Efficacy of nitrogen on growth and medicinal value of periwinkle (*Catharanthus roseus*) in an inceptisol of Varanasi

Abstract

Periwinkle (Catharanthus roseus), is a perennial herbaceous, belongs to the Apocynaceae family. The content of the economically important metabolite is more important than the yield of the plant. The main aim of this study was the evaluation of the effects of nitrogen on growth of the *Catharanthus roseus* and the presence or absence of alkaloid vinblastine. The experiment involved a pot experiment conducted during Rabi season 2021-2022 in the net house of the Department of Soil Science and Agricultural Chemistry season at Agricultural Research Farm of the Institute of Agricultural Sciences followed by soil and leaves samples were analysed in the laboratory of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Plants fed with recommended dose of P: K (40:40) and nitrogen fertilizer according to treatment. Higher dose of nitrogen i.e., 10.0 g/urea (T5) showed the greatest increase in plant height, number of branches, flowers per plant and SPAD value while number of leaves per plant was maximum at 2.5 g urea/pot (T2) whereas the lowest growth were exhibited in control treatment (T1). The presence or absence of vinblastine was tasted by Thin Layer Chromatography (TLC) and the result showed that at all the levels of nitrogen treatment, vinblastine was present except at control treatment(T1) till a month after transplanting.

Key words: Nitrogen, Alkaloid, Vinblastine, Thin Layer Chromatography, Growth

INTRODUCTION

Periwinkle (*Catharanthus roseus),* is a perennial herbaceous subshrub with stems that root at the nodes and short ascending blooming shoots, belongs to the Apocynaceae family. The content of vinblastine economically important metabolite is more important than the yield of the plant component containing the metabolite in industrial crops, such as medicinal plants, because it dictates the cost of its extraction (Levy, 1982). Researchers have been paying close attention to these alkaloids because of their high price but low presence in the plant. To improve the yield of these alkaloids, researchers are looking into the cell and tissue culture, as well as biotechnological components of the plant (Moreno *et al.,* 1995; Jacobs *et al.,* 2000).). In recent years, medicinal alkaloids such as indole alkaloids and opiates have been the subject of extensive research. Vinblastine, vincristine, catharanthine, ajmalacine, and vindoline are the plant's main alkaloids (Abdolzadeh *et al.,* 2006), as stated by (Van der Heijden *et al.,* 2004), *Catharanthus* alkaloids are a category of approximately 130 terpenoid-indole-alkaloids. Vinblastine and vincristine are leaf-specific bisindole alkaloids. *Catharanthus roseus* (periwinkle) has been employed in the majority of alkaloid research. The biosynthetic pathways and alkaloids involved have been a major focus of research for many years, but producing the dimeric indole alkaloid vinblastine, which is used in cancer chemotherapy, has proven to be extremely challenging (Farnsworth, 1984; DeLuca, Balsevich, Tyler, Eilert, Panachuk, and Kurtz, 1986).

Nitrogen is one of the primary nutrients required by plants to complete their life cycle, primary nutrients are those nutrients which require by plants in huge amounts. It provides energy to plants for growing and producing fruits and vegetables. It also helps in the photosynthesis process for creating food. It is the main constituent of protein, chlorophyll, and nucleic acids. It gives vigorous growth to plants. It gives vegetative growth and delays maturity. It makes the plant succulent.

The deficiency of nitrogen causes chlorosis in plants *i.e*., yellowing of lower leaves. The deficiency of nitrogen increases the starch content but decreases the protein content. Deficiency symptoms of nitrogen are purple coloration appears in the shoot axis, suppresses or delays the flowering. It has been noted that the application of nitrogen increased the vegetative growth of plants. Mineral nutrition can increase the yield and the alkaloid content in periwinkle (Jana *et al.,*1996). Nitrogen fertilization has been found to increase leaf and root yields, significantly. Alkaloids are nitrogenous compounds;

therefore, nitrogen may play an important role in the biosynthesis and accumulation of alkaloids in plants. (Sreevalli *et al.,*2004).

MATERIALS AND METHODS

The investigation was carried out in a pot experiment under net house at the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. (India). The soils of Varanasi are formed on alluvium deposited by the river Ganges. Pots having 5 kg capacity were taken and labelled. The experiment was laid out in a completely randomized design (CRD) with five treatments and three replications. The treatments detailed are given below: T_1- Control (RDF), T_2 - 2.5g urea/pot, T_3- 5.0g urea/pot' T_4- 7.5g urea/pot T_5- 10.0g urea/pot. The seedlings of periwinkle were sown on 16th November, 2021.The recommended dose of fertilizer (RDF) for periwinkle (*Catharanthus roseus*) was N: P_2O_5:K_2O: 120:40:40 kg ha^{-1}. The nutrient solution was prepared using urea as N source, analytical grade Diammonium phosphate (DAP) as the source of phosphorus and potassium chloride (KCl) as the source of potassium. Water was added to the soil samples to raise the moisture content to 50% of the field capacity. The moist samples were then transferred in the pots and kept in the net house. One seedling was sown in each pot and the upper layer was moistened with water to ensure proper growth. The plants were maintained in the pot culture and care was taken to ensure proper growth. Irrigation was given as and when required. The initial soil analyzed using standard methods like available nitrogen (Subbiah and Asija 1956), phosphorus (Olsen *et al*. 1954), potassium (Hanway and Heidal 1952), organic carbon (Walkley and Black 1934), soil pH and electrical conductivity (Jackson1973). The soil of experiment field was low in organic carbon (0.45%) and available nitrogen (194.51kg/ha), moderate in phosphorus (19.8kg/ha), potassium content (211.61kg/ha), Fe (12.8 mg/kg), Cu (1.5mg/kg), Mn (16.40mg/kg), Zn (0.52mg/kg) with a neutral in soil reaction (pH 7.31) and safe electrical conductivity (0.36 dS/m). Morphological characteristics like plant height, number of branches per plant, number of flowers per plant, number of leaves per plant and SPAD value were observed at different growth stages of periwinkle plant. For analysis of alkaloid content, leaves sample were collected at different growth stages of periwinkle. The collected leaves were washed thoroughly with tap water to remove dirt and impurity. Then crushed the leaves sample properly, and transfer the crushed leaves sample of each treatment into a separate sample bottle. Dip this crushed leaves sample into methanol, shake this for 1 to 2 minutes and leave them for 3 to 4 days at room temperature. After 3 to 4 days collected extract from the sample bottle into Eppendorf and leave them to dry at room temperature for 3 to 4 days and then store them in the refrigerator for further analysis. The method used for alkaloid analysis was Thin Layer Chromatography. At maturity, the plants were harvested. The analysis which was analysed in the laboratory were chemical properties like soil pH, Electrical Conductivity (EC) and soil organic carbon, available soil micronutrients (N, P, and K) and available soil micronutrients (Fe, Zn, Cu and Mn). Plant sample containing leaves were grinded and grinded samples were digested with concentrated H_2SO_4 and digestion mixture (K_2SO_4:$CuSO_4$:Se powder in the ratio of 10:1:0.1). The total nitrogen content in plant was then estimated by micro-kjeldahl method as suggested by Tendon (2001).

Statistical analyses

Data from the laboratory and pot experiments were assessed by Duncan's Multiple Range Tests (DMRT) with a probability $p<0.05$ by using SPSS program (SAS version 17.0).

Results and Discussions

Morphological growth parameters

Plant height

At all phases of growth, no significant differences in plant height were seen after nitrogen administration. Treatment T5 (10g urea pot^{-1}) had the maximum plant heights of 6.60,10.50,14.10 and 16.00 cm at 30, 60, 90 and 120 DAT, respectively.

All urea treatments increased plant height significantly over the control, but without significant difference between them.

Table 1: Effect of nitrogen on plant height (cm)

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
T1	5.53^{a}	9.01^{a}	12.01^{a}	14.40^{a}
T2	5.76^{b}	9.40^{a}	12.30^{b}	14.80^{b}
T3	5.90^{b}	9.80^{a}	12.70^{b}	15.20^{b}
T4	6.03^{b}	10.01^{a}	13.30^{b}	15.70^{b}
T5	6.60^{b}	10.05^{a}	14.10^{b}	16.01^{b}

Different letters for each parameter show significant differences $p<0.05$

Number of branches per plant

The number of branches shows significant increment at 120 DAT. The highest number of branches was recorded at T5 treatment. At 120 DAT smallest number of branches was seen at RDF treatment. All urea treatments increased the number of branches significantly over the control, but without significant difference between them. Yadav and Singh (1997) investigated the effects of 0, 60, 120, 180, 240, and 300 ppm N on African marigold growth and fresh flower yield in a pot experiment, finding a consistent increase in plant height and spread up to 120 ppm N, as well as an increase in the number of branches and fresh flower yield up to 180 ppm N.

Table 2: Effect of nitrogen in number of branches on periwinkle

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
T1	0.33^{a}	3.00^{a}	5.33^{a}	8.33^{a}
T2	0.66^{b}	2.33^{b}	6.00^{b}	9.00^{b}
T3	0.66^{b}	3.00^{b}	7.33^{b}	11.00^{b}
T4	1.00^{b}	3.33^{b}	7.66^{b}	11.33^{b}
T5	1.66^{b}	4.66^{b}	8.00^{b}	11.66^{b}

Different letters for each parameter show significant differences $p<0.05$

Number of leaves per plant

Application of 10 g urea treatment produced more leaves per plant, as compared to the control, 2.5 and 5.0 g urea application, but not substantially different from the 7.5 g application of urea. There was a significant increment in the number of leaves along with the increment with nitrogen doses over the control treatment. The smallest number of leaves were observed at 30 DAT in T1. Chadha et al., (1999) at 150 days after transplanting, plants treated with 60 kg N/ha had the most leaves per plant, branches per plant, and plant height, (DAT).

Table 3: Effect of nitrogen on the number of leaves per plant on periwinkle.

Treatment	30 DAT	60 DAT	90DAT	120 DAT
T1	21.33^{c}	62.00^{c}	82.00^{c}	91.66^{c}
T2	32.00^{b}	72.00^{b}	91.00^{b}	109.66^{b}
T3	33.00^{b}	73.66^{b}	91.00^{b}	111.00^{b}
T4	44.33^{a}	94.33^{a}	119.00^{a}	136.66^{a}
T5	45.66^{a}	96.66^{a}	121.00^{a}	138.66^{a}

Different letters for each parameter show significant differences $p<0.05$

Number of flowers per plant

The application of 5g urea produced the greater number of flowers, while the application of 2.5 g urea came at the second place. The number of flowers increases with an increment in nitrogen application. The highest number of flowers was seen at T3 treatment (5g urea/pot) which was 22, 33, 35, and 42 at 30, 60, 90 and 120 DAT. The smallest number of flowers was found in the control treatment which was 8, 15, 20, 25, 33 at 30, 60, 90 and 120 DAT. According to Gupta et al., (1999), flowers per plant, and flower production (q/ha) increased owing to 100 kg N/ha in marigold.

Table 4. Effect of nitrogen on number of flowers per plant on periwinkle.

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
T1	8.00^{d}	15.00^{d}	20.00^{c}	25.30^{d}
T2	13.00^{c}	23.00^{cd}	28.31^{c}	32.00^{c}
T3	22.00^{c}	33.00^{c}	35.00^{c}	41.00^{c}
T4	10.30^{b}	18.00^{b}	23.40^{b}	32.00^{b}
T5	10.00^{a}	18.00^{a}	22.00^{a}	28.00^{a}

Different letters for each parameter show significant differences $p<0.05$

Greenness Index

Greenness index of leaves as a function of nitrogen application levels. All treatments had a significant increase in SPAD value over RDF (T1). Application of 10g urea had the highest SPAD value, which was 86.20. The lowest value was observed in T1 (RDF). Having an adequate supply of nutrients, particularly nitrogen, throughout the growing season boosts the pace of leaf growth and nitrogen content, resulting in higher chlorophyll levels. Sonam *et al.,* (2018), Chanchal *et al.,* and Sonam *et al.*, (2020) found that increase dose of nitrogen application, increased the SPAD value.

Table 5: SPAD value of plant leaves

Treatment	30 DAT	90 DAT	120 DAT
T1	40.33^{e}	61.83^{e}	62.15^{e}
T2	51.56^{d}	70.80^{d}	70.76^{d}
T3	60.76^{c}	76.20^{c}	76.60^{c}
T4	74.10^{b}	82.50^{b}	83.56^{b}
T5	78.86^{a}	86.50^{a}	86.20^{a}

Different letters for each parameter show significant differences $p<0.05$

Effect of nitrogen on alkaloid content in periwinkle.

At all the levels of nitrogen treatment, vinblastine was present except at 30 DAT, under T1 treatment (control).

According to Golamhosseinpour *et al.,* (2011) who claimed a positive correlation between nitrogen and vegetative growth coupled with an increase in alkaloids content of the Periwinkle plants and N fertilization up to 150 kg/ha boosted total alkaloid levels in periwinkle genotypes leaves and roots, according to Sreevalli *et al*., (2004).

Table 6: Presence or absence of vinblastine (alkaloid) at different levels of nitrogen.

Treatment	30 DAT	60 DAT	80 DAT	100 DAT	120 DAT
T1	A	P	P	P	P
T2	P	P	P	P	P
T3	P	P	P	P	P
T4	P	P	P	P	P
T5	P	P	P	P	P

(A – Absent, P – Present)

Effect of nitrogen application on post-harvest soil of nitrogen-treated soil -

pH

The data on the pH of the soil as a result of nitrogen treatment is shown in table 7. When different amounts of nitrogen were added, the pH of the soil reduced non significantly. Similar results found were by Aquino *et al.,* (1976).

Electric Conductivity

According to the data, which is shown in table 7 there were no significant differences in the EC of soil at T2, T3 and T4 doses of nitrogen treatment. It was in the 0.41 – 0.43 dS m^{-1} range. T5 had the greatest EC of the soil 0.45 dS m^{-1}, whereas T1 had the lowest at 0.38 dS m^{-1}. Badyal (1980) and Sharma (1994) found the similar findings.

Organic Carbon

When periwinkle was treated with varied doses of N, organic carbon in the post-harvest soil increased, according to the data in Table 4.22. Organic carbon percentages in soil ranged from 0.466 to 0.53. Treatment T5 had the highest level of organic carbon (0.53%), while treatment T1 had the lowest (0.46%). The amount of nitrogen in the soil has significant effect on the amount of organic carbon in the soil. Joon (1989) and Sharma (1994) found the similar result.

Available Nitrogen

There was increase the available nitrogen in soil with the increased nitrogen application. The highest available nitrogen was found at T5 treatment which was 221.46 kg ha^{-1} and the smallest amount of available nitrogen was found at T1 treatment which was 200.7 kg ha^{-1}. there was a significant increment in available nitrogen at T5 over control. Muthuvel *et al.,* (1979) and Jhoon (1989) recorded similar finding.

Available Phosphorus

There was decrease the available phosphorus in soil with the increased amount of nitrogen application. The highest available phosphorus was found at T1 treatment which was 19.2 kg ha^{-1} and the smallest available nitrogen was found at T5 treatment which was 14.67 kg ha^{-1}. there was a significant decrement in available phosphorus at T5 over control. Biswal (1979) and Chandel (1985) recorded similar findings.

Available Potassium

There was increase the available potassium in soil with the increased amount of nitrogen application. The highest available potassium was found at T5 treatment which was 254.4 kg ha^{-1} and the smallest available potassium was found at T1 treatment which was 216 kg ha^{-1}. there was a significant increment in available potassium at all levels of nitrogen. Sharma (1984) and Jhoon (1989) recorded similar findings.

Micronutrients (Zn, Cu, Mn and Fe)

The findings in Table 7, demonstrated that the Zn content in the soil ranged from 0.55 to 1.03 mg kg^{-1} depending on the amount of nitrogen present. The use of nitrogen gave significant results. T5 has the highest Zn concentration in comparison to other treatments.

The data on Cu content in soil demonstrated that a graded dose of nitrogen application resulted in a non-significant increase of Cu content in the soil. Cu concentrations in the soil ranged from 2.0 to 2.7 mg per kilogram. T5 had the highest Cu concentration, at 2.7 mg kg^{-1}.

Mn levels in soil ranged from 17.2 to 17.8 mg kg^{-1}, according to the results. T2 had the highest Mn concentration, with 17.8 mg kg^{-1}.

The data on Fe content in the post-harvest soil demonstrated that a graded dose of nitrogen application resulted in a non-significant increase in Fe content in the experimental soil. T5 had the highest Fe which was 15 mg kg^{-1}.

Table 7: Effect of nitrogen application on properties of post-harvest soil.

Treatment	pH	EC (dSm-^{1})	O.C (%)	Available N (Kg ha^{-1})	Available P (Kg ha^{-1})	Available K (Kg ha^{-1})	Available Fe ($mgKg^{-1}$)	Available Cu ($mgKg^{-1}$)	Available Mn ($mgKg^{-1}$)	Available Zn ($mgKg^{-1}$)
T1	7.2^{a}	0.38^{c}	0.46^{c}	200.70^{c}	22.00^{d}	216.11^{e}	13.66^{c}	2.31^{c}	17.26^{b}	0.55^{e}
T2	7.1^{a}	0.41^{b}	0.47^{c}	200.21^{c}	19.20^{cd}	220.92^{d}	13.63^{c}	2.33^{c}	17.80^{ab}	0.62^{d}
T3	7.0^{a}	0.42^{b}	0.49^{b}	209.90^{b}	17.50^{bc}	229.26^{c}	14.26^{bc}	2.01^{bc}	16.93^{ab}	0.71^{c}

T4	6.8^{a}	0.43^{b}	0.51^{ab}	218.70^{ab}	15.50^{b}	234.51^{b}	14.76^{ab}	2.50^{ab}	17.10^{ab}	0.85^{b}
T5	6.7^{a}	0.45^{a}	0.53^{a}	221.46^{a}	14.67^{a}	254.4^{a}	15.01^{a}	2.70^{a}	17.60^{a}	1.03^{a}

Different letters for each parameter show significant differences p<0.05

Effect of nitrogen application on periwinkle leaves

Nitrogen content in plant

There was significant increment was found in the nitrogen content of plant leaves from T1 to T5 with application of higher doses of nitrogen. The highest nitrogen was found at T5 treatment which was 2.54 % and the smallest content of nitrogen was found at T1 treatment which was 1.31%. There was a significant increment in the nitrogen content at T5 over control. Zankat (1992), Nieuwhof and Jansen (1993) all observed the similar findings.

Table 8: Nitrogen content in post-harvest leaves.

Treatment	Nitrogen (%)
T1	1.31^{e}
T2	1.56^{d}
T3	1.79^{c}
T4	2.1^{b}
T5	2.54^{a}

Different letters for each parameter show significant differences p<0.05

Micronutrients (Fe, Cu, Mn, Zn) in plant leaves

There was no any significant increment or decrement found in the leaves of periwinkle's micronutrient content after nitrogen application except Mn. The highest Mn found at T5 treatment which was 39.9 mg/kg and the smallest Mn content in leaves was found at T1 which was 33 mg/kg.

Martin (1980) reported only increased leaf Mn concentrations with increased N but no effect on K, P, Ca, Fe, Cu, B or Zn and B leaf concentrations.

Table 9: Effect of nitrogen application on Fe, Cu, Mn, Zn content of plant leaves

Treatment No.	Fe	Cu	Mn	Zn
T1	250.1^{a}	5.1^{a}	33.0^{e}	55.0^{a}
T2	250.2^{a}	5.1^{a}	35.3^{d}	55.1^{a}
T3	250.1^{a}	5.2^{a}	37.8^{c}	55.0^{a}
T4	250.6^{a}	5.0^{a}	39.4^{b}	55.3^{a}
T5	250.5^{a}	5.1^{a}	39.9^{a}	55.1^{a}

Different letters for each parameter show significant differences p<0.05.

CONCLUSION

Nitrogen, had an impact on plant growth and development in *Catharanthus roseus*. Maximum growth which includes plant height, number of branches per plant, and number of leaves per plant was found at highest level of nitrogen treatment and maximum number of flowers obtained at T2(2.5g urea/pot). The results of the present study reveal that *Catharanthus roseus* crop requires complete fertilizer having nitrogen as the predominant constituent since it has a predominant influence on the growth of plant, and alkaloid content of leaves and roots.

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