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PHCOG MAG.: Research Article

Role of Plant Growth Regulators for Improving Andrographolide in *Andrographis Paniculata*

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ABSTRACT

The present study was aimed at improving the content of therapeutically active principle, andrographolide and whole plant biomass of *Andrographis paniculata* (Burm. F.) Wall.Ex Nees, by foliar application of plant growth regulators. The pot cultured plants were treated with Gibberellic acid (GA_3), Indole acetic acid (IAA), Naphthalene acetic acid (NAA) and Benzyl amino purine (BAP), in the concentration of 25, 50, and 100mg/l each. The plants sprayed with distilled water were maintained as control. The foliar application of these growth regulators was initiated at 30 DAT and continued upto flowering stage at an interval of 10 days. The HPLC analysis revealed that, application of IAA-50mg/l was very effective to enhance the andrographolide content (45%), which was followed by NAA-50mg/l (37%) over control. The whole plant biomass (Panchang) of *Andrographis* is used in medicine and hence fresh and dry biomass was also determined. The results indicated that NAA (Fresh Weight: 221%, Dry Weight: 155%) and IAA (Fresh Weight: 215%, Dry Weight: 120%) emerged out as the best treatments for improving fresh and dry biomass over control. The treatments of IAA and NAA (50mg/l) were found to be most effective for improving whole plant biomass as well as andrographolide content which can be recommended for the growers.

Keywords: *Andrographis, Andrographolide, Plant Growth Regulators, Whole Plant Biomass.*

INTRODUCTION

Since ancient times, *Andrographis paniculata* is used as an effective drug in traditional Siddha and Ayurveda. Tribal people from India and some other countries use this multipurpose drug for clinical applications. Extract of this plant exhibits antityphoid and antifungal activities. It is also reported to possess antibiotic, antimalarial (1), hepatoprotective (2), antipyretic and anti-inflammatory (3), and cancerolytic (4, 5) properties and used as an immunostimulant. A recent study conducted at Bastyr University, USA confirmed its anti-HIV activity (6).

Among the various medicinal plants, *Andrographis paniculata* is in pressing demand because of its anti-HIV property. But the biological yield and production of secondary metabolites is very low in this plant. The estimated demand of this drug in India is 1000 tonnes per year. The average rate of the dry herb, in Indian market is Rs. 1800/- per quintal and average income is Rs.5000/-Kg dry herb per hectare. The cost of cultivation per hectare is Rs.20750/- and gross returns is Rs.75000/- whilst net return is Rs.54250/- there is no authentic data on export of this drug from India.

The plant is mainly collected from wild resources. Suitable areas for its cultivation are plains of UP, Bihar, West Bengal, Assam, Orissa, Gujarat, Madhya Pradesh, Maharashtra, Andhra Pradesh, Tamil Nadu, Kerala and Karnataka(7).

OBJECTIVE

To improve the andrographolide content in *Andrographis paniculata* (Burm. F.) Wall.Ex Nees, through foliar

application of different plant growth regulators (PGRs) in various concentrations.

MATERIALS AND METHODS

Plant material:

Seedlings of *Andrographis paniculata* were procured from Regional Research Institute (Ay), Kothrud, Pune-411028. Seedlings were potted in medium sized earthen pots containing soil and FYM (3:1). As the young seedlings are highly sensitive to direct sunlight, they were first stabilized in shade net conditions and then transferred to natural conditions. After their acclimatization to natural conditions (30 DAT), the treatment schedule of PGRs was initiated.

Treatment details:

Foliar application of GA_3, IAA, NAA and BAP in the concentration of 25, 50, and 100mg/litre each was given up to 90 DAT at the interval of ten days. Plants sprayed with distilled water were considered as control. The analyses of plant biomass and andrographolide content were carried out after completion of treatment schedule at anthesis stage.

Estimation of whole Plant Biomass:

Fresh weight of control and treated plants with all plant parts (root, shoot, leaves, flowers and pods) was taken as fresh biomass. After shade drying for 10 to 15 days, they were again weighed for dry biomass.

HPLC analysis of andrographolide: Chemicals:

Methanol and HPLC water were obtained from Merck (Mumbai). Standard for the experiment was obtained from Sigma Aldrich Pvt. Ltd. (Mumbai).

Sample preparation for HPLC:

The powder of shade-dried plant material (2g) was extracted exhaustively for five hours in soxhlet with methanol. It was evaporated to dryness using rota vapor and dissolved in mobile phase (methanol: water) to obtain the solution of known strength (50ug/ml).

Standard Preparation

The solution in the range of 10–100 µg/ml of andrographolide was prepared in methanol.

HPLC Equipment and Conditions

Andrographolide content was analyzed by using a reverse-phase high- performance liquid chromatographic method. The andrographolide samples were chromatographed on a C_{18} Hypersil BDS column with UV detection at 254 nm.

HPLC was performed with a DIONEX (Germany) chromatograph equipped with P680 HPLC pump, a 2ml injection loop (Rheodyne, U.S.A), and a UVD170U detector, controlled with Chromeleon software (DIONEX Softtron GmbH, Germany). Compounds were separated on a 250 mm × 4.6 mm i.d., 5 µm particle size (Thermo Electron Corporation). The amount of sample injected was 20 µl.

The mobile phase was isocratic type prepared from methanol and water in proportion of 65:35(v/v). The flow rate was 0.7ml/min and detection wavelength was 254 nm.

RESULTS AND DISCUSSION

Andrographolide content:

From the Table I and Fig I to V, it was seen that all the plant growth regulator treatments resulted in to increased andrographolide content as compared to control (6 mg/g). Amongst all the PGRs, IAA 50mg/litre had a pronounced effect on the andrographolide content (8.7mg/g) which was followed by NAA 50mg/litre (8.2mg/g).

Similar results were reported in *Solanum nigrum* by Bhatt et al (8). They noted positive influence of IAA on alkaloid production. Reports in *Solanum jaminoides* (9), *Solanum kahasianum* (10), had also shown positive influence of IAA on secondary metabolite production.

In this investigation, the plants treated with NAA (50mg/litre) also showed comparatively higher content of andrographolide (Fig 2). Different species of *Solanum* like *S. khasianum* (11), *S. lanciniatum* (12), had shown a similar trend with application of NAA.

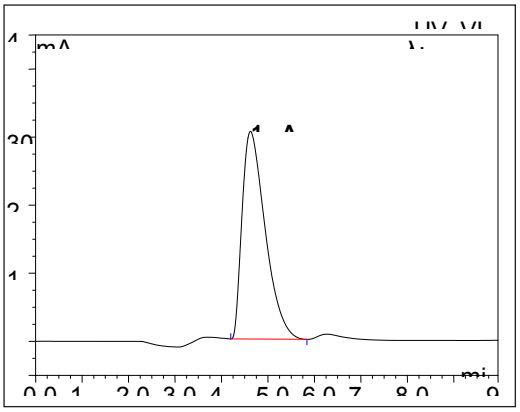

Figure I: Quantification of Andrographolide in Control plants of *Andrographis paniculata*

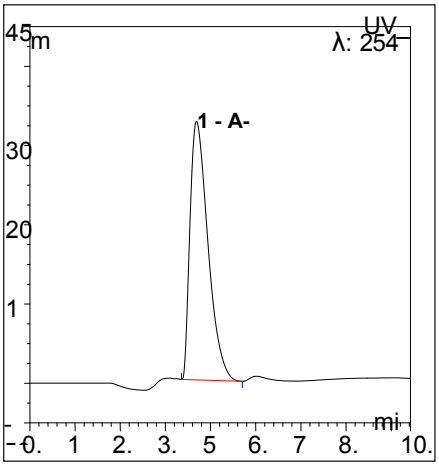

Figure II: Quantification of Andrographolide in GA_3 treated plants of *Andrographis paniculata*

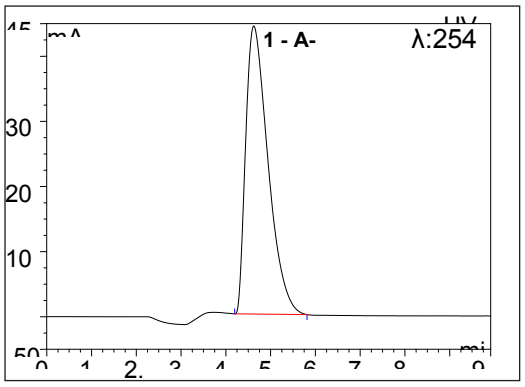

Figure III: Quantification of Andrographolide in IAA treated plants of *Andrographis paniculata*

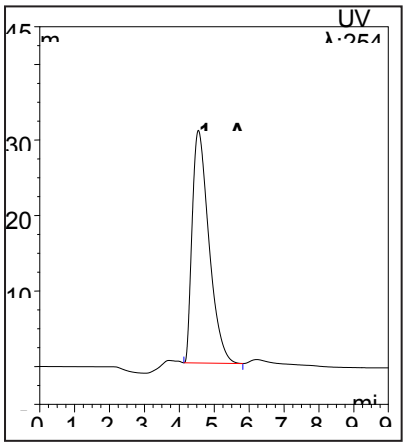

Figure V: Quantification of Andrographolide in BAP treated plants of *Andrographis paniculata*

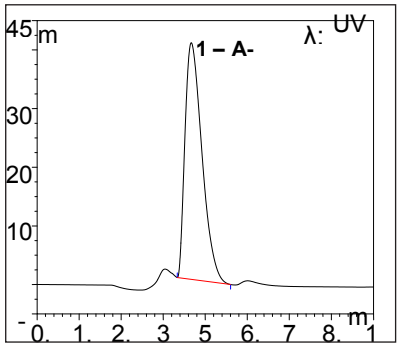

Figure IV: Quantification of Andrographolide in NAA treated plants of *Andrographis paniculata*

Table 1:Effect of different PGRs on Andrographolide content (mg/g dry weight) in *Andrographis paniculata*

Parameter Treatments (50mg/l)	Andrographolide
Control	6.0±0.42
GA_3	7.2±0.87
IAA	8.7±0.65
NAA	8.2±0.50
BAP	6.6±0.73
$LSD_{0.05}$	0.86
Significance	**

Data are means (n=5) followed by ±standard deviation.;
**represents significance at $p<0.05$

The treatments of GA_3 and BAP were also quite effective for enhancing the andrographolide content (Fig 4 and 5). GA_3 showed 20% increase in andrographolide content over control while BAP showed only 10% increase in andrographolide content over control. The influence of GA_3 on biosynthesis and accumulation of alkaloid content was recorded by various workers in *Solanum khasianum* (13). The impact of GA_3 on andrographolide content might be due to enhanced biosynthetic pathway of andrographolide.

Improved growth, biomass, chlorophylls, photosynthetic rate, leaf area are responsible to enhance the production of various secondary metabolites(14). The results of present investigation are in agreement with the above findings. The treatments of IAA and NAA emerged as best treatments for enhancing the andrographolide content by 45% and 37% respectively over control. The same treatments were also effective for improving fresh and dry biomass of the whole plant (Table I). This suggests that the improvement in quality as well as quantity can be achieved with the use of foliar application of PGRs, which can be a good approach of secondary metabolite improvement for growers.

Whole plant biomass:

All the data regarding the influence of PGRs treatments on whole plant biomass is statistically significant at $p<0.05$ (Table 2). As indicated in fig VI, comparatively increase in fresh and dry biomass per plant was recorded in the plants treated with NAA (FW 80.03gm, DW 40.09gm), which

Figure VI: Plants of *Andrographis* treated with different PGRs

Table 2: "Effect of PGRs on whole plant biomass in *Andrographis paniculata"*

PGR	Fresh weight (gm)	Dry weight (gm)
Control	24.63±1.02	15.69±0.95
GA_3	42.76±2.18	16.83±0.69
IAA	77.63±5.49	34.52±2.44
NAA	79.07±2.50	40.09±1.27
BAP	46.81±2.85	19.14±0.98
$LSD_{0.05}$	4.18	1.86
Significance	**	**

Data are means (n=5) followed by ±standard deviation.
**represents significance at $p<0.05$

was followed by IAA (FW 77.63gm, DW 34.52gm), GA_3 (FW 46.81gm, DW 19.14gm) and BAP (FW 42.76gm, DW 15.69gm) treatments as compared to control (FW 24.63gm, DW 6.83gm).

Plant growth regulators are known to have their positive effect on growth, translocation and flowering (15, 16). The effect of gibberellic acid on growth, photosynthesis, enzyme activities and productivity have been well studied (16, 17, 18).

The increase in fresh and dry biomass of *Andrographis paniculata* might be due to the improvement in the primary metabolites like carbohydrates, reducing sugars, proteins, starch, etc., due to PGRs treatments. This improvement might have caused the improvement in growth. The treatments of various growth regulators might be responsible for increased transport of these assimilates from source (leaves) to sink (pods and seeds) and ultimate conversion into final yield. The whole process of manipulation of source-sink relation might have favoured the yield increase. The use of PGRs in agriculture has increased only because of this property of PGRs. GA_3 enhances the growth through mobilization of reserve starch for root and shoot growth and their elongation (19). The effect of growth regulators for improving the growth and productivity in *Asparagus racemosus* have indicated that combination of growth regulators can improve the productivity in *Asparagus* (20). The significant increase in morphine yield of opium per plant (104%) using foliar application of GA_3 and triacontanol in combination have been reported (21).

Similarly, the significant enhancement with NAA as compared to control, in growth and yield of menthol oil in *Mentha arvensis* using 40 mg/l GA_3 has been reported (22). However, Hudge *et al* (23) have reported NAA (25 and 50ppm) as the best treatment for improving number of fruits per plant, biological yield and seed yield per plant in *Abelmoschus moschantus* . The increase in growth and yield of *Artemisia annua* due to application of IAA has been obtained (24).

CONCLUSION

The overall results have confirmed that PGRs treatments are effective for improving the fresh and dry biomass as well as andrographolide content in *Andrographis paniculata*. The final objective of sellers and buyers of medicinal plants is to get sufficient quantity of active secondary metabolites in them. Not only this efficacy and efficiency of medicinal plants for therapeutic use is mostly depending on the same. From the results of present investigation on this aspect, it was found that IAA and NAA at the concentration of 50mg/l were highly useful to improve the content of andrographolide by 45% and 37% respectively over control. With the support of multilocation trials at commercial level these treatment are recommendable to the growers intending to improve andrographolide content.

Thus, the application of PGRs if used judicially will help to get better production of whole plant biomass and best quantity of andrographolide with minimum inputs. This methodology will definitely help to reduce the burden of heavy doses of chemical fertilizer and inorganic manures on the soil and farmers.

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