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Effect of Dikegulac on Growth and Alkaloid Production in *Catharanthus roseus* (L.) G. DON. (Pink flowered)

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Application of sodium-dikegulac reduced plant height with associated increase in branch and leaf number and root biomass in *C. roseus* (L.) G. DON. Chlorophyll content reduced significantly after first month of 100 and 250 μg/ml DK application. However, such reduction was replaced by significant rise after forth month in 250 μg/ml DK application and fifth month in 100 μg/ml DK application followed by appreciable decline only in 250 μg/ml DK treatment but 100 μg/ml DK maintained higher level till harvest. Total sugar content was significantly high during forth and fifth month stage of growth after DK application. Amino acid content was higher during third to fifth month in 100 μg/ml DK treatment and during third to forth month in 250 μg/ml DK treatment. Tryptophan, on the other hand showed higher content at the fifth month stage of growth after application of DK in both the concentrations. Leaf and root dry weight as well as total alkaloid content were highest in 100 μg/ml DK application. DK, therefore, appears to be a potential chemical for increasing biomass and alkaloid content in *C. roseus*.

Keywords: *Catharanthus roseus*, plant growth, dikegulac, alkaloid

INTRODUCTION

Catharanthus roseus or Madagascar periwinkle belongs to the family Apocynaceae and produces a class of secondary metabolites termed terpenoid indole alkaloids. This plant has now gained considerable reputation in the therapeutic world for its wide assemblage of over 100 alkaloids (Datta, 1981). The leaves of *C. roseus* (L.) G. DON. contain vincristine and vinblastine alkaloids which are used in a wide variety of human neoplasam, whereas its root contains high percentage of ajmalicine and serpentine alkaloids. The latter are used in treatments of high blood pressure and related maladies. Several studies have shown that plant secondary metabolites including alkaloids increase under different kinds of stresses (Saenz *et al*, 1993). On the other hand, some recent studies have shown that plant growth regulators, like cytokinin and BA, stimulate alkaloid synthesis in cell cultures of *C. roseus* (Decendit *et al*, 1992). Fungal elicitors were also found to induce the synthesis of catharanthine and ajmalicine (Godoy-Hernandez and Loyola-Vargas, 1991). With regard to plant growth retardants, however, only CCC has been studied on biomass production and alkaloid yield in *C. roseus* (Choudhury and Gupta, 1996) and in *C. pusillus* (Basu, 1992). A plant growth retardant like Na-dikegulac (DK) produces a number of effects on crop plants of which increase in branch and leaf number (Bocion and de Silva, 1977; Bhattacharjee and Gupta, 1984) and yield are most important. Their effect on increase of root biomass was also shown earlier (Purohit, 1979). The present study analyses the effect of DK on growth modification, especially the biomass production and alkaloid yield in *C. roseus*. Some major biochemical changes were also followed to correlate alkaloid biosynthesis and precursor availability.

MATERIALS AND METHODS

Fresh seeds of *C. roseus* (L.) G. DON. (pink flowered) were collected from the Botany Department, Burdwan University. They were sown in nursery beds after superficial decontamination for 15 minutes in 0.1% $HgCl_2$. Uniform seedlings of 20-25 day-old were transplanted in the field at 45 cm × 30 cm spacing (plant to plant as well as row to row). The experiment

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was conducted for three consecutive years (from March-April to September-October). The field was divided into three blocks that served as main plots. Each block (main plot) was divided into three subplots of 2 m × 1.5 m, containing 25 plants in each subplot. Plants were grown in consecutive three years in subplots of three blocks in randomised block design. Foliar application of freshly prepared 100 and 250 µg/ml DK in 0.5% tween-80 (surfactant) was made for three consecutive days starting from 60 day-old plants. Control plants were sprayed with distilled water containing 0.5% tween-80. The treatments were repeated at fortnightly interval and continued upto 150 days (each plant received six sprays). The crop was harvested at 180th day. Cultural practices were followed as described by Datta (1981). After harvesting, the foliage and roots were separated and air-dried to 6-8% moisture content.

Growth Analyses

Plant height, number of branches, number of abscised and non-abscised leaves were recorded at regular monthly intervals and values of 25 plants were recorded for each treatment and mean values of the results of 3 years were incorporated in tables and figures. Similarly, dry weight of leaves and roots after harvest were taken by oven-drying method (60°C for 72 hrs.) and preserved in desiccators at room temperature.

Biochemical Analyses

For chlorophyll estimation, leaves of fifth node of primary branch from apex of all 60-day old plants were tagged for sample collection and 1-2 leaves per plant were collected randomly from all 25 plants at regular monthly interval till 180th day. Chlorophyll was estimated following the method of Litchenthaler (1987). For other biochemical parameters, leaf samples were collected from 60-day old plant and such collections were made of consecutive 5 months till 180-day old of plant age. Such samplings were made with the leaves of fifth node of primary branch having same maturity status (area) and estimation of total carbohydrate (soluble+insoluble, Mc Cready, 1950) and free amino acid (Moore and Stain, 1948) were done. Total tryptophan content (free+conjugated) was estimated following the method of Mertz *et al* (1975).

Alkaloids of leaves and roots were extracted and purified by acid-base partition according to Kutney *et al* (1980) with the modification as done by Godoy-Hernandez and *Loyola-Vargas* (1991).

Statistical Analysis

Data on growth parameter and biochemical analyses were collected and analysed statistically. Analysis of Variance-F-test was done and Critical Difference was computed whenever significant difference was obtained. Correlation Coefficient was found out for growth parameters to examine if the relationship between two variables was actual or apparent (Zar, 1974).

RESULTS AND DISCUSSION

Increase of total biomass by chemical manipulation is essential for higher productivity of alkaloids. Application of 100 and 250 µg/ml DK in *C. roseus* respectively reduced the height, but significantly increased the number of branches and leaves per plant (Table 1). It is known that DK quickly disrupt apical dominance owing to its rapid acropetal mobility (Arzee *et al*, 1977). Its effect, on the production of profuse axillary branches of many ornamental plants is amply documented (Bocion and de Silva, 1977; Bhattacharjee and Gupta, 1984). It is also evident from the observation that the changes in root : shoot length per plant increased in both the concentrations of this retardant and retardant induced increased root growth is reported elsewhere (Purohit and Chandra, 1981; Adedipe and Ormrod, 1977). Number of non-abscised leaves per plant

Table 1. Effect of DK on growth parameters of *C. roseus* (at 180 day after the initiation of treatment)

Treatment of DK (µg/ml)	Plant Height (cm)	Root length: Shoot length/ plant	No. of Branch/Plant	Total leaves/ Plant	Non-abscised leaves/plant	Abscised: Nonabscised leaves/plant
Control	127.72	0.189	52.36	1259.64	856.72	0.451
100	99.32	0.355	95.76	1442.16	1071.44	0.345
250	86.84	0.337	100.72	1583.56	1115.28	0.492
	*6.304	*0.125	*2.729	*55.734	*51.527	*0.125

* L.S.D. at 5%.

Table 2. Effect of DK on correlation coefficient for agro-morphological characters in *C. roseus* plant

Treatment of DK (μg/ml)	Height vs. Branch No.	Height vs. Total leaf No.	Branch No. vs. Total leaf No.	Branch No.vs. Abscised leaf No.
Control	0.726*	0.145*	0.175*	0.226*
100	-0.061 N.S.	0.811*	-0.224 N.S.	-0.036 N.S.
250	-0.879*	-0.007 N.S.	0.087 N.S.	0.700*

* Significant at 5% level. N.S.: Not significant.

at the time of harvest when considered, showed significant difference against control and abscised : non-abscised leaf ratio per plant decreased with the application of 100 μg/ml DK (Table 1).

The simple correlation analyses (Table 2) showed that with increasing concentrations of DK, plant height was reduced with increase in branch number. But reduction in height increased the total leaves only at higher concentration. The positive correlation between branch number and total leaf number indicated increase in leaf biomass in 250 μg/ml DK application. But it was negatively correlated in 100 μg/ml DK application. On the other hand though the correlation between branch number and abscised leaf number was found to be insignificant, the negative value indicated the inhibitory effect of DK on leaf abscission i.e. increase in number of branches decreased the rate of leaf fall in 100 μg/ml DK application. But the significant positive correlation betweenm these two characters indicated increase of abscission rate at the higher concentration of 250 μg/ml DK.

DK application appreciably reduced the chlorophyll content at both the concentrations only after first month of application as against control (Fig. 1). But higher level of chlorophyll was noted during forth month stage of 250 μg/ml DK application and during fifth and sixth month stage of growth in 100 μg/ml DK application. Therefore, present study showed that chlorophyll resynthesizing ability was actually the function of time and concentration. Revival of chlorophyll level after initial decline as an effect of DK application is reported earlier (Bhattacharjee and Gupta, 1983). Decline of chlorophyll in leaves at later stage of growth in 250 μg/ml DK appears to be related with acceleration of leaf fall (Nooden and Leopold, 1978).

The level of sugar increased in both the concentrations of DK and this was maintained till harvest (Fig. 2). This is probably because of enhancement of enzymatic activities and such effect of growth retardants is well documented (Bhattacharjee and Gupta, 1981; Bhatnagar and Bisaria, 1979). DK treatment also significantly increased the amino acid at third month stage and maximum rise was noted at the forth month stage followed by significant decline at both the concentrations (Fig. 3). DK effect on increase in RNA and protein content is reported in literature (Gressel and Cohen, 1977), but such effect was

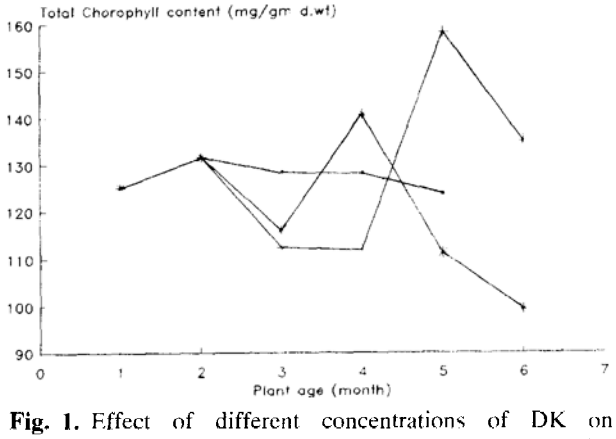

Fig. 1. Effect of different concentrations of DK on changes in chlorophyll content of *C. roseus* at monthly interval. The concentrations of DK are represented by the symbols - 100 μg/ml (+), 250 μg / ml (*) and the control (.). L.S.D. at 5%-6.573.

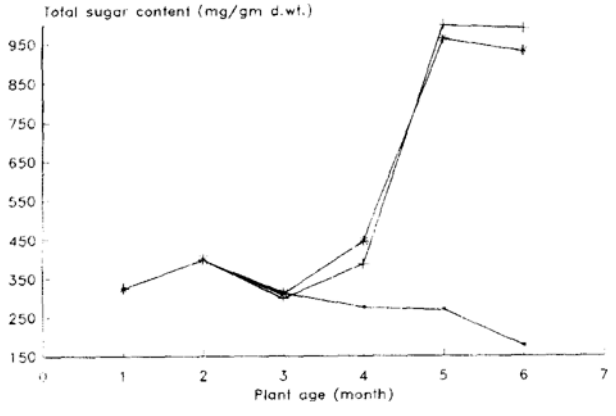

Fig. 2. Effect of different concentrations of DK on changes in total sugar content of *C. roseus* at monthly interval. The symbols are same as in Fig. 1. L.S.D. at 5% - 39.364.

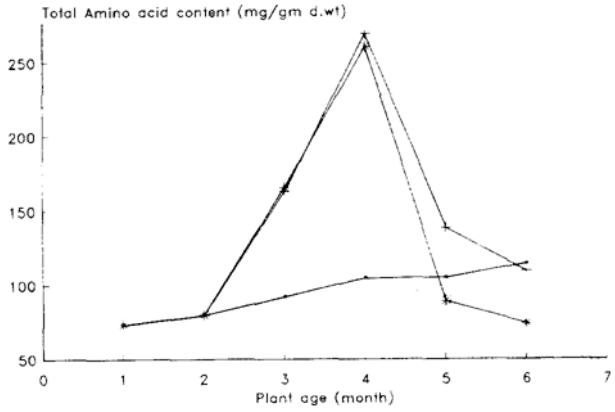

Fig. 3. Effect of different concentrations of DK on changes in free amino acid content of *C. roseus* at monthly interval. The symbols are same as in Fig. 1. L.S.D. at 5% - 13.193.

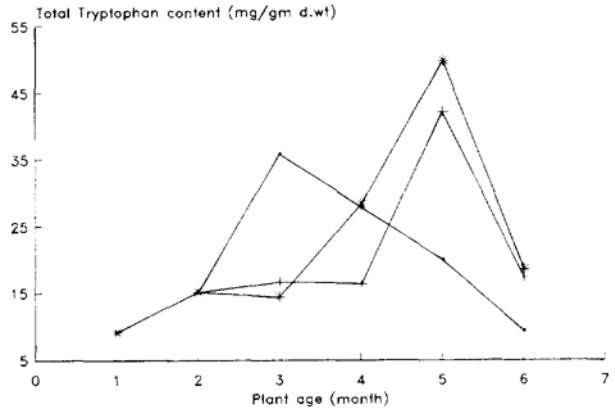

Fig. 4. Effect of different concentrations of DK on changes in tryptophan of *C. roseus* at monthly interval. The symbols are same as in Fig. 1. L.S.D. at 5% - 4.782.

inhibitory in higher concentration (Bhattacharjee and Gupta, 1983). Growth retardant induced increase of some amino acid are also reported (Naylor and Stephen, 1993). Tryptophan content declined after third month in control plants whereas higher level of tryptophan was maintained at fifth month stage and even at six month stage in DK treatments (Fig. 4). From biochemical analysis, it therefore appears that DK rejuvenated the plants by way of enhanced metabolism during later periods of growth.

Dry matter of the leaves of treated plants increased in both the concentrations of DK (Table 3). But dry matter of root increased more in lower concentration. Alkaloid content of leaf and root tissue significantly increased in both the concentrations but it was more in 100 µg/ml DK treatment (Table 3). Increase of total alkaloid production with concomitant increase of leaf and root biomass in 100 µg/ml DK treatment was likely. From commercial view point, such results are quite encouraging. It was reported earlier that tryptophan, the mother amino acid of indole alkaloid, when added in the culture medium, the callus tissue contained more alkaloids (Grogger, 1980). Increase of alkaloid level therefore shown to be related with the availability of amino acid precursor in treated plants. DK also affects the integrity of plasmalemma (Gressel, 1980). But this occurs only at the higher concentration (Zilkah and Gressel, 1980). Many enzymes of indole alkaloid biosynthesis are membrane-bound and for their efficient activity, they need membrane integrity. Application of 250 µg/ml DK reduced the alkaloid content against 100 µg/ml DK, but the value was significantly higher against control. At higher concentration 250 µg/ml DK, alkaloid precursor like sugar and tryptophan increased significantly against untreated plants. Therefore, it appears that this concentration partially affected the integrity of membrane, thereby, lowering the enzymatic activity for efficient production of alkaloids.

An ideal plant type of *Catharanthus* should have bushy growth, producing higher biomass and having a much-branched root system. The root system may be shallow with short and thinner roots which contribute towards higher ajmalicine+serpentine content, since the alkaloids are concentrated in the thin root bark (Singh *et al*, 1992). So, DK appears to be a very potential chemical from commercial view point as it increased the total biomass of plant to a significant extent and concentration of 100 µg/

Table 3. Effect of DK on yield of leaf and root biomass and alkaloid production of *C. roseus* plant

Treatment of DK (µg/ml)	Dry leaves/plant (gms)	Total alkaloid content in leaves (mg/kg of d.w.)	Dry roots/ plant (gms)	Total alkaloid content in roots (mg/kg of d.w.)
Control	17.31	45	4.42	24
100	23.59	150	13.15	132
250	26.11	84	6.56	51
	*4.278	*4.64	*1.627	*5.350

*L.S.D. at 5%.

ml DK found to be highly effective in increasing alkaloid content when estimated even on per kg dry weight basis.

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