Journal of Ornamental Plants
https://sanad.iau.ir/en/Journal/jornamental/
ISSN (Print): 2821-0093   ISSN (Online): 2783-5219

Research Article
Volume 14, Number 1: 1-10, March, 2024

Investigating the Effect of Different Levels of Nitrogen Fertilizer on Quantitative and Qualitative Characteristics of *Chrysanthemum* (cv. Borna)

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Received: 04 November 2023   **Accepted:** 27 January 2024

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Abstract

Chrysanthemum is one of the five main cut flowers in Iran. Knowledge about the optimal range of macro-nutrientsespecially nitrogen (N), for the best quantitative and qualitative characteristics of chrysanthemum is of great importance. Randomized complete blocks design in three replications was implemented in this research. Five levels of N included 0, 75, 150, 225 and 300 kg/ha (ammonium nitrate) has been applied to chrysanthemum "Borna" cultivar in Mahalat city of Iran. These traits were measured: Score of life after harvest, branch number, flower numbers, flower longevity, days to flowering, chlorophyll, dry weight, shoot fresh weight, crown diameter, stem diameter and flower diameter. The results showed that maximum crown diameter, flower longevity, chlorophyll index, fresh and dry weight of the plant were obtained at the level of 150 kg/ha, compared to the control. Also, the highest total absorption of macro-nutrients (nitrogen, phosphorus and potassium (NPK)), and micro-nutrients (iron, manganese, zinc and copper) was observed at the level of 150 kg/ha fertilization. According to the results, N application in the level 150 kg/ha can be recommended to have best growth condition for the "Borna" cultivar of chrysanthemum in Mahalat city.

Keywords: Chrysanthemum, Growing and flowering indices, Macro-and micro-nutrients, N fertilizer.

INTRODUCTION

Chrysanthemum is the second most popular flower in the world, and one of the five main cut flowers in Iran (Taghipour *et al*., 2019). Cultivation area of chrysanthemum in 2019 was 253 hectares in Iran, and 15 hectares of which are in Markazi province and mainly in Mahalat city (Taghipour *et al*., 2019). One of the factors affecting the quality of chrysanthemum cultivars is the management of plant nutrition, especially macro-nutrients such as nitrogen (N). N is one of the main macro-nutrients for plant growth, and it is a growth bottleneck in plant nutrition and a key element (Vojodi Mehrabani, 2017). Also, the excessive use of application of chemical fertilizers such as N fertilizers in order to increase the amount of horticultural and agricultural products, along with the industrialization of countries, put the world in the risk of environmental pollutions (Aldhous, 2000).

Chrysanthemum is one of the fast-growing plants with high nutritional requirements especially N, that the environmental conditions including balanced and optimal nutritional status could lead to increase in photosynthesis in this plant (Teja *et al*., 2017). The availability of N during the first seven weeks of growth is very vital for the plant, however for example N fertilizer should not be given to the plant when the diameter of the flower bud reaches 1-1.5 cm (Vojodi Mehrabani, 2017). Satar *et al*. (2016) recommended the application of N and P for chrysanthemum in 100 to 200 kg/ha concentration, respectively. Rajan *et al*. (2019) studied different amounts and sources of N in the form of nitrate, ammonia, and urea in chrysanthemum (cv. Thai Chen). They found that the best form of N was nitrate at a level of 200 kg/ha, which caused the highest plant height, stem diameter, dry and wet weight shoot of the plant, and the maximum number of flowers. Chopde *et al*. (2015) studied the growth, flowering and yield of chrysanthemum cultivars, under the influence of N fertilizers. They reported the application of 300 kg/ha of N caused the most appropriate growth and quantitative and qualitative performance of the product. Also, one of more recent researches showed that application of 280 mg/kg of N increased the number of flowers, diameter of flower stalk, diameter of stem compared to the control (Ali and Mjeed, 2017).

Most of the literature reported that application of N fertilizers had a significant positive effect on plant height, number of flowers, flower diameter, stem diameter, bud length and flower stalk diameter in different cultivars of chrysanthemum. However, lack of research on the effect of N fertilizer application on Borna cultivar of chrysanthemum in Iran was our reason to conduct this research. Moreover, the use of chemical fertilizers, especially N, in chrysanthemums in the Mahalat city has not been subjected to special recommendations, which causes no-application or in some cases excessive application of fertilizers in this region.

The objective of this research was to investigate the effect of N fertilizer (ammonium nitrate) in five levels (0, 75, 150, 225 and 300 kg/ha) on the quantitative and qualitative characteristic of Borna cultivar of chrysanthemum. Furthermore, ability of the plants to absorb macro-nutrients (NPK) and micro-nutrients (iron, manganese, zinc and copper) from the soil was monitored in this study.

MATERIALS AND METHODS

Study site and the experiment

The experiment was conducted on Borna cultivar of chrysanthemum, in a farm in the National Research School of Flowers and Ornamental Plants (1747 m above sea level, longitude E 30˚ 27´ 50´´ and latitude N 33˚ 54´ 30´´), Mahalat, Iran. According to table 1, the average temperature during the experiment were 22/10 ºC (day/night) and relative humidity of

43%. The soil texture was sandy loam and the soil classification was determined as Entisols. Characteristics of the soil are given in table 2, and also the result of irrigation water analysis was shown in table 3.

Table 1. Climatic conditions.

Average minimum temperature (°C)	Average maximum temperature (°C)	Average relative humidity (%)	Total hours of sunshine (h)	Total precipitation (mm)
10.2	22.1	43	3146.6	280.6

Table 2. Average results of soil analysis (depth of 0-30 cm) before planting in the experimental area.

Sand	Silt	Clay	EC	pH	OC	TN	P	K	Fe	Zn	Mn	Cu	B	CEC
	%		dS/m		%					mg/kg				$Cmol^+$/kg
56	32	12	1.35	7.9	0.51	0.05	13	293	2.58	0.62	4.46	0.58	0.82	9.6

Table 3. Irrigation water properties.

EC	pH	SAR	Carbonate	Bicarbonate	Sulphate	Chlorine
(dS/m)				($Cmol^+$/kg)		
0.89	7.9	1.11	0	4.1	2.67	2.11

The experiment was implemented in a randomized complete block design in three replications. Nitrogen treatment was applied at five levels of 0, 75, 150, 225 and 300 kg/ha of N from the source of ammonium nitrate (NH_4^+) fertilizer (from N1 to N5, respectively).

Rooted chrysanthemum were planted in plots of 1×1 m, at distances of 20×25 cm (20 plants per m^2). Other fertilizers included triple superphosphate, potassium sulfate, magnesium sulfate, sequestrin138 (with 6% iron), manganese sulfate, zinc sulfate, copper sulfate, and boric acid have been applied in concentrations of 200, 100, 100, 40, 30, 40, 20, and 20 kg/ha, respectively, in all treatments. Irrigation was done according to the needs of the plant, once a week, using basin irrigation method.

N fertilizer was added into the soils in four stages; the first time a quarter of the fertilizer was applied just before planting (June 20th), second quarter 15 days after planting, third quarter 30 days after planting, and the last quarter 45 days after planting. The first fertilizer is mixed with the soil and the fertilizers after planting are spread on the soil.

Soil and plant properties

Soil properties included soil texture, pH, EC, CEC, organic carbon (OC), concentration of macro-nutrients (NPK) and micro-nutrients (Fe, Mn, Zn, Cu, B) were determined before plant cultivation in depth of 0-30 cm (Tekaya *et al.,* 2014; Ogunkunle *et al.,* 2020). The source of irrigation water supply of the National Flower and Ornamental Plant Research Institute was an aqueduct, and the water properties were analyzed included EC, pH, sodium adsorption ratio (SAR), carbonate, bicarbonate, sulphate, and chlorine.

Plant growth properties were determined in the pre-flowering stage, by taking newly matured leaves. Chlorophyll index was measured using a chlorophyll meter (Spad 502, Minolta, Japan) in five new mature leaves per plant (Lu *et al.,* 2022). Vegetative plant properties were determined included; plant height, stem diameter with digital caliper, branch number, and fresh and dry weights by digital balance. The absorption of NPK and micro-nutrients (Fe, Mn, Zn, Cu) by leaves were also measured (Tekaya *et al.,* 2014; Ogunkunle *et al.,* 2020). The measurement of elements, including iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), was done by atomic absorption, based on previous studies (Tekaya *et al.,* 2014; Ogunkunle *et al.,* 2020). Nitrogen was also measured by the Kjeldahl method (Tekaya *et al.,* 2014; Roussos *et al.,* 2017).

During the flowering period, measurements were included; time to start flowering, flower longevity, number of flowers, flower diameter, crown diameter, flower quality and life after harvest score.

Statistical analysis

The collected data has been statistically analyzed using SAS software version 9.2. Mean comparison and analysis of variance of the data of soil and plant properties was performed using LSD test at level of 5%. Data was normalized by min and max normalization.

RESULTS AND DISCUSSION

Soil characteristics before planting and irrigation water properties

Based on table 2, the soil texture was sandy loam and light enough to cultivate ornamental flowers, and also good for root development. The nutritional status of the soil showed that N concentration was low, and therefore application of N fertilizer was necessary for the best plant growth. However, according to table 2, other nutritions concentration has also been considered to apply P, K and micro-nutrients fertilizers into the soil to have better nutritional balance for plant growth and flowering. Table 3 showed that the irrigation water was neither salty nor sodic, therefore it was suitable to cultivate ornamental flower of chrysanthemum.

Plant properties and nutrients absorption

Based on table 4, the effect of N levels on all of the plant properties was statistically significant according to the LSD test. table 5 showed the plant growth and flowering properties affected by different N levels. Some of the plant growth properties like crown diameter and dry weight shoot had higher amounts in N2 and N3 levels (75 and 150 kg/ha N fertilizers, respectively) compared to the control (N1=0 kg/ha N fertilizer). Plant height, flower diameter and numbers were highest in N2 level (75 kg/ha N fertilizer) compared to the control. Crown diameter, plant fresh and dry weights shoot, chlorophyll, flower longevity and score of life after harvest were highest in N3 level (150 kg/ha N fertilizer). Therefore, it can be concluded that N application at the level of ≤150 kg/ha increased the quantitative and qualitative properties of the plant compared to the control (N1).

Table 4. The results of analysis of variance of the plant properties.

S.o.V	df	Plant height	Flower diameter	Stem diameter	Crown diameter	Fresh weight shoot	Dry weight shoot
Replication	2	87.816*	0.147^{ns}	26.62*	1.517^{ns}	0.181^{ns}	844.7^{ns}
Nitrogen (N)	4	19.47*	0.647**	11.69*	1.500*	0.060*	474.8*
CV (%)		19.78	11.97	33.02	16.61	11.26	48.92

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test.

Continued Table 4. The results of analysis of variance of the plant properties.

S.o.V	df	SPAD	Days to flowering	Flower longevity	Flower numbers	Branch number	Score of life after harvest
Replication	2	1.641^{ns}	0.017^{ns}	0.355^{ns}	19.16^{ns}	135.27**	1.35^{ns}
Nitrogen (N)	4	17.637*	9.33*	0.031*	7.90*	34.608*	0.942*
CV (%)		8.25	2.86	27.55	25.76	46.85	26.40

*, ** and ^{ns}: Significant at P < 0.05, P < 0.01 and insignificant based on the LSD test.

Table 5. Comparison of mean values and the effect of different levels of nitrogen fertilizer on quantitative and qualitative characteristics of chrysanthemum.

N level	NH_4NO_3	Plant height	Flower diameter	Stem diameter	Crown diameter	Fresh weight shoot	Dry weight shoot
	kg/ha	cm	cm	cm	cm	g	g
N1	0	33.97 abc	2.68 b	1.73 a	16.17 b	104 d	24.81 d
N2	75	37.5 a	3.44 a	1.5 ab	15.83 b	164 b	38.38 b
N3	150	34.43 ab	3.17 ab	1.8 ab	17.33 a	223 a	50.78 a
N4	225	31.4 abc	3.11 ab	1.1 b	10.33 d	98 e	22.52 c
N5	300	28.23 c	2.70 b	1.57 ab	12.33 c	114 c	25.21 cd

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

Continued Table 5. Comparison of mean values and the effect of different levels of nitrogen fertilizer on quantitative and qualitative characteristics of chrysanthemum.

N level	NH_4NO_3	SPAD	Days to flowering	Flower longevity	Flower numbers	Branch number	Score of life after harvest
	kg/ha	mg/100 g FW					
N1	0	57.13 a	134.7 b	8.66 a	6.66 b	7.33b	2.33 bc
N2	75	57.17 a	134 b	8 ab	7.33 a	8.6 ab	3 ab
N3	150	57.63 a	134.7 b	8.67 a	6.33 b	8.00 b	3.33 a
N4	225	54.83 b	137.3 a	7.66 b	4.66 c	9.33 a	2.00 c
N5	300	54.93 b	135 b	7.66 b	6.66 b	5.00 c	2.00 c

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the LSD test.

The increase in fresh and dry weight shoot of the plant may be due to increase in nutrient availability with enhancing N levels up to 150 kg/ha, this is in agreement with the results reported by Gangwar *et al.* (2012). The increase in fresh and dry weight shoot of the plant might also be due to an increase in plant spread, number of branches and leaf area, which in turn could increase number of flowers. It might be because of increased carbohydrate reserve for the development of floral primordia apart from the structural development of the plant (Teja *et al.,* 2017). It was also stated that increase in flower numbers and flower yield might be due to higher levels and balanced application of nitrogen and phosphorus (Satar *et al.,* 2016). Teja *et al.* (2017) found that plant height increased with application of nitrogen up to 200 kg/ha, and they considered it as essential role of nitrogen in the biosynthesis of nucleic acids, therefore it plays a vital role in promoting the plant growth. Further, nitrogen has been identified as an important constituent of chlorophyll, proteins and amino acids, thereby enhancing the rate of photosynthesis. The increase in plant height also might be due to greater uptake of nutrients into the plant system through soil application, which involved in the cell division, cell elongation as well as protein synthesis, and finally enhanced the stem length and vegetative growth (Teja *et al.,* 2017). According to table 5, The application of N fertilizer up to 150 kg/ha increased the life of flower after harvest, which may cause the lack of N and therefore led to reproductive growth overcomes the vegetative growth and extended life after harvest (Grewal and Tanya, 2016). Photosynthetically produced carbohydrates are transported to the points of growth are used predominantly in the synthesis of nucleic acids and proteins, hence the application of nitrogenous fertilizers during the vegetative growth phase to the plants controls the growth of the plant to a larger extent (Teja *et al.,* 2017).

The application of all nitrogen levels had significant effect on the vegetative and floral parameters like plant height, number of primary branches, leaves, root suckers, flowers per plant, size of flower and delayed days taken to full bloom (Grewal and Tanya, 2016).

N application in the range of 75 to 225 kg/ha caused the highest branch numbers compared to the control (N1) (Table 5). Nitrogen in the range of 75 to 225 kg/ha (150 kg/ha in average) may increase the photosynthetic activity by increasing the size of the photosynthesizing resources (chlorophyll, number of branches and leaf surface). Teja *et al.* (2017) also reported that increase in the branch numbers could be due to the basic role of nitrogen in photosynthesis, thereby resulting in more number of leaves and branches per plant. Therefore, it facilitates the development of flowers with more photosynthesis, and improves conditions for increasing cell division and expanding the number of flower tissue cells and flower size.

However, at N4 and N5 levels (225 and 300 kg/ha N fertilizers, respectively) most of the plant properties had lowest amounts, which revealed that application of higher N fertilizers (> 150 kg/ha) in our work did not show positive effect on plant growth properties.

Plant nutrients in leaves were measured included NPK as macro-nutrients and Fe, Mn, Zn and Cu as micro-nutrients (Fig. 1). The results showed that N3 level (150 kg/ha N fertilizer) had maximum concentrations of all nutrients compared to the control (N1=0 kg/ha N fertilizer). It revealed that 150 kg/ha concentration of N fertilizer for chrysanthemum, Borna cultivar, led to the best nutrient absorption by plants and it was in accordance with the increase trend of most of the growing and flowering properites of chrysanthemum (Table 5). Moreover, according to fig.1, K among the macro-nutrients and Fe and Mn among the micro-nutrients had the highest concentrations. It was due to the initial concentration of the K, Fe and Mn in the soil before planting and fertilization (see table 2), which were maximum among the macro- and micro-nutrients, respectively. Therefore, absorption of K and Fe and Mn by plants increased in comparison to the other nutrients.

The increase of NPK, absorption by plants in application of N in level of 150 kg/ha compared to the control was in consistent with the results reported by Rajan *et al.* (2019). The demand for nutrients absorption by plant could be increased due to the increase in photosynthesis sources as mentioned earlier.

Cluster analysis of chrysantemum based on physical and chemical properties of the leaves was shown in fig. 2. Cluster I included nitrgent treatment at 0, 75 and 300, had medium values for days to flowering. Cluster II included nitrogen treatmet at 150 through 225, had lowest values for days to flowering.

To determine the dispersion of chrysantemum cultivars, principle components analysis (PCA) was used (Fig. 3). According to the fig. 3, the variances explained by the first two components, PC1 and PC2, were 49.2 and 30.7%, respectively. In addition, the strongest positive correlations with leaf N, P, K, Fe, Mn, Zn and Cu, plant height and numbers, stem and crown diameter,fresh and dry weight shoot belonged to PC1. It also showed the strongest positive correlations with flower longevity, flower diameter and chlorophyll and branch number. Days to flowering and were strongly and negatively correlated with PC1 and PC2 (Fig. 3).

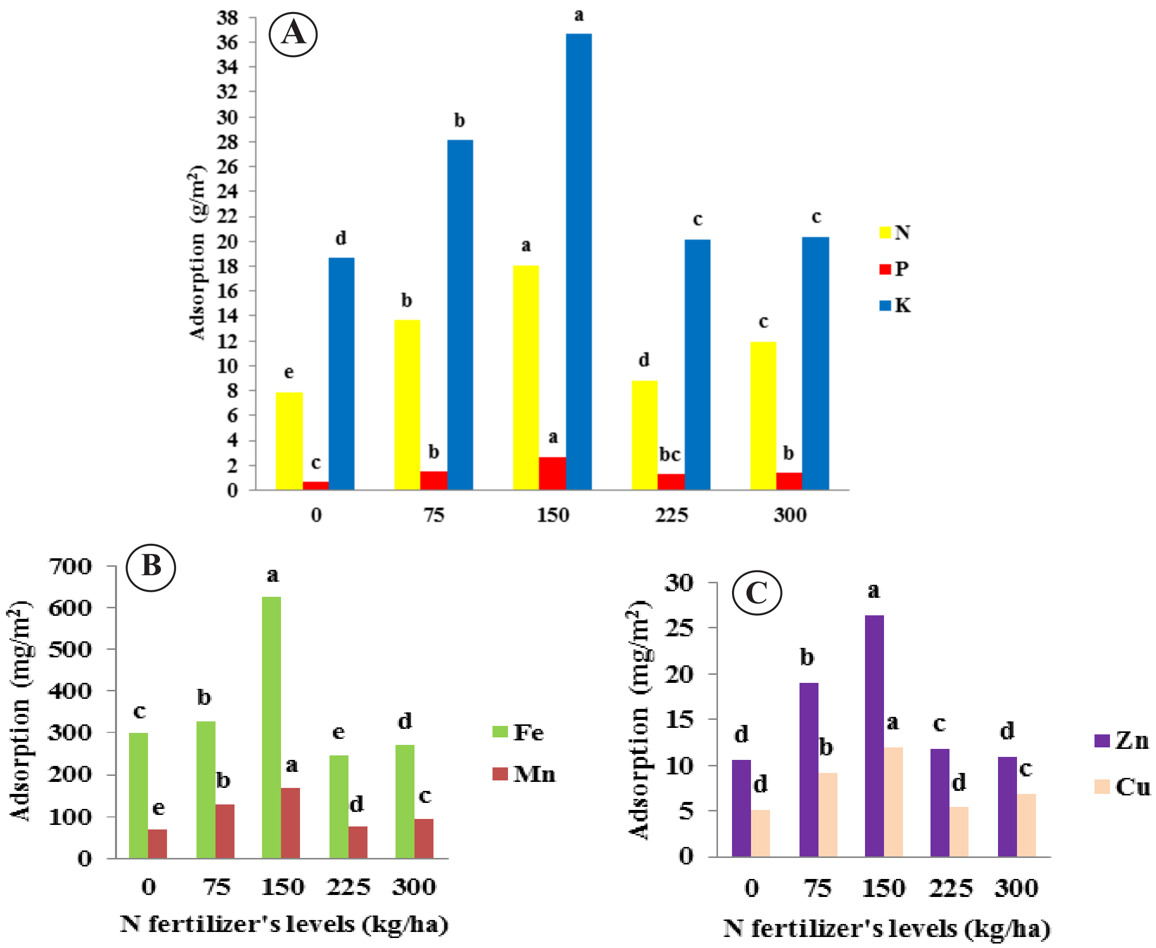

Fig. 1. The effect of different N levels on mean adsorption of macro-nutrient (a), micro-nutrients of Fe and Mn (b), and Zn and Cu (c) by plant leaves.

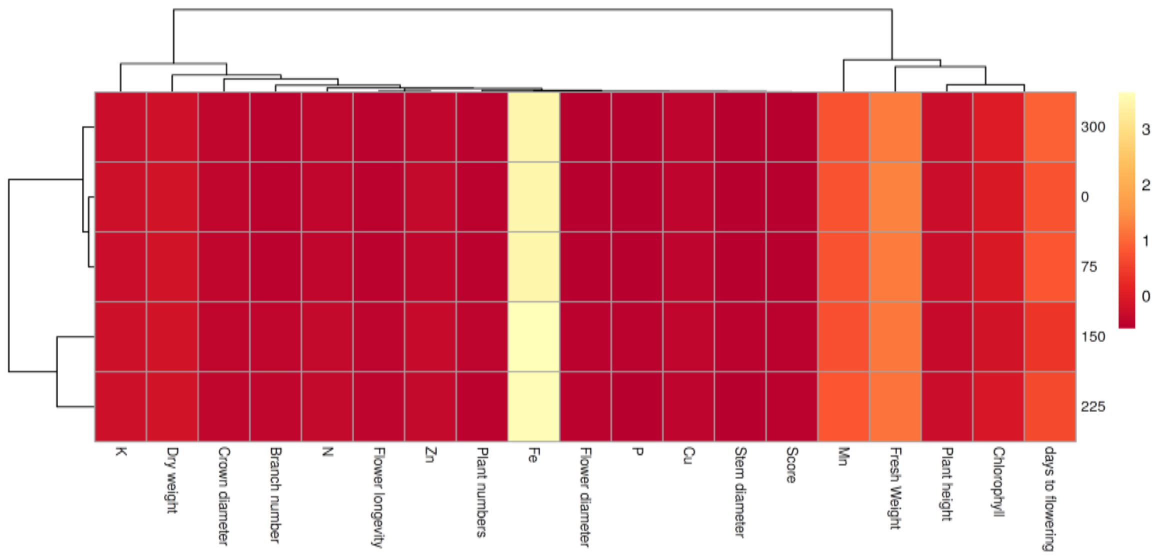

Fig. 2. Clustering heatmap analysis of chrysantemum based on physical and chemical properties of leaf (color gradient from low (dark red), medium (red) to high (yellow). Nitrogen levels: 0, 75, 150 and 225 kg/ha).

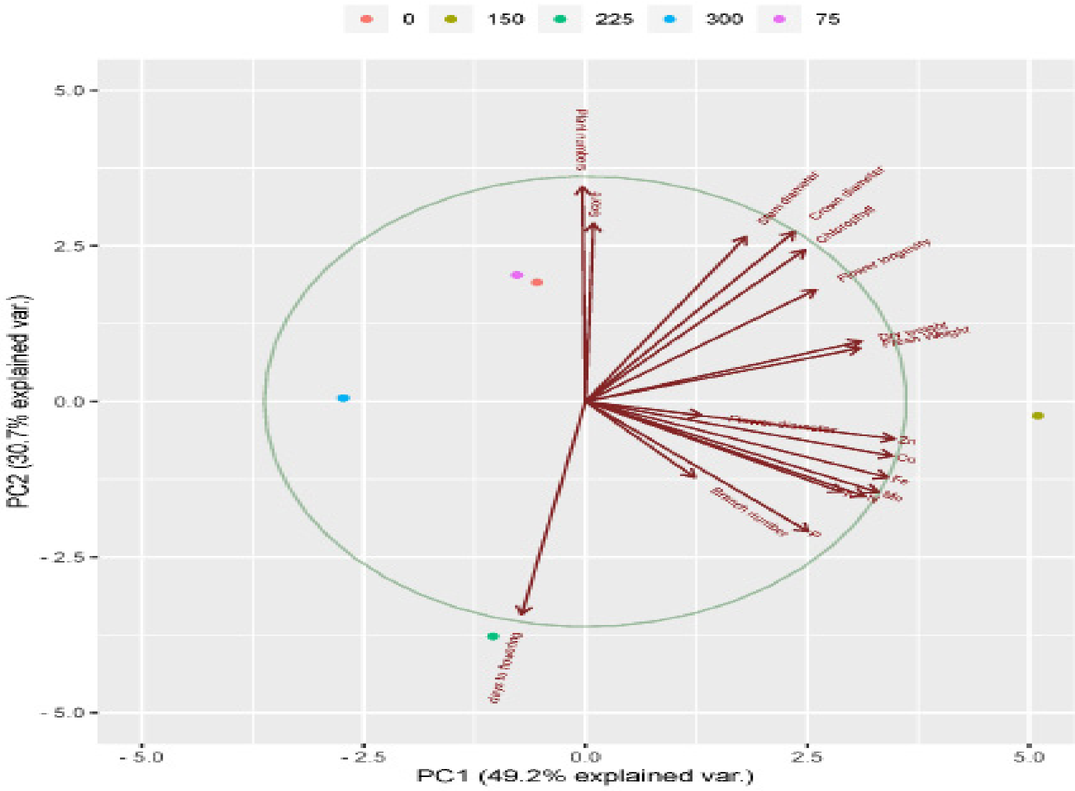

Fig. 3. The principle cluster analysis (PCA) of chrysantemun based on physical and chemical properties of leaf according to the first and the second principal components (PC1/PC2):7

Soil NPK concentrations after harvest

Based on table 6, the effect of N levels on all of Soil NPK concentrations after harvest was statistically significant according to the LSD test. The soil NPK after harvest was affected by N levels of fertilizers, and was higher in N1, N2 and N3 levels than N4 and N5 levels (Table 7). The amount of available P and K remaining in the soil after harvest decreased due to the increase in the levels of N application (N4 and N5, 225 and 300 kg/ha, respectively). The possible reason for this finding could be an increase in soil microbial activity due to high concentration of N, which led to increase in P and K absorption by the soil microorganism (Gu *et al*., 2021; Guo *et al*., 2022). Therefore, NPK at high levels of N application mostly consumed by soil microbes and the remaining amounts in the soil samples decreased.

Also, the soil NPK after harvest were lower in N3 (150 kg/ha) than N2 and N1 (75 and 0 kg/ha, respectively) (Table 7), which means the higher plant growing and flowering indices in N3 level (see table 7) led to the greater NPK adsorption by plants and low remaining amounts of NPK in the soil. Liu *et al*. (2022) showed that nitrogen application (between 100-200 kg/ha) improved plant growth, increased grain yield and regulated amino acid and mineral nutrient concentration delivery rates in plant roots, which also led to higher P and K uptake and translocation ability.

Table 6. The results of analysis of variance of Soil NPK concentrations after harvest.

S.o.V	df	N	P	K
Replication	2	0.002**	120.313*	39.699 ^{ns}
Nitrogen (N)	4	0.001*	112.98*	3828.86*
CV (%)		32.34	28.01	14.13

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the LSD test.

Table 7. The effect of different N levels on soil NPK concentration after harvest.

N levels	N	P	K
	%	mg/kg	
N1	0.068a	24.37a	261.6a
N2	0.064a	25.83a	244.6ab
N3	0.061a	20.36bc	244.5ab
N4	0.052b	19.82bc	223.9b
N5	0.048b	18.82c	217.2b

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

CONCLUSION

According to the increasing effects of different levels of N fertilizers on most of the observed quantitative and qualitative plant properties compared to the control (no N fertilizer application), it should be mentioned that N was an essential element for Borna cultivar of chrysanthemum growth. Normally, increase in amount of nitrogen fertilizers causes an increase in cell division, which leads to higher plant height, the number of branches in the plant, the leaf area, and the wet and dry weight of the plant. Among different applied N levels (0-300 kg/ha), N level of 75 and 150 kg/ha had the best effect on growing and flowering properties of chrysanthemum. Plant height, flower diameter and numbers were highest in N2 level (75 kg/ha N fertilizer) compared to the control. Crown diameter, plant fresh and dry weights shoot, chlorophyll, flower longevity and score of life after harvest were highest in N3 level (150 kg/ha N fertilizer). Increase in plant chlorophyll and fresh and dry weights shoot led to more photosynthesis by plants and therefore better plant growth in N3 level (150 kg/ha N fertilizer), which enhanced macro- and micro-nutrients absorption by plant. Also, higher N levels (225 and 300 kg/ha N fertilizers) had not positive effects on plant growth and could not be recommended. However, NPK concentrations in soil after harvest were lowest in the higher N levels in this study (225-300 kg/ha), which revealed that N application in high concentrations may increase soil microbial activity, and therefore the macro-nutrients have been consumed mostly by the soil microorganisms and remaining amount of the elements in the soil decreased.

ACKNOWLEDGMENT

The financial support of the Ornamental Plants Research Center is gratefully acknowledged.

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How to cite this article:

Khalaj, M.A., Bani Jamali, S.M., Shafiei, M.R., Farahani, E., Javan, M. & Sayyad-Amin, P. (2024). Investigating the Effect of Different Levels of Nitrogen Fertilizer on Quantitative and Qualitative Characteristics of *Chrysanthemum* (cv. Borna). Journal of Ornamental Plants, 14(1), 1-10.

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