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Effect of Nitrogen and Potassium Fertilization on Growth, Yield and Alkaloidal Content of Periwinkle (*Catharanthus roseus* G. Don)

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ABSTRACT

Two field experiments were conducted at the farm of Saft El-Laban, Giza, Egypt, during two successive seasons (2005 and 2006) to study the effect of nitrogen (N) and potassium (K) fertilization on growth, yield and alkaloidal content of periwinkle (*Catharanthus roseus* G. Don). Periwinkle seedlings were transplanted from the nursery to a permanent field in April in both seasons. The trials included three doses of N (50, 100 and 150 Kg/fed (4200 m^2). as ammonium nitrate) and three doses of K (25, 50 and 75 Kg/fed as potassium sulfate) and their combinations. Plant height (cm), number of branches per plant, fresh and dry weights of plant, as well as the herb yield were determined. These applications produce a significant increase in all the different characters under investigation. The high level of both N and K resulted in the highest values of all parameters. The yield of total *C. roseus* alkaloids under different fertilization treatments was determined with TLC densitometry analysis, where the highest percentage were recorded in plants fertilized with 150 kg/fed of N and 25 kg/fed of K. Special attention had been paid in this paper to the indole alkaloids, specifically vincristine and vinblastine.

Keywords: fertilizers, indole-alkaloids, vincristine, vinblastine

INTRODUCTION

Periwinkle (*Catharanthus roseus* (L.) G. Don, syn. *Vinca rosea* L., Lochnera rosea Reich.) is a tropical perennial plant, native to Madagascar and belonging to the Apocynaceae family. Presently, it is grown in many tropical and subtropical regions. The phytochemistry, biology and methods of the culture of *C. roseus* have been intensely investigated (Moreno *et al*. 1994). The main reason of the great interest in *C. roseus* is due to its ability of synthesize a wide range of terpenoid indole alkaloids (TIAs) which are valued due to their wide spectrum of pharmaceutical effects (reviewed by Vazquez-Flota and Miranda-Ham 2006). Three types of action are particularly important: diuretic (vindoline, catharanthine), hypotensive (ajmalicine, serpentine), and anticancer (vinblastine and vincristine) (Kohlmüzer 1968).

The plant contains more than 100 alkaloids, distributed in all parts of plant, but in different proportions (Lata 2007). Total alkaloid concentration in root amounts to 2–3% or reaches up to 9% in fibrous roots, whereas leaves contain *ca.* 1% of alkaloids with a very small amount of these compounds in flowers (Lata 2007). The anticarcinogenic indoles, vinblastine (VLB) and vincristine (VCR) used in leukemia and lymphomas are present in leaves while ajmalicine, used as antihypertensive (for the circulatory disorders, high blood pressure and related maladies) or sedative agents, is contained in roots. VLB and VCR occur in very low concentrations of 1 g t^{-1} and 20 mg t^{-1} of plant material, respectively (Tyler 1988; Lata 2007). They are still considered as the most interesting chemotherapeutic compounds currently accessible for clinical use (Pezzuto 1997).

VLB and VCR are derived from the condensation of the monoterpenoid indole alkaloids, catharanthine and vindoline. Other compounds synthesized by *C. roseus*, such as vindoline and catharanthine, which possess diuretic properties, are present in leaves and roots, respectively (Pareek 1985).

Although cell and tissue cultures of *C. roseus* have been widely investigated as an alternative source of TIA production, still plants grown in the field are the only commercial source for the production of these drugs (DiCosmo and Miasawa 1995; reviewed by Hedhili *et al.* 2006).

Adequate selection of soil and agricultural practices, such as sowing, planting, fertilization, irrigation, harvest date etc., may influence alkaloid yield.

In general, the fertilization of medicinal plants increases the yield of bioactive compounds. It is commonly known that under field conditions, nitrogen (N) is the main yield-determining factor. The other macroelements, as phosphorous (P) and potassium (K) have a smaller effect on plant yield. Thus, this study aimed to investigate the effect of N and K fertilization on growth, yield and alkaloid content of periwinkle.

MATERIALS AND METHODS

Experimental

Two field experiments were carried out at the farm of Saft El-Laban, Giza, Egypt during two successive seasons (2005, 2006) to study the effect of N and K on growth, yield and alkaloid content of *C. roseus*. Chemical properties of experimental soil are shown in **Table 1**. Soil was classified as clay in texture.

Nursery preparation

Periwinkle seeds were provided by the Horticulture Department, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Nursery soil was ploughed and divided into six plots. Seeds were sown on the 10th February and all agricultural practices were done at the proper time. Seedlings were thinned to reduce competition for sunlight, water and added minerals. Healthy seedlings were

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Short Communication

Table 1 Chemical properties of experimental soil.

Sample No.*	Field capacity (%)	pH	Total soluble salts (ppm)	Organic carbon (%)	Organic matter (%)	Ammoni ate (ppm)	Nitrate (ppm)	Organic nitrogen (ppm)	Total nitrogen (ppm)	Available phosphorus (ppm)	Total phosphorus (ppm)	Total Fe (ppm)	Total Mn (ppm)	Total Zn (ppm)
1	60.0	8.07	365	1.00	1.72	36.4	48.13	895	980	64	417	351	82	43
2	69.3	8.01	352	0.11	0.19	35.8	56.88	637	730	73	449	402	70	35
3	62.1	8.20	384	0.15	0.26	34.4	56.35	789	980	85	547	394	76	41
Mean	63.8	8.01	367	0.42	0.72	35.5	53.79	774	897	74	471	382	76	40

transplanted from the nursery to the permanent field on the 13th of April of both seasons on a hill with a 40 cm intra- rows and 60 cm inter-row distance.

Field experiment

The experiment included three levels of N fertilization (50, 100 and 150 kg/fed) in the form of ammonium nitrate (33.5% N), and three levels of K fertilization (25, 50 and 75 kg/fed) in the form of potassium sulphate (48.5% K_2O) and their all possible combinations. The nine treatments were arranged in a complete randomized block design (CRBD) with four replicates. The experimental unit area was 4 m^2 (2 × 2 m).

N fertilizers were added in two portions, the first one was added at the beginning of vegetative growth (two months from transplanting) while the second portion was applied after one month from the first transplant. K fertilization was carried out before the flowering stage (four months from transplanting).

The following parameters were recorded at harvest time (six months from transplanting): plant height (cm), number of branches per plant, fresh and dry weights of herb per plant; total yield (ton/fed).

Plant samples from each of the previously mentioned treatments were separately air dried and ground for chemical analyses. ANOVA analysis was used for comparison between means and L.S.D at the 5% level according to Snedecor and Cochran (1980).

Determination of the alkaloid content

About 75 g of each of the nine samples were percolated with ethanol (96%) at room temperature until exhaustion. The ethanol extract of each treatment was separately concentrated *in vacuo* at 40°C to about 100 ml and dissolved with 10% HCl. The acidic solutions were left overnight in a refrigerator and filtered from the resinous matter. Successive extractions with petroleum ether were carried out to remove the fatty substances.

The aqueous acidic solutions were shaken separately with chloroform (2 × 100 ml) and the aqueous layer of each sample was rendered alkaline with (0.5 N) NaOH solution (pH = 10), the total alkaloids were extracted with chloroform (2 × 100 ml) till exhaustion. The combined chloroform extract of each sample was washed with distilled water (2 × 100 ml), dehydrated over anhydrous sodium sulfate and distilled off *in vacuo* at 40°C. A yellowish brown residue of each was obtained which represented the total alkaloids. The percentage of total alkaloids in the different prepared samples was gravimetrically calculated on the basis of plant samples dry weights.

Total alkaloids of each sample were dissolved in different volumes of methanol to give the same concentration in all samples for chromatographic analysis using TLC densitometry.

Sample solutions

Sample solutions were prepared by dissolving known mgs of extracted samples in the same volume of MeOH to obtain 1% stock solutions. Standard stock solutions of both vincristine and vinblastine were prepared by dissolving 10 mg of each one in 10 ml MeOH.

TLC densitometry analysis

A CAMAG Automatic TLC Scanner with CATS evaluation software was used with the following settings: wavelength scans: 289 nm and scanning speed: 20 mm/s, multilevel calibrations via peak area by linear regression and silica gel ready-made plate (Merck 60 F 254, 20 × 20 cm). Samples were applied in band wise using TLC sampler III, distance from lower edge was 10 cm, band length was 8 mm, track distance was 15.5 mm and 11 bands represented all samples were applied. The plate developed with EtOAc: EtOH: C_6H_6: NH_4OH (100: 5: 5: 3).

The obtained chromatograms were integrated and the concentration of both vincristine and vinblastine was calculated using WINCATS4.

RESULTS AND DISCUSSION

The mean values of growth parameters and yield of dry weight of *C. roseus* as affected by N and/or K are shown in **Table 2**.

Increasing N fertilization from 50 Kg N to 100 Kg N/fed significantly increased plant height, number of branches, fresh and dry weights of plant and yield (dry weight ton/fed). These increments reached 20.9, 19.5, 18.7, 19.6 and 21.2%, respectively. Increasing nitrogen doses from 100 Kg to 150 Kg increased these values to 7.9, 12.8, 19.5, 20.3 and 27.5%, respectively. It is clear that fertilization with 150 Kg N/fed gave the highest values of the studied characteristics. These results are in agreement with those of Ibrahim *et al*. (1993) who mentioned that application of NPK at the rates of (100 Kg N, 50 Kg P_2O_5 and 25 Kg K_2O) with foliar spray resulted in highest fresh yield per hill of *Ocimum basillicum* L., and Ezz El-Din (1995) who mentioned that seed and oil yield of *Silybum marianum* reached the maximum values at the rates of 50 Kg N/fed and 30 Kg P_2O_5/fed.

K fertilization with 50 Kg/fed significantly increased all growth parameters and fresh and dry weights of *Catharanthus* compared to 25 Kg treatment. Increasing potassium dose from 50 to 75 Kg/fed increased all parameters except plant height which significantly decreased. These findings agree with Csizinszky (1999) who mentioned that fresh weight yields of sweet marjoram and Italian parsley in the

Table 2 Effect of N and K fertilization on growth and yield of *Catharanthus roseus* plants.

Characters / Treatments	Plant height (cm)	No. of branches /plant	Fresh wt (g)/plant	Dry wt (g)/plant	Dry wt (ton)/fed
N_1K_1	37.67	30.74	185.61	40.21	0.600
N_1K_2	42.00	37.81	215.00	45.60	0.675
N_1K_3	45.10	34.20	225.19	48.80	0.720
Mean N_1	41.59	34.20	208.50	44.80	0.660
Mean K_1	46.13	37.97	233.69	50.64	0.755
N_2K_1	48.12	39.27	231.17	50.10	0.750
N_2K_2	50.97	40.19	242.40	52.50	0.780
N_2K_3	51.81	43.20	268.90	58.20	0.870
Mean N_2	50.30	40.88	247.40	53.60	0.800
Mean K_2	48.72	41.03	249.70	53.73	0.800
N3K1	52.60	43.90	284.30	61.60	0.915
N_3K_2	53.18	45.10	291.70	63.10	0.945
N_3K_3	57.11	49.30	311.20	68.90	1.200
Mean N_3	54.29	46.10	295.70	64.50	1.020
Mean K_3	44.67	42.23	268.43	58.63	0.930
L.S.D. 5%	1.61	2.07	14.06	3.67	0.41

wt = weight

N_1, N_2 and N_3 = 50, 100 and 150 kg/fed, respectively.

K_1, K_2 and K_3 = 25, 50 and 75 kg/fed, respectively.

Table 3 The percentage of total alkaloids, vinblastine and vincristine in the prepared samples.

Sample	% TAC*	TAC* (mg/plant)	TAC* (g/feddan)	Vincristine (%)	Vinblastine (%)
N_1K_1	0.07	28.15	420.00	0.005432	0.004620
N_1K_2	0.07	31.92	472.50	0.003941	0.004865
N_1K_3	0.11	53.68	792.00	0.002068	--
Main N_1	0.08	35.84	528.00	0.0038136	0.0031616
Main K_1	0.19	96.22	1434.50	0.0361456	0.011145
N_2K_1	0.05	25.05	375.00	--	0.00195
N_2K_2	0.02	10.50	156.00	--	--
N_2K_3	0.24	139.68	2088.00	0.047856	--
Main N_2	0.10	53.60	800.00	0.047856	0.00195
Main K_2	0.05	26.87	400.00	0.002685	0.00198
$N3K_1$	0.45	277.20	4117.50	0.103005	0.026865
N_3K_2	0.05	31.55	472.50	0.004115	0.00108
N_3K_3	0.14	96.46	1680.00	0.011634	--
Main N_3	0.21	135.45	2142.00	0.0395846	0.0139725
Main K_3	0.16	90.61	1488.00	0.0205193	--

* TAC: total alkaloid content

first two harvests of 1993-1994 growth seasons were maximized at 40 or 80 Kg K/ha.

The interaction effect between nitrogen and potassium was significant for all characters. Increasing potassium doses under any of N rates increased all studied parameters except number of branches which decreased with increasing potassium from 50 to 75 Kg less than the 50 Kg N treatment. The highest values of all parameters were observed from combined treatment of 150 Kg N and 75 Kg K.

Determination of the total alkaloid contents of the collected plant samples in **Table 3** shows that the alkaloid percentage increased with an increase in the level of N and reached its maximum at the maximum level of N. This was expected as it is commonly known that under field conditions, N is the main yield-determining factor (Lata 2007). Also, the results of thin layer densitometry analysis revealed that the highest percent of vincristine (0.103005%) was found to be in the plant sample treated with highest level of N. The heights yield of alkaloid as well as vincristine and vinblastine were obtained with the lowest concentration of K.

Catharanthus roseus, as an alkaloidal plant, can be classified as a high N demanding plant, but obviously also other nutrients are needed to obtain a high yield of herb and a high content of biologically active substances.

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