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Plant Growth Regulators and Fungicides Alters Growth Characteristics in *Catharanthus roseus*; Comparative Study

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Abstract: The effect of different plant growth regulators (PGR) and fungicide treatments on the growth characteristics of *Catharanthus roseus* (L.) G. Don. (Madagascar periwinkle, Family: Apocynaceae) was investigated in the present study. The PGR used were paclobutrazol (PBZ), gibberellic acid (GA_3) and *Pseudomonas fluorescens* elicitors (PF Elicitors) by soil drenching on 38, 53, 68 and 83 days after planting (DAP) by soil drenching. The plants were taken randomly on 45, 60, 75 and 90 DAP and used for estimating the growth and anatomical characteristics changes. The total height of the plant increased with the age in the control, gibberellic acid and *P. fluorescens* treated *Catharanthus roseus* plants, but it decreased significantly under PBZ treatments. Our results have good significance, as these increases the secondary metabolites of this traditional medicinal plant.

Key words: *Catharanthus roseus*, Apocynaceae, Paclobutrazol, Gibberellic acid, *Pseudomonas fluorescence,* Growth

INTRODUCTION

Catharanthus roseus (L.) G. Don. (Madagascar periwinkle, Family: Apocynaceae) is a perennial tropical plant that produces more than 100 monoterpenoid indole alkaloids (MIAs) including two commercially important cytotoxic dimeric alkaloids used in cancer chemotherapy [1-3]. Roots of this plant are the main source of an anti-hypertension alkaloid ajmalicine [4]. *C. roseus* is also a popular ornamental plant. Three distinct varieties based on the flower colour viz., the pink flowered 'rosea', the white flowered 'alba' and the white with a pink or yellow ring in the orifice region 'Ocellata' are found in *C. roseus*. Pink flowered cultivar gives higher yield of foliage and roots and total alkaloids [5].

Triazole compounds are systemic fungicides having plant growth regulating properties. The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including Gibberellic acid, ABA and Cytokinins [6]. Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-trizol-1-yl)-pentan-3-ol] is a triazolic group of fungicide which have plant growth regulating properties. The growth regulating properties of PBZ are mediated by changes in the balance of important plant hormones including the Gibberellins, ABA and cytokinins [7,8]. PBZ has been proved as an agent in stress amelioration in medicinal plants [4,6] and crop plants [9,10].

Gibberellic Acid (GA_3), which comes from a naturally occurring growth hormone, is a member of a type of plant hormone called Gibberellins, which regulate the growth rate of plants[11]. Gibberellins are involved in several plant development processes and promote a number of desirable effects including stem elongation, uniform flowering, reduced time to flowering and increased flower number and size [12]. In plants, certain secondary metabolite pathways are induced by infection with

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microorganisms. It was reported that, arbuscular mycorrhizal symbiosis maintained more normal water relations in plants [13].

The objectives of the present study are to understand the effect of plant growth regulators such as PBZ, GA_3 and *Pseudomonas fluorescence* elicitors on the growth and anatomical characteristics changes of *C. roseus* plants under field conditions.

MATERIALS AND METHODS

Plant Materials and Growth Regulators: Medicinally important plant species, *Catharanthus roseus* (L.) G. Don. (Family: Apocynaceae) was selected for the present investigation. The seeds were obtained from Herbal Folklore Research Centre, Tirupati andhra Pradesh, India. The triazole compound paclobutrazol was obtained from Syngenta, India Ltd., Mumbai. The plant growth regulator Giberellic acid (GA_3) was purchased from Himedia India Ltd., Mumbai. The elicitor, *Pseudomonas fluorescens* was obtained from Krishi Care Bioinputs, Chennai, India.

The plants were raised in Botanical Garden of Department of Botany, Annamalai University. The seeds were sown separately in raised seedbeds by broadcasting method and covered with fine soil to ensure proper germination. The nursery beds were watered twice a day and weeded regularly in order to ensure healthy growth of the seedlings.

Treatments and Samplings: Seven plots were selected by randomized block design (RBD). 10 mg L^{-1} paclobutrazol, 5 ìM gibberellic acid and 1 mg *Pseudomonas fluorescens* concentrations were used for the treatments and control plants were irrigated with well water. The treatments were given on 38, 53, 68 and 83 DAP by soil drenching. The plants were taken randomly on 45, 60, 75 and 90 DAP and separated into root, stem, leaves and flowers and used for determining growth, anatomical characteristics, mineral composition, pigments and biochemical constituents, antioxidant potentials and alkaloid contents.

Growth Parameters

Height of the Plant and Root Length: The plant height was measured from the soil level to the tip of the shoot and expressed in cm. The plant root length was measured from the point of first cotyledonary node to the tip of longest root and expressed in cm.

Number of Leaves and Total Leaf Area: The number of fully developed leaves were counted and expressed as number of leaves per plant. The total leaf area of the plants was measured using LICOR Photoelectric Area Meter (model LI-3100, Lincoln, USA) and expressed in cm^2 per plant.

Determination of Fresh and Dry Weight: After washing the plants in the tap water, fresh weight was determined by using an electronic balance and the values were expressed in grams. After taking fresh weight, the plants were dried at 60°C in hot air oven for 24 hours. After drying, the weight was measured and the values were expressed in grams.

Statistical Analysis: Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for six samples in each group. p values ≤ 0.05 were considered as significant.

RESULTS

Effect of Growth Regulators on Height of the Plant (Table 1, Plate 1): The total height of the plant increased with the age in the control, gibberellic acid and *P. fluorescens* treated *Catharanthus roseus* plants, but it decreased significantly under PBZ treatments. The increase was higher in gibberellic acid treated when compared to *P. fluorescens* treatments. Highest plant height was noted in 45 DAP under gibberellic acid treatments and it was nearly 122.58 percent over control. The lower plant height was observed in PBZ treated plants on 90 DAP and it was 67.60 percent over control.

Effect of Growth Regulators on Root Length (Table 1, Plate 2): The total root length of the plant increased with the age in control, gibberellic acid and *P. fluorescens* treated plants, but it significantly decreased ($P \leq 0.05$) under gibberellic acid treatments. The increase was higher in PBZ treated when compared to *P. fluorescens* treatments. There was highest increase of root length in PBZ treated plants on 60 DAP (133.33) and a maximum decrease was found in gibberellic acid treated (88.89) *C. roseus*.

Effect of Growth Regulators on Total Leaf Area (Table 1): The total leaf area of the plant increased with the age in control and *P. fluorescens* treated plants, but it decreased under gibberellic acid and PBZ treatments. The decrease was more prominent in gibberellic acid treated and it was 74.03 percent over control on 90 DAP when compared

Table 1: Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on morphology of *Catharanthus roseus* on different growth stages

Growth Stages (DAP)	Control	Paclobutrazol	Gibberellic acid	*P. fluorescens*
Plant height				
45	31±1.192^{a}	28±0.966^{b}	38±1.251^{d}	36±1.201^{c}
60	43±1.593^{a}	31±1.107^{b}	48±1.715^{c}	47±1.603^{d}
75	56±1.867^{a}	40±1.481^{b}	60±1.911^{c}	58±1.899^{d}
90	71±2.290^{a}	48±1.714^{b}	78±2.785^{c}	72±1.291^{a}
Root length				
45	13±0.500^{a}	17±0.586^{b}	12±0.489^{a}	16±0.583^{b}
60	18±0.642^{a}	24±0.607^{b}	16±0.582^{c}	21±0.701^{d}
75	21±0.700^{a}	27±0.932^{b}	20±0.689^{a}	24±0.606^{c}
90	27±0.931^{a}	36±1.241^{b}	26±0.867^{a}	35±1.238^{b}
Total leaf area				
45	142.8±4.760^{a}	130±4.642^{a}	119±4.250^{a}	151±5.800^{a}
60	186±7.184^{a}	181.8±6.734^{a}	152±5.846^{a}	190±6.334^{a}
75	245.4±8.180^{a}	234±7.800^{a}	189±6.097^{a}	249±8.330^{a}
90	295±10.172^{a}	240±9.231^{a}	218.4±7.280^{a}	309±9.968^{a}

Values are given as mean±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT)

Table 2: Effect of paclobutrazol, gibberellic acid and *P. fluorescens* on fresh and dry weight of *Catharanthus roseus* on different growth stages

Fresh weight					Dry weight				
DAP	CON	PBZ	GA	PF	DAP	CON	PBZ	GA	PF
45	08.60±0.331^{a}	10.55±0.362^{b}	9.40±0.349^{c}	11.10±0.380^{d}	45	1.43±0.049^{a}	1.72±0.066^{b}	1.69±0.065^{c}	1.75±0.067^{b}
60	11.24±0.388^{a}	12.73±0.412^{b}	11.68±0.389^{a}	13.52±0.499^{c}	60	1.60±0.057^{a}	1.80±0.064^{b}	1.73±0.067^{c}	1.93±0.068^{d}
75	16.32±0.544^{a}	18.26±0.725^{b}	16.54±0.559^{a}	19.65±0.812^{c}	75	2.33±0.078^{a}	2.41±0.083^{a}	2.38±0.079^{a}	2.52±0.084^{b}
90	28.16±0.908^{a}	31.47±1.015^{b}	29.23±0.912a	31.61±1.158^{c}	90	3.92±0.151^{a}	3.98±0.132^{a}	3.96±0.152^{a}	4.00±0.138^{a}

Values are given as mean±SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d) differ significantly at $P \leq 0.05$ (DMRT)

Plate 1: Variations in growth of *Catharanthus roseus* under treatment with paclobutrazol, gibberellic acid and *Pseudomonas fluorescens*. (a) Field view on 30 DAP, (b) and field view on 45 DAP.

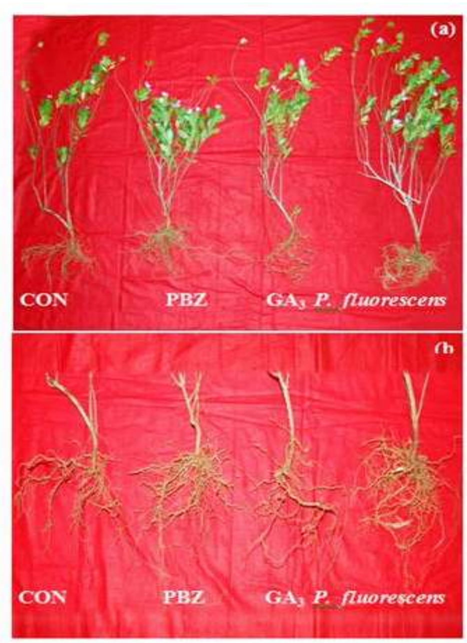

Plate 2: Variations in growth of *Catharanthus roseus* under treatment with paclobutrazol, gibberellic acid and *Pseudomonas fluorescens*. (a) individual plants and (b) roots from control and treated plants on 90 DAP.

to PBZ treatments. Treatment with *P. fluorescens* significantly increased ($P \leq 0.05$) the total leaf area at all stages of growth and the maximum increase was observed in 45 DAP which is 106.34 percent over control.

Effect of Growth Regulators on Whole Plant Fresh and Dry Weight (Table 2, Fig. 5): The fresh weight increased with the age in control and treated plants. Gibberellic acid treatment slightly increased the fresh weight on 75 DAP but it increased to a significant level under *P. fluorescens* treatments, in which a maximum increase was noted on 45 DAP, upto 129.07 percent over control. PBZ also increased the fresh weight of plants than that of *P. fluorescens* treatments. The dry weight also increased in control and treated plants, but increased only upto a slight extent, not significant ($P \leq 0.05$) in gibberellic acid and PBZ treatments on 75 and 90 DAP. The dry weight of the plants increased to a significant level under *P. fluorescens* treatments with a maximum increase of upto 122.38 percent over control on 45 DAP.

DISCUSSION

The plant height reduced under PBZ treatments in *Catharanthus roseus*. The treatment with gibberellic acid and *P. fluorescens* increased the plant height. Triazole treatments reduced stem elongation and plant height in *Plectranthus forskholii* [14], Cassava [15] and *Catharanthus roseus* [16]. GA exerts profound effects on fundamental processes of plant growth and development. GA is widely regarded as a growth promoting compound that positively regulates processes such as seed germination, stem elongation, leaf expansion, flower and fruit development and floral transition [11]. Gibberellins are involved in several plant development processes and promote a number of desirable effects [17]. It was apparent that treating plants with GA_3 increased height of the plant over the control, which may be attributed to the growth promotion effect of GA_3 in stimulating and accelerating cell division, increasing cell elongation and enlargement [18]. The plant hormone GA and ABA exert profound effect on fundamental processes of plant growth and development, in which GA and ABA has mostly antagonistic effects of GA promoting and ABA inhibiting the processes [19].

There found an increase in plant height in *C. roseus* under treatment with PGPR *P. fluorescens*. Similar results were reported in *C. roseus* under different PGPR treatments [20]. *P. fluorescens* increased plant height in *C. roseus* [13]. PGPR produced high quantities of extracellular IAA and tryptophol in culture medium supplemented with tryptophan, a precursor of IAA. Auxin production by PGPR is believed to play a major role in plant growth promotion, although little new evidence with plants has been published in recent years.

The root length was increased with PBZ and *P. fluorescens* treated plants to a higher extent on the other hand, GA inhibited root growth in *C. roseus*. Triadimefon treatment increased the root growth in *Withania* [8]. An increase in root length was reported in paclobutrazol and triadimefon treated *C. roseus* [4]. Triazole compounds increased the root growth, which was associated with increased the endogenous cytokinin levels [1]. This stimulation of root growth may be related to the increased partitioning of assimilates towards the roots due to a decreased demand on the shoot [21]. Inhibition of GA and increase of cytokinin and ABA may be the reason for the increased root length in the triadimefon treated plants. It would be associated with larger parenchyma cells and the promotion of radial cell expansion [22].

Yan *et al.* [23] reported that the population of PGPR strains *Bacillus Pumilus* and *P. fluorescens* colonizing tomato roots after application into the soil less medium showed higher population on the whole roots and lateral roots than on the tap roots. Inoculation of wheat with *Asospirillum brasilense* wild strains increased in root hair formation. A mutant of *Asospirillum brasilense* with production of phytohormones, but with high nitrogenase activity did not enhance root over uninoculated controls. Increased root growth was reported in *C. roseus* under treatment with PGPR [24]. In general, increased plant biomass and N_2-fixation were recorded in strains having increased production of indole compounds which might be the reason for the increased root length of *C. roseus* plants under elicitation.

The total leaf area was reduced under paclobutrazol and GA treated *Catharanthus* plants while it increased under *P. fluorescens* treatments. Paclobutrazol reduced the leaf area in *Vigna unguiculata* [9]. The treatments with triadimefon and hexaconazole reduced leaf area in *Solenostemon rotundifolius* [25]. Propiconazole reduced the leaf area in *C. roseus* [6]. The reduced leaf area in triazole treatments may be due to the increased ABA content and reduced gibberellin biosynthesis induced by triazoles.

GA_3 caused a significant decrease in leaf area in all stages of growth in *C. roseus*. The application of gibberellic acid has the potential to control growth and flowering by reduced leaf area and induce earliness in strawberry. The response of strawberry to exogenous GA_3 is similar to that caused by certain natural environmental factors [26].

The increase of leaf area under *Pseudomonas* treatments might be due to the hormone producing ability of this elicitor, which is evident from the findings of Tiwari *et al.* [27] who found that the *Pseudomonas* isolates of pearl millet were able to produce IAA under *invitro* conditions and reduced acetylene to ethylene. The treated *C. roseus* plants showed an increase in fresh weight when compared to control. The main reason for increase in fresh weight is due to the increased root growth under paclobutrazol treatments. Triazole compounds inhibited gibberellin biosynthesis cytokinin and abscisic acid stimulated tuberization and reduced stolen length in potato by counteracting gibberellin action [1]. Cytokinin and abscisic acid content induced by these triazoles might be the cause for increased root growth and in turn increased fresh weight [6].

The PGPR strains of *Pseudomonas* are known to produce IAA and GA in the rhizosphere of plants and stimulated the crop growth as evidenced by increased seedling emergence, vigour, seedling weight root system development and yield [28]. Gopal [29] revealed that the inoculation of PGPR increased the plant height, number of leaves, number of laterals and root diameter and increased in fresh and dry weight and seed yield in Ashwagandha. The treatments with paclobutrazol, GA and *P. fluorescens* increased the whole plant dry weight of *C. roseus.* The increase in dry weight was significant when compared to control. The mode of action of triadimefon can be explained by the inhibitory effect of triazoles on gibberellic acid levels and increases the ability of partitioning of assimilates to tuberous organs as observed in potato and gladiolus which confers increased plant biomass. It can be interferred that the higher chlorophyll content in the leaves, leading to higher photosynthesis might have increased total dry weight in the triazole treated *C. roseus.*

It was apparent that treating plants with GA_3 increased height of the plant over the control, which may be attributed to the growth promotion effect of GA_3 in stimulating and accelerating cell division, increasing cell elongation and enlargement or both [17,18] which in turn increased the dry weight of the plants. Two fold increases in shoot dry weight and three fold increase in root dry weight were observed due to the inoculation of stem cutting of potato with *P. fluorescens*. Gopal [29] reported that *Pseudomonas* treatment significantly increased plant growth, dry matter production, yield of Ashwagandha.

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