International Journal of Plant & Soil Science

Volume 35, Issue 19, Page 954-962, 2023; Article no.IJPSS.105302
ISSN: 2320-7035

Impact of Nitrogen Levels on the Growth and Medicinal Properties of Periwinkle (*Catharanthus roseus*) in an Inceptisol of Varanasi, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i193630

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/105302

Original Research Article

Received: 18/06/2023
Accepted: 23/08/2023
Published: 29/08/2023

ABSTRACT

Aims: Periwinkle (*Catharanthus roseus*), is a perennial herbaceous, belongs to the Apocynaceae family. The medicinal poperties are more economically important than the yield of the plant. Nitrogen is the main nutrient affect the medicinal properties of the plant. The main aim of this study

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was the evaluation of the effects of nitrogen on growth of the *Catharanthus roseus* and the presence or absence of alkaloid vinblastine.

Place and Duration of Study: The experiment involved a pot experiment conducted during *Rabi* season of 2021-2022 in the net house, at Agricultural Research Farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Methodology: The experiment was laid out in a completely randomized design (CRD) with five nitrogen levels i.e. Control (RDF), 2.5g urea/pot, 5.0g urea/pot, 7.5g urea/pot and 10.0g urea/pot in three replications. Plants fed with recommended dose of P: K (40:40) and nitrogen fertilizer according to treatment.

Results: Higher dose of nitrogen *i.e.,* 10.0 g/urea (T_5) 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 (T_2) whereas the lowest growth were exhibited in control treatment (T_1). 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 (T_1) till a month after transplanting.

Keywords: Nitrogen; alkaloid; vinblastine; thin layer chromatography; growth.

1. 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 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 [1-2] 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 [3] as stated by [4] *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 [5-6].

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 [7]. 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 [8].

2. MATERIALS AND METHODS

2.1 Experimental Site and Treatments

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:

Table 1. Treatment details

Treatment Symbol	Treatment (Nitrogen levels)
T_1	Control (RDF)
T_2	2.5 g urea/pot
T_3	5.0 g urea/pot
T_4	7.5 g urea/pot
T_5	10.0 g 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.

2.2 Research Methodology

The initial soil analyzed using standard methods like available nitrogen Subbiah and Asija [9], phosphorus Olsen et al. [10], potassium Hanway and Heidal [11], organic carbon Walkley and Black [12], soil pH and electrical conductivity Jackson [13]. 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 Tendon [14].

2.3 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).

3. RESULTS AND DISCUSSION

3.1 Morphological Growth Parameters

3.1.1 Plant height

At all phases of growth, no significant differences in plant height were seen after nitrogen administration. Treatment T_5 (10 g 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.

3.1.2 Number of branches per plant

The number of branches shows significant increment at 120 DAT. The highest number of branches was recorded at T_5 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 [15] 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.

3.1.3 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 T_1. Chadha et al. [16] 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).

3.1.4 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 T_3 treatment (5g urea/pot) which was

Table 2. Effect of nitrogen levels on plant height of periwinkle

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
T_1	5.53^a	9.01^a	12.01^a	14.40^a
T_2	5.76^b	9.40^a	12.30^b	14.80^b
T_3	5.90^b	9.80^a	12.70^b	15.20^b
T_4	6.03^b	10.01^a	13.30^b	15.70^b
T_5	6.60^b	10.05^a	14.10^b	16.01^b

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

Table 3. Effect of nitrogen levels on number of branches of periwinkle

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
T_1	0.33^a	3.00^a	5.33^a	8.33^a
T_2	0.66^b	2.33^b	6.00^b	9.00^b
T_3	0.66^b	3.00^b	7.33^b	11.00^b
T_4	1.00^b	3.33^b	7.66^b	11.33^b
T_5	1.66^b	4.66^b	8.00^b	11.66^b

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

Table 4. Effect of nitrogen levels on the number of leaves per plant on periwinkle

Treatment	30 DAT	60 DAT	90DAT	120 DAT
T_1	21.33^c	62.00^c	82.00^c	91.66^c
T_2	32.00^b	72.00^b	91.00^b	109.66^b
T_3	33.00^b	73.66^b	91.00^b	111.00^b
T_4	44.33^a	94.33^a	119.00^a	136.66^a
T_5	45.66^a	96.66^a	121.00^a	138.66^a

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

Table 5. Effect of nitrogen levels on number of flowers per plant on periwinkle

Treatment	30 DAT	60 DAT	90 DAT	120 DAT
T_1	8.00^d	15.00^d	20.00^c	25.30^d
T_2	13.00^c	23.00^{cd}	28.31^c	32.00^c
T_3	22.00^c	33.00^c	35.00^c	41.00^c
T_4	10.30^b	18.00^b	23.40^b	32.00^b
T_5	10.00^a	18.00^a	22.00^a	28.00^a

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

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. [17], flowers per plant, and flower production (q/ha) increased owing to 100 kg N/ha in marigold.

3.1.5 Greenness index

Greenness index of leaves as a function of nitrogen application levels. All treatments had a significant increase in SPAD value over RDF (T_1). Application of 10g urea had the highest SPAD value, which was 86.20. The lowest value was observed in T_1 (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. [18] and Chanchal et al. [19] found that increase dose of nitrogen application, increased the SPAD value.

3.2 Effect of Nitrogen Levels on Alkaloid Content in Periwinkle

At all the levels of nitrogen treatment, vinblastine was present except at 30 DAT, under T_1 treatment (control).

According to Golamhosseinpour et al. [20] 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. [8].

3.3 Effect of Nitrogen Application on Post-Harvest Soil of Nitrogen-Treated Soil

3.3.1 pH

The data on the pH of the soil as a result of nitrogen treatment is shown in Table 8. When different amounts of nitrogen were added, the pH of the soil reduced non significantly. Similar results found were by Aquino et al. [21].

3.3.2 Electric conductivity

According to the data, which is shown in Table 8 there were no significant differences in the EC of soil at T_2, T_3 and T_4 doses of nitrogen treatment. It was in the 0.41 – 0.43 dS m^{-1} range. T_5 had the greatest EC of the soil 0.45 dS m^{-1}, whereas T_1 had the lowest at 0.38 dS m^{-1}. Badyal [22] and Sharma [23] found the similar findings.

3.3.3 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 8. Organic carbon percentages in soil ranged from 0.466 to 0.53. Treatment T_5 had the highest level of organic carbon (0.53%), while treatment T_1 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 [24] and Sharma [23] found the similar result.

Table 6. Effect of nitrogen levels on SPAD value of plant leaves on periwinkle

Treatment	30 DAT	90 DAT	120 DAT
T_1	40.33^e	61.83^e	62.15^e
T_2	51.56^d	70.80^d	70.76^d
T_3	60.76^c	76.20^c	76.60^c
T_4	74.10^b	82.50^b	83.56^b
T_5	78.86^a	86.50^a	86.20^a

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

Table 7. Effect of nitrogen levels on presence or absence of vinblastine (alkaloid) in periwinkle

Treatment	30 DAT	60 DAT	80 DAT	100 DAT	120 DAT
T_1	A	P	P	P	P
T_2	P	P	P	P	P
T_3	P	P	P	P	P
T_4	P	P	P	P	P
T_5	P	P	P	P	P

(A – Absent, P – Present); DAT- Days after Transplanting

Table 8. Effect of nitrogen levels on properties of post-harvest soil of periwinkle

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 ($mgKg^{-1}$)
T_1	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}
T_2	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}
T_3	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}
T_4	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}
T_5	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; DAT- Days after Transplanting

3.3.4 Available nitrogen

There was increase the available nitrogen in soil with the increased nitrogen application. The highest available nitrogen was found at T_5 treatment which was 221.46 kg ha^{-1} and the smallest amount of available nitrogen was found at T_1 treatment which was 200.7 kg ha^{-1}. there was a significant increment in available nitrogen at T_5 over control. Muthuvel et al. [25] and Jhoon [24] recorded similar finding.

3.3.5 Available phosphorus

There was decrease the available phosphorus in soil with the increased amount of nitrogen application. The highest available phosphorus was found at T_1 treatment which was 19.2 kg ha^{-1} and the smallest available nitrogen was found at T_5 treatment which was 14.67 kg ha^{-1}. there was a significant decrement in available phosphorus at T_5 over control.Chandel [26] recorded similar findings.

3.3.6 Available potassium

There was increase the available potassium in soil with the increased amount of nitrogen application. The highest available potassium was found at T_5 treatment which was 254.4 kg ha^{-1} and the smallest available potassium was found at T_1 treatment which was 216 kg ha^{-1}. there was a significant increment in available potassium at all levels of nitrogen. Sharma [23] and Jhoon [24] recorded similar findings.

3.3.7 Micronutrients (Zn, Cu, Mn and Fe)

The findings in Table 8, 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. T_5 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. T_5 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. T_2 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. T_5 had the highest Fe which was 15 mg kg^{-1}.

3.4 Effect of Nitrogen Application on Periwinkle Leaves

3.4.1 Nitrogen content in plant

There was significant increment was found in the nitrogen content of plant leaves from T_1 to T_5 with application of higher doses of nitrogen. The highest nitrogen was found at T_5 treatment which was 2.54 % and the smallest content of nitrogen was found at T_1 treatment which was 1.31%. There was a significant increment in the nitrogen content at T_5 over control. Zankat [27], Nieuwh of and Jansen [28] all observed the similar findings.

3.4.2 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 T_5 treatment which was 39.9 mg/kg and the smallest Mn content in leaves was found at T_1 which was 33 mg/kg.

Martin [29] 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 levels on nitrogen content in post-harvest leaves of periwinkle

Treatment	Nitrogen (%)
T_1	1.31^e
T_2	1.56^d
T_3	1.79^c
T_4	2.1^b
T_5	2.54^a

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

Table 10. Effect of nitrogen levels on Fe, Cu, Mn, Zn content of plant leaves of periwinkle

Treatment	Fe	Cu	Mn	Zn
T_1	250.1[a]	5.1[a]	33.0[e]	55.0[a]
T_2	250.2[a]	5.1[a]	35.3[d]	55.1[a]
T_3	250.1[a]	5.2[a]	37.8[c]	55.0[a]
T_4	250.6[a]	5.0[a]	39.4[b]	55.3[a]
T_5	250.5[a]	5.1[a]	39.9[a]	55.1[a]

Different letters for each parameter show significant differences p<0.05; DAT- Days after Transplanting

4. 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 T_2 (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.

ACKNOWLEDGEMENTS

This paper and the research behind it would not have been possible without the exceptional support of my supervisor, Dr. Ramawatar Meena and Dr. Shreyans Kumar. His enthusiasm, knowledge and exacting attention to detail have been an inspiration and kept my work on track. I thank Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, and Department of Pharmaceutical Engineering and Technology, IIT, Banaras Hindu University, Varanasi for providing the resources for completion of my research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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