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EFFECT OF PLANT GROWTH REGULATORS ON SECONDARY METABOLITES ACCUMULATION AND ANTIOXIDANT ACTIVITY OF *CATHARANTHUS ROSEUS* L.

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ABSTRACT: Secondary metabolites not only play a vital role in plant defense but also had shown various medicinal properties which provide a scientific base for using herbs and different plants as alternative medicines that were used by ancient communities. Many of the drugs today are simple synthetic modifications of the natural compounds. The developing commercial requirements of secondary metabolites in recent years has resulted in a great interest in secondary metabolism. Different strategies have been studied with the objective of improving the production of secondary metabolites. Many studies proved the role of plant growth regulators in the accumulation and production of many of these active and therapeutic compounds. Short term experiment was carried out to investigate the response of *Catharanthus roseus* L. plant to the foliar spray of GA_3, IAA and 6-bezylaminopurin at (0, 30, 60, 90 ppm) concentrations during the blooming stage. Three parameters were studied, phenols content, alkaloids content and the antioxidant activity in leaves extracts of this plant. Results showed there were significant increments in all studied parameters, that was obvious at high concentrations of all plant growth regulators treatments. The significant increase in antioxidant activity percentage was in correlation with the significant increases in phenols and alkaloids content. This study is encouraging economically to produce high amounts of these secondary metabolites that had proved their antimicrobial and antitumor properties for decades.

INTRODUCTION: Medicinal plants were considered as effective and safer alternatives to synthetic antibiotics. Plant secondary metabolites are natural products that have an important role in plant defense system against pathogenic attacks and environmental stresses. According to its remarkable activities, plant secondary metabolites were widely used as medicines and food additives [1, 2].

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Different strategies have been widely studied to improve the production of secondary metabolites in plants, one of these strategies included using plant growth regulators to stimulate secondary metabolites biosynthesis [3, 4, 5, 6].

Catharanthus is a genus from the family Apocynaceae that has eight species of herbaceous perennial plants, six endemics to the island of Madagascar, the seventh and eighth are native to the Indian subcontinent in southern Asia. *Catharanthus roseus* plant is one of the most important medicinal plants, produces anticancer alkaloids particularly vinblastine and vincristine in concentrations of 0.0003 to 0.0004 % dry weight in leaves and accumulate antihypertensive alkaloids

such as ajmalicine and serpentine in roots [7]. Other alkaloids extracted from this plant were used for the treatments of blood pressure and urinary disorders [8]. Vincristine and vinblastine are synthesized by monomeric alkaloids catharanthine and vindoline present in the vacuole of leaves and stem cells of *Catharanthus roseus* [9].

Effect of plant growth regulators on secondary metabolites accumulation in *Catharanthus roseus* was studied in many researches, it was found that using ethylene, chlormequat and salicylic acid resulted in the significant increase of vincristine, vinblastine and catharanthine content [10]. Treating this plant during the blooming period with salicylic acid and ethephon gave an increase in vindoline, vinblastine, and catharanthine content, while ABA and GA_3 retarded it [11]. Using cytokinins [12], auxins [13], methyljasmonate [14] and GA_3 [15] had influenced onindol alkaloids acceleration in *Catharanthus roseus*. Many of plants possess as radical scavengers which protect the human body against pathological conditions, some of these medicinal plants have chemopreventive and therapeutic effects on human pathogens [16, 17].

Studies related this to secondary metabolites accumulation as phenols, alkaloids, and volatile oils [18, 19]. *Catharanthus roseus* plant has a wide spectrum of phenolic compounds, such as cinnamic acid derivatives, flavonoids and anthocyanin's that have antioxidant activities. Effects of gibberellins, auxins, and cytokinins on phenol content and the antioxidant activity in plants have been widely studied [20, 21, 22, 23]. From this premise, the aim of the present research is to study the effect of plant growth regulators foliar spray on phenols and alkaloids contents and the antioxidant properties of this plant during the blooming period.

MATERIALS AND METHODS: *Catharanthus roseus* plant was classified and authenticated at the Department of Biology herbarium, College of Science, University of Baghdad (Authentication number 50845).

Catharanthus roseus seeds were germinated in Petri dishes using distilled water, after germination, the seedlings were transferred to trays containing soil and organic manure mixture in a greenhouse at Department of Biology for two weeks at 25 ± 3 °C for acclimation, then shifted to pots arranged in a randomized complete blocks design with 10 treatments, each treatment consist of 3 replicates. Mean of replicates were compared using (LSD) at 5% level [24]. When the plants began to bloom with an average height of 50 cm, short term treatments of three concentrations of GA_3, IAA and 6-benzylaminopurinewere carried out by foliar spray. The control treatment was the untreated plants with PGR_s. the average temperature was 20-25 °C and relative humidity was 65-70% during the period of the treatments. The PGRs were sprayed on aerial parts of the plants at (0, 30, 60 and 90 ppm concentrations), the spray repeated after two days. Leaves samples were collected after five days from the second treatment for further experiments.

Estimation of Total Phenols: Total phenols were estimated according to [25]. 500 mg of fresh leaves was grounded in a pestle and mortar with 10 ml of 80% ethanol. The homogenate was centrifuged at 10000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved with 5 ml distilled water and used as an extract. To 2 ml of this extract, 0.5 ml of Folin-Ciocoalteau reagent was added in a test tube. After 3 min, 2 ml of Na_2CO_3 solution was added and mixed thoroughly. The mixture was kept in a beaker containing boiling water for one minute, and after cooling, the absorbance was read by spectrophotometer at 650 nm. Total phenols were determined by using standard curve prepared with different concentrations of gallic acid, the results were expressed in milligrams per gram fresh weight.

Estimation of Alkaloids: Ten grams of small pieces of fresh leaves were digested in 10% HCl in a conical flask (v.100 ml), then placed in a hot water bath at 70 °C for 4 h. The extract was filtered and the filtrate was supersaturated by using Bouchardat's reagent (iodine potassium iodide) in order to bind the alkaloids present. After addition of the reagent, a dark-brown precipitate of alkaloids was formed. The precipitate was filtered by using pre-weighed Whatman filter paper (no. 1) then the filter paper allowed to dry and weighed again using analytical balance [26].

Antioxidant Activity Assay: Fine powder (30 gm) of leaves of each plant treatment was extracted with (100 ml) ethanol (99%) in conical flask (v.250 ml)

and put in the thermo shaker at 150 rpm for 24 h at 30 °C, then filtered by using Whatman filter paper (no. 1) and concentrated by rotary evaporator. 50 mg/ml was made for each studied treatment. The free radical scavenging activity was measured by DPPH assay, this included equal volumes of DPPH (60µM) and plant extract of each treatment at the (0, 30, 60, 90 ppm) concentrations of GA_3, IAA and 6-benzyladenine, that were mixed in a cuvette and allowed to stand for 30 min at room temperature. The absorbance was red at (517 nm) using UV spectrophotometer, the control was DPPH (60µM) [27], the percentage of DPPH discoloration was according to the formula:

% Discoloration = Absorbance of control – Absorbance of test ×100 / Absorbance of control

RESULTS AND DISCUSSION: Foliar spray of PGR_S (GA_3, IAA, and 6-Benzylaminopurine) at the concentrations (30, 60, 90 ppm) increased phenols content, alkaloids content and antioxidant activity in leaves of *Catharanthus roseus* plant compared with control treatment.

TABLE 1: EFFECT OF PLANT GROWTH REGULATORS FOLIAR SPRAY-ON PHENOL CONTENT, ALKALOID CONTENT AND ANTIOXIDANT ACTIVITY % IN *CATHARANTHUS ROSEUS* LEAVES, (MEAN OF THREE REPLICATES)

PGR_S	Phenol content (mg/g fresh weight)	Alkaloid content (mg/g fresh weight)	Antioxidant activity %
GA_S Con.	2.33 a	1.85 a	71.689 a
GA_3 30 ppm	5.24 b	3.39 b	75.515 b
GA_3 60 ppm	6.31 b	4.28 b	80.324 c
GA_3 90 ppm	8.27bc	5.86 c	82.120 cd
IAA Con.	2.33 a	1.85 a	71.689 a
IAA 30 ppm	5.16 b	3.43 b	76.969 a
IAA 60 ppm	6.84 b	3.97 b	82.410 b
IAA 90 ppm	11.91c	4.88 bc	84.281 bc
6-BAP Con.	2.33 a	1.85 a	71.689 a
BAP 30ppm-6	5.28 b	3.72b	78.422 b
6-BAP 60ppm	7.22 b	4.08 b	81.561 b
6-BAP 90ppm	12.07c	5.15 bc	83.242 bc
LSD P ≤ 0.05	2.42	1.35	3.35

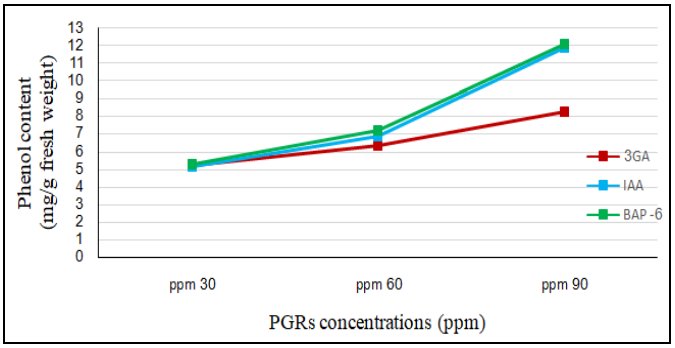

FIG. 1: EFFECT OF PGRS FOLIAR SPRAY-ON PHENOL CONTENT IN *CATHARANTHUS ROSEUS* LEAVES EXTRACTS

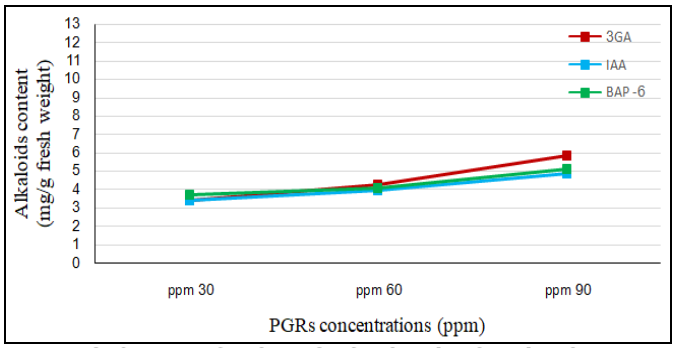

FIG. 2: EFFECT OF PGRS FOLIAR SPRAY ON ALKAOIDS CONTENTIN *CATHARANTHUS ROSEUS* LEAVES EXTRACTS

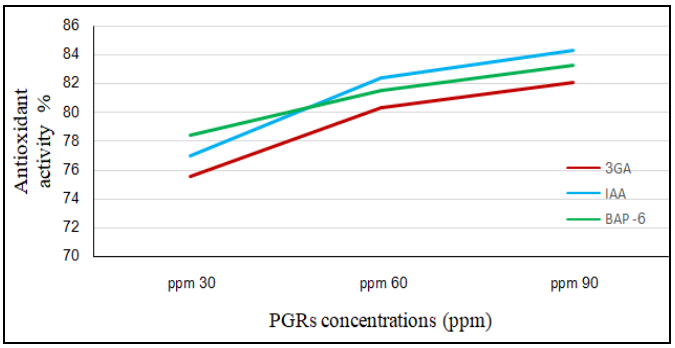

FIG. 3: EFFECT OF PGRS FOLIAR SPRAY ON ANTIOXIDANT ACTIVITY % OF *CATHARANTHUS ROSEUS* LEAVES EXTRACTS

Results, as shown in **Table 1** and **Fig. 1, 2, 3**, revealed that there were significant increases in all studied parameters that were obvious at the higher concentrations of PGR_S. The effect of IAA and 6-benzylaminopurineon alkaloids biosynthesis may be due to influencing peroxidase activity [28].

A study revealed that auxins had a role in methylerthtid phosphate (MEP) pathway, leading to biosynthesis of TIA (terpenoidindol alkaloids) at a gene expression regulation level [29].

Cytokinins enhanced the accumulation of alkaloids in *Catharanthus roseus* cultures, experiments showed that cytokinin greatly enhanced the gene expression of geranil 10-hydroxlase (GIOH) gene [30]. IAA and GA enhanced phenols accumulation and increased the antioxidant activity, these result may be due to increasing peroxidase activity, ascorbic acid, α-tocopherol and glutathione that had antioxidant properties [31].

The effect of GA_3 on antioxidant activity may be due to the enhancement of antioxidant enzymes such as ascorbate peroxidase, superoxide dismutase, catalase, peroxidase and polyphenol oxidase in this plant [32]. The effect of phenols production on antioxidant properties of this plant was extensively studied [33, 34, 35]. It was found that different leaves extracts of this plant had shown antitumor and antioxidant activity and that was related to the content of alkaloids and phenols [36, 37].

Results of this study are encouraging to the continuity of further experiments on this plant to obtain high contents of secondary metabolites that proved its chemotherapeutical properties.

CONCLUSION: This research showed that *Catharanthus roseus* leaves extracts proved its richness in active compounds that considered antioxidants, and treatment with plant growth regulators by foliar spray at the blooming period is an effective strategy to have considerable amounts of these antioxidants for medical uses.

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CONFLICTS OF INTEREST: The author declared no conflict of interest.

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