence interval and degree of freedom = 12 and Critical value for 't' is 1.78. There are four treatments, each treatment considers of 3 replications and plant age was recorded at the interval of 15 days. All data was subjected to ANOVA and comparisons of mean were made with least significant difference test at the 5% level probability. Statistical F-test was evaluated at P 0.05.

## RESULTS

The study revealed that application of IAA, GA<sub>3</sub> and Kinetin at a particular concentration that is 10<sup>-5</sup>, 10<sup>-5</sup> and



**Figure 3.** Variation in fresh weight of tuber in g/ plant due to hormonal treatment at the interval of 15 days.



**Figure 4.** Variation in dry weight of tuber in g/ plant due to hormonal treatment at the interval of 15 days.

**Table 2.** Analysis of variance (No. of tubers *Coleus barbatus*).

Days	45	60	75	90	105	120	135
SEM	0.45	0.78	1.32	0.56	1.08	0.89	1.33
CD at 5%	0.80*	1.39*	2.36*	0.99*	1.92*	1.58*	2.37*
CV	22.88	18.83	15.22	4.43	6.24	3.93	5.23

\*Significance level for F-test at 5% P ns- not significant.

**Table 3.** Analysis of variance (tubers fresh wt. *C. barbatus*).

Days	45	60	75	90	105	120	135
SEM	0.44	0.66	2.51	2.32	3.72	6.14	5.74
CD at 5%	0.78*	1.17*	4.47*	4.13*	6.62*	10.94*	10.22*
CV	17.71	11.47	9.19	3.76	4.05	4.57	3.21

\*Significance level for F-test at 5% P; ns- not significant.

**Table 4.** Analysis of variance (tubers dry wt. of *C. barbatus*).

Days	45	60	75	90	105	120	135
SEM	0.41	0.36	1.09	2.08	1.92	9.21	4.93
CD at 5%	0.73*	0.65*	1.94*	3.71*	3.42*	16.39*	8.78*
CV	31.00	11.54	8.51	7.05	4.21	12.74	5.63

\*Significance level for F-test at 5% P; ns- not significant.



**Plate. 1.** Tuber of *C. barbatus* before hormonal treatment.



**Plate 2.** Tuber of *C. barbatus* after hormonal treatment.

$10^{-7}$  M respectively was most promotive for number of tubers, fresh weight and dry weight, in comparison to control (Plates 1 and 2). However, the effect of IAA was

comparatively vigorous, followed by GA<sub>3</sub> and Kinetin. The data/observations recorded were analyzed as factorial analysis. The main factors causing variations in growth

parameter are plant growth hormones and time interval. For analysis, SPSS version 10.0 was used which gave the effect of single factorial analysis.

The combined effect of growth regulators and time interval on growth of tuber reveals that treated plants was significantly higher than those without treatment. Growth hormones causes increase in number of tubers, which in turn manifests itself in the form of more fresh and dry matter. The number of tubers, fresh weight and dry weight (g/ plant) at different growth stage at control and different hormonal treatment with their statistical analysis were presented in Figures 2, 3 and 4, with their statistical analysis in Tables 2, 3 and 4. In the present study, the number of tubers starts appearing from 45th day up to maturity that is 135th day of growth stage due to hormonal impact as compared to control. These values showed significant variance till last sampling. During control, the values for number of tubers, fresh weight and dry weight was studied at 60th day of growth stage and an average number of tubers, fresh weight, dry weight (g/ plant) noted were 2.33 t/ plant, 2.27 g/ plant and 1.08 g/ plant. When plot B, C and D were subjected to hormonal treatment that is IAA ( $10^{-5}$  M), GA<sub>3</sub> ( $10^{-5}$  M), and Kinetin ( $10^{-7}$  M) conc., it was observed that IAA showed maximum level of significance in no. of tubers, fresh weight and dry weight at 60<sup>th</sup> day stage and was increased by ca. 143, 380 and 443%. Similar stimulatory effects of GA<sub>3</sub> and Kinetin on the aforesaid parameters can be likewise accounted by the promotion of c.a. 186, 215, 315, 143, 246, and 294% respectively as compared to that of control. The level of significance goes on increasing linearly.

IAA was found to outperform GA<sub>3</sub>, and Kinetin, with  $10^{-5}$  M concentration, proving relatively more effective till last sampling viz. 135th day growth stage. It was observed that on last sampling due to the effect of IAA on number of tubers, fresh and dry weight of tubers in g/plant was reported to be increased by c.a. 52, 30 and 37% respectively as compared to control. While, due to GA<sub>3</sub> it was increased by c.a. 28, 14 and 13% respectively whereas, due to Kinetin it was promoted by c.a. 16%, 19% and 25% respectively. This postulate is supplemented by the observed increment in fresh and dry weight with the increase in number of tubers, due to the effect of plant growth hormones. The influence of plant growth hormones on the aforementioned parameters may be ascribed to the stimulation of growth causing an overall increase in yield.

The crop is ready for harvest after about 135 - 150 days of planting. During the growing period if any flowers are produced they should be nipped off to obtain more biomass. The crop is harvested manually by uprooting the individual plants. The tubers are separated, cleaned chopped into pieces and shade dried yielding about 12 % of the dry matter and 0.44% coloneol. On an average, a yield of 95.83 g/tuber of dried tubers per hectare may be obtained. However, if proper cultivation practices with the

spray of IAA are applied a yield of 130.76 g/tuber of dried tuber can be easily obtained per hectare.

## DISCUSSION

Hence, the present study revealed that application of plant growth hormones viz. IAA, GA<sub>3</sub> and Kinetin in their physiological ranges that is  $10^{-7}$  to  $10^{-5}$  M on growth of tubers of *C. barbatus* have been found promotory in comparison to control. These findings were in agreement with the findings of Mukhtar (2004) and Chauhan et al. (2009). The promotory effect of growth regulators in a particular concentration is a well-known feature. The IAA has more pronounced effect on underground part that is root, whereas GA<sub>3</sub> and Kin on aboveground parts that is plant height, flowering etc. Auxins were probably the best hormone for general use because they are generally non-toxic to plants over a wide range of concentration and effective in promoting rooting of large number of plant species. These findings are supported by the results of Hartman et al. (1990) and Davies (1995). It was also observed that in treated plots, rooting appeared from 45th day stage whereas in control it started appearing from 60th day stage, also reported by Weaver (1972). This may be because auxins are known to stimulate the root development by including root initial that are different from the young secondary phloem, cambium and pith tissues. Early rooting promoted due to the effect of growth regulators and these findings were in agreement with the findings of Gurumurthi et al. (1984) and Klass et al. (1987).

Likewise, IAA also exerts an influence on plant growth by enlarging leaves and increasing photosynthetic activities in plants. It also activates the translocation of carbohydrates during their synthesis (Rietenour et al., 1996). It was resulted from present research work that an increase in dry matter production, observed by the application of growth regulators on both the species. Similar findings were reported by Leben and Barton (1957), Castro and Bergann (1973) on bean crop and Agarwal et al. (1994) on *Trifolium*. While, GA<sub>3</sub> also promote rooting but to some extent, it showed more pronounced effect on shoot development, as GA<sub>3</sub> is a well known causative of wall extensibility, leading to cell expansion, elongation of internodes and ultimately increased plant height. These findings were in agreement with the findings of Liu and Loy (1976), Moore (1989), Huttly and Phillips (1995) and Kende and Zeevaart (1997). GA<sub>3</sub> induced wall extensibility (Huttly and Phillips, 1995) and expansion, elongation of internodes (Moore, 1989) and expansion of leaf area which in turn manifests itself in the form of more dry matter. At the time of cell division, the decisive cells need more nutrients, which are made available by the efficient manipulation, absorption and utilization of the available nutrients triggered by the GA<sub>3</sub> spray.



Reports are available that application of PGRs ultimately affects the endogenous level of auxins (Andreae and Andreae, 1953; Kuraishi and Muir, 1962; Wort, 1964), which finally affects the growth and development of plant. Auxins (IAA) interact with one or more components of the biochemical system involved in the protein synthesis. However, it has been identified the proper step where auxins exerts an effect. According to popular concept, auxins do act through influence upon enzyme involved in growth. Roychaudhary and Sen (1964) found that application of auxins to peas resulted in an enhanced RNA synthesis.

On the contrary, Kinetic also showed promotory effect on the growth of tubers which leads to increased fresh and dry weight. This was characterized by stimulation of cell division and enlargement (Jablonski and Skoog, 1954; Miller, 1955). Application of growth regulators might have contributed to increased availability of growth promoters at critical stages and thus increasing the yield. Similar findings were also reported by Biswas and Chaudhari (1981) and Malik et al. (1986) carried out experiments to study the effect of growth hormones on *Arachis hypogea*.

## ACKNOWLEDGEMENTS

The authors are thankful to the Principal, Pt. L.M.S. Government, P.G. College, Rishikesh (Dehradun), for giving support and advices whenever necessary. RKG and GKD are thankful to UGC, New Delhi and UCOST, Dehradun for financial support.

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