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Effect of RDN combined with foliar spray of nano urea affects vegetative parameters of gaillardia (*Gaillardia pulchella*) cv. Local

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Abstract
This study investigated the application of RDN (recommended dose of nitrogen) combined with foliar spray of Nano Urea affects vegetative growth of gaillardia (*Gaillardia pulchella*) cv. Local, conducted at the Horticulture Polytechnic, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat, during Summer 2024. The experiment employed seven treatments involving different combinations of recommended dose of nitrogen (RDN) and Nano Urea. Among the treatments, combination of 75% RDN with Nano Urea @ 4 ml/lit recorded maximum plant height (27.9, 53.0 and 65.9 cm), number of leaves per plant (102.25, 272.67 and 421.13) and number of branches per plant (11.33, 15.38 and 18.20) at 60, 90 and 120 days after transplanting. Maximum leaf area (17.49 and 19.88 cm²) was recorded at 90 days and 120 DAT, along with leaf chlorophyll content (3500.37 mg/100 g).

Keywords: Nano urea, nitrogen, gaillardia, foliar spray

Introduction
Floriculture is the flourishing discipline of cultivating and marketing of flowers and ornamental plants, emerged as a promising industry in India. Flowers are an integral part of human life due to their diversity in beauty, colour, form, texture and fragrance. Without flowers, the world would not have been so beautiful, as charming and as cherishing today. They have a high aesthetic value in our daily lives because they represent purity, beauty, tranquillity, love and passion. Nowadays, Floriculture is fast growing and highly competitive industry with the continuous growing of new varieties and introduction of new flower crops. Our country currently occupies about 285 thousand ha area for floriculture with loose flower production around 22.84 Lakh MT and cut flower production around 9.47 Lakh MT during 2024-25. Total floriculture export for India during 2024-25 was 717.82 crore and major importing countries are USA, Netherlands, UAE, UK and Germany. Currently, India has more than 300 export-oriented units (EOUs) working in floriculture. (Anon., 2024[a]) [1].
Gaillardia (*Gaillardia pulchella*) flower commonly referred to as 'Fire Wheel' or 'Blanket Flower. It is an important flower crop of the *Asteraceae* family and is indigenous to the Central and Western United States. The 18th century French botanist Mr. Gaillard de Marentoneau, suggested the generic name gaillardia. Morinaaga *et al*. (1929) [10] reported that *Gaillardia pulchella* is diploid (2n=36). Numerous factors, including like, soil fertility, irrigation availability, plant density and plant protection measures, are necessary for the successful commercial production of the gaillardia flower. Proper fertilization, particularly nitrogen is one of the decisive factors influencing the growth and flowering of annual plants. Nitrogen deficiency reduces stem elongation, leaf area, photosynthesis rate, dry matter accumulation and leaf chlorophyll content (Bar-Tar *et al*., 2001 and Zhao *et al*., 2003) [2, 16]. While, excessive application of N usually increases input cost, pest incidence can diminish crop and poor quality flowers.
Interest in nanotechnology has grown as a result of the increasing difficulties facing in Indian agriculture, with the aim of boosting crop yield and improving resource use efficiency.

Utilizing nanomaterials of smaller than 100 nm size, nanotechnology has become a cutting edge science to develop concentrated plant nutrients sources with increased absorption rates, improved utilization efficacy and reduced losses (Kumar *et al*., 2021) [7].

Nano fertilizers have large surface areas and particle sizes that are smaller than the pore size of stomata's, which allows for easily penetration into plant tissues from the applied surface and improved nutrient absorption and use efficiency. Plant cell walls have pores that range in diameter from 5 to 50 nm. As a result, only aggregates of nanoparticles with a diameter smaller than the plant cell wall pore diameter could easily penetrate and reach the plasma membrane. Nanoparticles with a size less than 5 nm go through the cuticular pathway, whereas those with larger sizes travel through the stomatal pathway before reaching the conducting system and they aid in the rapid and simple absorption of nutrients by leaves (Dimkpa *et al*., 2015 and Qureshi *et al*., 2018) [3, 14].

Nano Urea is more efficacious in terms of nutrient absorption owing to lesser losses which contains 4% total nitrogen (w/v). The size of nano-nitrogen particles ranges from 20-50 nm. The water contains an even distribution of these particles. It contains 40,000 mg/L of nitrogen in a 500 ml bottle, which is same amount of nitrogen nutrient as one bag (45 kg) of conventional urea. It enters through stomata and other openings on plant leaves during critical growth stages and is absorbed by the plant cells. It is dispersed from the source to sink inside the plant wherever it is needed due to phloem transport. For the plant to grow and develop properly, Unutilized nitrogen is stored in the vacuole and released gradually for proper growth and development of the plant (Kantwa and Yadav, 2022) [5].

Materials and Methods

Experimental setup: The present study was conducted at Horticulture Polytechnic, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat, India (20º 55' North latitude and 72º 54' East longitude, at an elevation of approximately 11.93 meters above mean sea level) from February 2024 to May 2024. The soil had a flat topography, a medium to poor ability for drainage and a dark greyish brown Montmorillonite-type clay minerals make up the majority of more than 50% clay concentration. The availability of "N" in the soil was generally medium fertility level was medium in available 'P_2O_5' as well as 'K_2O' with pH of 7.4-7.8 and E.C. of 0.32-0.40 dSm^{-1}. After one month, healthy seed was planted on the raised bed to main field at a spacing of 30 x 30 cm. Seven treatments were replicated three times in the Randomized Block Design that was used. Among the treatments were included T_1: 100% RDN (No Nano Urea spray), T_2: 50% RDN + Nano Urea @ 2ml/lit (2 spray), T_3: 75% RDN + Nano Urea @ 2ml/lit (2 spray), T_4: 50% RDN + Nano Urea @ 3ml/lit (2 spray), T_5: 75% RDN + Nano Urea @ 3ml/lit (2 spray), T_6: 50% RDN + Nano Urea @ 4 ml/lit (2 spray) and T_7: 75% RDN + Nano Urea @ 4 ml/lit (2 spray). The recommended dose of NPK fertilizers @ 100:50:100 kg ha^{-1}. Nitrogen was applied in two split doses with Nano Urea spray. whereas phosphorus and potash full dose were applied as basal dose, respectively. Nano Urea quantity should be dissolved in water Nano Urea was applied as foliar spray to the plants at 25 and 55 day after transplanting.

The observations recorded were the plant height by measuring the height from ground level to the growing tip of five randomly selected plants from net plot after 60, 90 and 120 days of transplanting with the help of meter scale and average was worked out and expressed in cm, The number of branches and number of leaves were recorded from five randomly tagged plants of net plot after 60, 90 and 120 days of transplanting, Leaf area recorded from the 3rd or 4th leaf the bottom of the tagged plants at 90 and 120 DAT with the help of digital leaf area meter and then average value were worked out, leaf chlorophyll content (mg/100 g) recorded After 30 days of transplanting, leaves from tagged plants were analyzed for chlorophyll estimation. Chlorophyll content was measured as per the method given by Sadasivam and Manickam (1997) [11] as per following details.

Principle: A spectrophotometer is used to measure the absorbances at 663 nm and 645 nm after chlorophyll is extracted in 80% acetone. The amount of chlorophyll was determined using the provided formula.

Equipment's, Glass wares and Reagents: electric weight balance, spectrophotometer, buchner funnel, centrifuge, mortar, pestle, filter paper and volumetric flask and analytical grade 80% acetone (prechilled).

Procedure

- One gram of mature finely cut leave was weighted and grounded using 20 ml volume of 80% acetone into a clean mortar.
- The sample was centrifuged (5000 rpm for 5 minutes) and the supernatant was transferred to a 100 ml volumetric flask.
- The process of centrifuging was repeated until the appearance of colourless residue.
- The mortar and pestle were washed thoroughly with 80% acetone to get the clear extract of leaves.
- The volume was made up to 100 ml with 80% acetone.
- Then the absorbance of the solution was taken at 645 and 663 nm against the blank solution of 80% acetone.
- The amount of chlorophyll present in extract was calculated using the following equations:

Chlorophyll "a" (mg/g tissue) = 12.7 (A_{663})-2.69 (A_{645}) × [V/1000 × W]
Chlorophyll "b" (mg/g tissue) = 22.9 (A_{645})-4.68 (A_{663}) × [V/1000 × W]
Total Chlorophyll mg g tissue 2 .2 (A_{645}) + 8.02 (A_{663}) × [V/1000 × W]

Where,
A = Absorbance at specific wavelength
V = Final volume of chlorophyll extract in 80% acetone
W = Fresh weight of tissue extracted

The experimental data for individual characters were subjected to statistical analysis as per Analysis of Variance technique for Randomized Block Design as described by Panse and Sukhatme (1985) [8]. The SEm (**±**) value for treatment was calculated for each character to understand the error in the treatment effect. The critical difference was carried out, where treatment effects found significant to compare the treatment means. The coefficient of variation

(%) was also worked out for each character to understand the nature of variability that existed in the experimental materials.

Results

Table 1 to 4 show the experiments findings and the effect of different levels of nitrogen in combination with Nano Urea on growth of gaillardia (*Gaillardia pulchella*) cv. Local.

1. Plant height (cm)

With respect to plant height in gaillardia, treatment T_7 (75% RDN + Nano Urea @ 4 ml/lit) recorded maximum plant height 27.9, 53.0 and 65.9 cm, respectively at 60, 90 and 120 DAT. Whereas, treatment T_1 100% RDN recorded minimum plant height 19.8, 40.3 and 51.7 cm, respectively at 60, 90 and 120 DAT.

Table 1: Effect of RDN combined with Nano Urea on plant height in gaillardia cv. Local

Treatments	Plant height (cm)		
	60 DAT	90 DAT	120 DAT
T_1:100% RDN	19.8	40.3	51.7
T_2: 50% RDN + Nano Urea @ 2 ml/lit	21.7	42.3	53.2
T_3: 75% RDN + Nano Urea @ 2 ml/lit	25.8	47.9	61.6
T_4: 50% RDN + Nano Urea @ 3 ml/lit	23.9	43.3	56.3
T_5: 75% RDN + Nano Urea @ 3 ml/lit	26.1	51.0	63.2
T_6: 50% RDN + Nano Urea @ 4 ml/lit	24.5	46.1	57.7
T_7: 75% RDN + Nano Urea @ 4 ml/lit	27.9	53.0	65.9
SEm (±)	1.42	2.25	2.13
CD at 5%	4.37	6.92	6.57
CV (%)	10.12	8.40	6.30

2. Number of Branches Per Plant

The treatment T_7 (75% RDN + Nano Urea @ 4 ml/lit) recorded highest number of branches plant^{-1} (11.33, 15.38 and 18.20, respectively) at 60, 90 and 120 DAT. Whereas, T_1 100% RDN recorded lowest number of branches plant^{-1} (8.82, 12.07 and 13.87, respectively) at 60, 90 and 120 DAT.

Table 2: Effect of RDN combined with Nano Urea on the number of branches per plant in gaillardia cv. Local

Treatments	Number of branches per plant		
	60 DAT	90 DAT	120 DAT
T_1:100% RDN	**8.82**	**12.07**	**13.87**
T_2: 50% RDN + Nano Urea @ 2 ml/lit	9.60	12.41	14.00
T_3: 75% RDN + Nano Urea @ 2 ml/lit	10.53	12.90	15.93
T_4: 50% RDN + Nano Urea @ 3 ml/lit	10.13	12.55	15.00
T_5: 75% RDN + Nano Urea @ 3 ml/lit	10.87	13.64	16.71
T_6: 50% RDN + Nano Urea @ 4 ml/lit	10.27	12.57	15.87
T_7: 75% RDN + Nano Urea @ 4 ml/lit	11.33	15.38	18.20
SEm (±)	0.30	0.54	0.52
CD at 5%	0.92	1.68	1.59
CV (%)	5.05	7.21	5.73

3. Number of Leaves Per Plant

Among the treatments, T_7 (75% RDN + Nano Urea @ 4 ml/lit) numerically, the highest number of leaves per plant (102.25, 272.67 and 421.13, respectively) at 60, 90 and 120 DAT. Whereas, treatment T_1 100% RDN recorded the least number of leaves per plant (83.11, 177.70 and 285.77, respectively) at 60, 90 and 120 DAT.

Table 3: Effect of RDN combined with Nano Urea on the number of leaves per plant in gaillardia cv. Local

Treatments	Number of leaves per plant		
	60 DAT	90 DAT	120 DAT
T_1:100% RDN	83.11	177.70	285.77
T_2: 50% RDN + Nano Urea @ 2 ml/lit	84.63	222.18	289.09
T_3: 75% RDN + Nano Urea @ 2 ml/lit	92.37	239.97	365.72
T_4: 50% RDN + Nano Urea @ 3 ml/lit	86.77	234.13	333.11
T_5: 75% RDN + Nano Urea @ 3 ml/lit	95.30	245.06	408.98
T_6: 50% RDN + Nano Urea @ 4 ml/lit	89.46	236.54	346.49
T_7: 75% RDN + Nano Urea @ 4 ml/lit	102.25	272.67	421.13
SEm (±)	3.76	9.09	25.97
CD at 5%	11.58	28.00	80.00
CV (%)	7.19	6.77	12.85

4. Leaf Area (cm^2)

Among the treatments, T_7 (75% RDN + Nano Urea @ 4 ml/lit) recorded significantly higher leaf area (17.49 and 19.88, respectively) at 90 and 120 DAT, whereas lowest leaf area was recorded in treatment T_1 100% RDN (12.53 and 14.00 cm^2, respectively) at 90 and 120 DAT.

5. Leaf Chlorophyll Content (mg/100 g)

The highest chlorophyll content (3500.37 mg/100 g) was recorded in treatment T_7 (75% RDN + Nano Urea @ 4 ml/lit), Meanwhile, the minimum leaf chlorophyll content (2614.32 mg/100 g) was recorded with treatment T_1 (100% RDN).

Table 4: Effect of RDN combined with Nano Urea on leaf area and leaf chlorophyll content in gaillardia cv. Local

Treatments	Leaf area (cm^2)		Leaf Chlorophyll Content (mg/100 g)
	90 DAT	120 DAT	
T_1:100% RDN	12.53	14.00	2614.32
T_2: 50% RDN + Nano Urea @ 2 ml/lit	14.01	16.60	2703.55
T_3: 75% RDN + Nano Urea @ 2 ml/lit	16.60	19.14	3067.21
T_4: 50% RDN + Nano Urea @ 3 ml/lit	15.94	17.16	2752.96
T_5: 75% RDN + Nano Urea @ 3 ml/lit	17.38	19.40	3367.15
T_6: 50% RDN + Nano Urea @ 4 ml/lit	16.21	18.00	2963.52
T_7: 75% RDN + Nano Urea @ 4 ml/lit	17.49	19.88	3500.37
SEm (±)	0.81	0.93	53.87
CD at 5%	2.50	2.86	165.96
CV (%)	8.93	9.06	3.11

Discussion

In the present study, improve plant height faster than conventional fertilizers. Their nano scale size particles enhances leaf absorption and increases nitrogen utilization, which leads to vigorous cell division as well as cell elongation. This generates more photosynthetic energy, which promote plant growth. Compared to conventional urea, the uptake of Nano Urea is slow, which minimizes nitrogen losses. It ensures a consistent and ample nitrogen supply during crucial growth and development phases, resulting in taller, more vigorous plants with enhanced health and productivity. These results are in close confirmatory with findings of Karavadia and Dhaduk (2002) [6] in annual chrysanthemum, Shinde *et al.* (2014) [13] in marigold.

The increase in the number of branches per plant may be attributed to the application of Nano Urea. Nano-sized particle dimension improves the absorption and transportation of nitrogen into plant system, that minimizes the wastage and improving the availability of nitrogen accumulation in the growing tissues. Nitrogen is an essential contributor to protein synthesis and cell division which also induces axillary bud development resulting in branch formation and vegetative growth. Nano Urea is not only reduces nitrogen deficiencies but also leads to overall vegetative growth due to a regular supply of nitrogen. The similar results were also reported by Sahithi *et al.*, (2023) [12] in marigold and Meena *et al.*, (2025) [9] in rose.

This might be due to Nano Urea pushes acceleration in phyllotaxis, that results in more number of leaves on plants due to its high nitrogen use efficiency and enhanced root uptake mechanism. Its minuscule particle size promotes rapid absorption through leaf stomata and cuticle also. Nano Urea minimizes nitrogen losses through leaching or volatilization, ensuring sustained nutrient availability for optimal leaf production and overall plant growth. Similar trends were reported by Vidyasree *et al.*, (2022) [15] in philodendron, Kalyani *et al.*, (2024) [4] in gerbera and Meena *et al.*, (2025) [9] in rose.

The Nano particles get absorbed more efficiently by leaves and provide a continuous supply of nitrogen throughout the plant, which enhance nutrient supply system within plant. It encourages more photosynthetic and metabolic activities which increases growth and larger sized leaves. The results obtained were conformity with the previous research of Vidyasree *et al.*, (2022) [15] in philodendron, Sahithi *et al.*, (2023) [12] in marigold and Kalyani *et al.*, (2024) [4] in gerbera.

Nitrogen, a crucial component of chlorophyll molecules, enhances quality and quantity of chlorophyll in plants by providing an efficient and consistent supply through Nano Urea. Nanoparticles of nitrogen may be quickly absorbed and translocated by the stomata, ensure its availability in leaf tissues. As a result, more chlorophyll and related proteins that are necessary for photosynthesis are produced. Consistent nitrogen availability, preserving ideal amounts of chlorophyll and encouraging the growth of healthy leaves. Furthermore, by lowering nitrogen losses from volatilization and leaching, Nano Urea guarantees that plants eventually get enough nutrients. More chlorophyll production enhance healthier and colourful leaves for better photosynthetic efficiency of plants. Similar observations are in close conformity with the findings of Sahithi *et al.*, (2023) [12] in marigold.

Conclusion

Based on the results of the present study, it can be concluded that application of 75% RDN combined with foliar application of Nano Urea @ 4 ml/lit water at 25 and 55 days after transplanting significantly increased plant height, number of branches per plant, number of leaves per plant, leaf area and leaf chlorophyll content in gaillardia cv. Local.

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