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Efficacy of nano urea in replacing conventional nitrogen application and its effect on fruit quality in strawberry

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Abstract

In a study conducted on strawberry cv. Winter Dawn, the combined application of nano urea and *Azotobacter* was investigated for its impact on quality and biochemical attributes under protected cultivation. The findings revealed a noteworthy response of strawberry plants to foliar nano applications compared to conventional soil application of urea. Despite the lower nitrogen dosage associated with nanoparticle supplementation, strawberries with nanoparticles exhibited significant improvements in qualitative and biochemical parameters. These results underscore the potential of urea nanoparticles as a viable nitrogen source, advocating for alternative approaches to nitrogen fertilization. By fine-tuning the dosage ratio, this strategy shows promise in fostering a more environmentally friendly, sustainable and modern method for cultivating strawberries. The enhanced quality and biochemical attributes observed in strawberries treated with nano urea highlight the efficacy of this innovative approach in optimizing plant growth and productivity. Furthermore, the utilization of nano urea in combination with *Azotobacter* demonstrates synergistic effects, potentially enhancing nutrient uptake and utilization efficiency in plants. This holistic approach improves crop quality and reduces the environmental impact associated with traditional fertilization methods. In conclusion, the findings of this study support the adoption of urea nanoparticles as a valuable tool in strawberry cultivation, offering a pathway towards sustainable agricultural practices. Future research efforts should focus on optimizing application protocols and assessing long-term effects to fully exploit the potential of nano-based fertilizers for strawberry.

Keywords

Azotobacter; biochemical; conventional urea; nano-urea; quality; strawberry; responsible consumption; production

Introduction

Strawberry (*Fragaria x ananassa* Duch.) is considered an exotic crop and is highly favoured by consumers primarily due to its taste and appearance (1). It is highly concentrated with vitamins, minerals, sugars, phenolics, anthocyanin, antioxidants, carotenoids and ascorbic acids (2). China leads in strawberry production and recorded 3.3 million tons of production during 2021 (3). Also, countries such as Mexico, Egypt, Russia, the Republic of Korea, Japan, Poland and Germany are employed in producing strawberries worldwide (4). In the last decade, the production of strawberries has recorded a two-fold increase worldwide owing to advancements in cultivation and nutrient application techniques (5). But still, India lags in area expansion under strawberry as well as production. It could be majorly attributed to the delicate nature of the fruit as well as irrational nutrient management strategies.

The judicious application of fertilizers and manures is imperative for achieving higher fruit yields of superior quality (6). Nano-fertilizers have emerged as a promising alternative, ensuring not only enhanced crop production but also the restoration of soil health. Sustainable intensification in agriculture has been identified as a possible goal using nano-fertilizers. Specifically, nano urea, a nanotechnology-based agricultural input, is pivotal in supplying nitrogen to plants. This unique fertilizer effectively meets the plant's nutrient requirements due to its bioavailability and desirable particle size, ranging from 20 to 50 nm. Nano urea significantly increased surface area, exceeding traditional 1 mm urea prills by a staggering factor of 10000 and a substantially higher number of particles, with approximately 55000 nitrogen particles per 1 mm urea prill. Additionally, the targeted and gradual release of nutrients (7) from NFs reduces their toxicity to plants (8). It lowers the amount of N lost through denitrification, fixation, volatilization, leaching and salt build up in the soil.

Consequently, the utilization of nano urea results in a remarkable enhancement of nitrogen availability to crops, surpassing 80 %, thereby enhancing nutrient use efficiency and ultimately leading to improved crop yields. Notably, nano urea exerts a substantial influence on both the qualitative and quantitative aspects of fruit cultivation. Keeping this in view, a field experiment was conducted to evaluate the efficacy of nano urea in conjunction with conventional urea and *Azotobacter* in promoting the qualitative traits of Strawberry cv. Winter Dawn.

Materials and Methods

The present study was conducted in the experimental field of Horticulture at Lovely Professional University, Punjab, India, during the year 2022-23. The experimental site is in Punjab's central plains, part of India's Trans-Gangetic agroclimatic zone. The experimental site was located at 31° 13' 26.4'' N and 75° 46' 14.9'' E. Runner-grown seedlings of the strawberry cv. Winter Dawn was obtained from Shimla. The experiment was laid out in a randomized block design (RBD) with 16 treatments. Each treatment was replicated thrice. Ten plants were maintained with a spacing of 45 cm x 30 cm. Data on quality and biochemical attributes was collected from the fruits of every individual plant within each replicated plot of respective treatment. This study used the recommended chemical fertiliser application dosage by Punjab Agricultural University for the experimental area as the control treatment (T_1). Other treatments were compared with the control treatment for various biochemical characteristics. Nano urea, having a particle size of 20-50 nm, was sourced from Indian Farmers Fertilizer Cooperative Limited (IFFCO), while *Azotobacter* (Utkarsh Ltd.) was administered at a constant concentration of 2 mL/L. The nano urea treatments varied in concentration, with 300 ppm (N_1) and 400 ppm (N_2) dosages. In this study, except for the treatment control (T_1), only the dosage of nitrogen through urea was varied, precisely at 25 %, 50 % and 75 % of the RDF. At the same time, phosphorus and potassium were applied at their recommended rates without modification. Nano urea was used as a foliar treatment for strawberry plants in conjunction with *Azotobacter* inoculation. The results of these treatments were then compared to those of conventional basal applications of chemical fertilizers (T_1).

Treatment Details

T_1: control [100 % RDF (PAU recommendation)], **T_2**: 25 % RDF + N_1, **T_3**: 25 % RDF + N_2, **T_4**: 50 % RDF + N_1, **T_5**: 50 % RDF + N_2, **T_6**: 75 % RDF + N_1, **T_7**: 75 % RDF + N_2, **T_8**: 25 % RDF + N_1+ *Azotobacter*, **T_9**: 25 % RDF + N_2+ *Azotobacter*, **T_{10}**: 50 % RDF + N_1+ *Azotobacter*, **T_{11}**: 50 % RDF + N_2+ *Azotobacter*, **T_{12}**: 75 % RDF + N_1+ *Azotobacter*, **T_{13}**: 75 % RDF + N_2+ *Azotobacter*, **T_{14}**: 25 % RDF + *Azotobacter*, **T_{15}**: 50 % RDF + *Azotobacter*, **T_{16}**: 75 % RDF + *Azotobacter*.

Note: RDF: (*44 kg N, 32 kg P_2O_5 and 40 kg K_2O/acre*); *N_1: 300 ppm Nano Urea and N_2: 400 ppm Nano Urea.*

Observation recorded

Fruit quality parameters recorded during the harvesting period included total sugars, non-reducing sugars, reducing sugars, total soluble solids, titratable acidity, TSS: acid ratio, ascorbic acid, anthocyanin and antioxidants. The total soluble solid of the juice was determined with an Erma-hand refractometer after extracting the fruit juice from 30 fruits. The same solution was titrated with 0.1 N sodium hydroxide to reach pH 8.1 (n = 10) and the total acidity was calculated. The results were expressed as a % of acid. Chemical analysis of fruits was done using the standard procedure (9). The anthocyanin pigment of berries was determined by the method (10). The antioxidant content of berries was estimated using the DPPH radical-scavenging activity method (11).

Statistical analysis

The collected data were subjected to analysis of variance in a randomized block design using SPSS v.23 (SPSS Inc. USA). The significance level was compared between the treatments using Duncan’s multiple range tests at a 5% significance level.

Results and Discussion

TSS (°brix), Titratable Acidity (%) and TSS: Acid ratio

There was a varied response to different doses of nano urea on the quality parameters of the strawberry fruit (Table 1). A significantly higher value to the tune of 9.10 °brix for TSS was obtained by applying 25 % RDF + N_2+ *Azotobacter* (T_9). However, the minimum TSS value was recorded with the application of 25 % RDF + *Azotobacter* (T_{14}) and was found to be at par with 50 % RDF +

Azotobacter (T_{15}) and 75 % RDF + *Azotobacter* (T_{16}) treatments. The lowest titratable acidity (Table 1), having a value of 0.42 %, was obtained under 25 % RDF + 400 ppm Nano Urea + *Azotobacter* (T_9) treatment. Highest titratable acidity (0.57 %) was recorded under 75 % RDF + 300 ppm Nano Urea (T_6), 75 % RDF + 400 ppm Nano Urea (T_7) and 75 % RDF + *Azotobacter* (T_{16}). The highest TSS: acid (Table 1) ratio of 21.73 was recorded with 25 % RDF + N_2+ Azotobacter (T_9) application. It may be ascribed to the better absorption of nano nitrogen particles into the plant, which results in faster growth and development. Sustained and optimum availability of nitrogen affects the sugar content in fruit crops, ultimately determining taste and flavour (12, 13). Since nano formulations can penetrate plant systems more effectively because of their nanoparticle size, their application has enhanced fruit TSS (6). Research demonstrated that the combination of *Azotobacter* along with the application of nitrogen sources through chemical fertilizer improved the yield and quality aspects of strawberry (14). It may be inferred from the data that inoculation of *Azotobacter* improved the quality attributes of strawberry. The plausible reasons include the fast metabolic conversion of starch and pectin into soluble compounds, the rapid movement of sugars from leaves to developing fruits and the increased accumulation of soluble components from protein hydrolysis and sugars and other components (14). Similar findings have been reported in strawberries (15).

Total Sugars, reducing sugars and non-reducing sugars

Application of 25 % RDF with 400 ppm Nano urea and *Azotobacter* recorded the highest total sugar content (8.77 mg/100 g) of strawberry, which was at par with 25 % RDF + 300 ppm Nano urea + *Azotobacter* application (Table 2) and is significant against control (T_1) which recorded a total sugar content of 6.83 mg/100 g. Fruits harvested from the plots, which were treated with nano urea combined with *Azotobacter*, produced a higher percentage of total sugar. The lowest total sugar content of 6.30 mg/100 g was recorded with the application of 25 % RDF + *Azotobacter* (T_{14}) and was on par with 50 % RDF + *Azotobacter* (T_{15}) and 75 % RDF + *Azotobacter* (T_{16}) treatments.

A higher value of 7.17 mg/100 g of reducing sugar was recorded in Treatment T_9. Amongst the various treatments, T_9 (25 % RDF + N_2 + *Azotobacter*) exhibited maximum content of non-reducing sugar (Table 2) and was lowest in control (T_{14}) with a value of 1.43 mg/ 100 g

Table 1. Effect of Nano Urea and *Azotobacter* inoculation on TSS, Titratable acidity and TSS: acid ratio in strawberry fruit.

Treatments	TSS (°brix)	Titratable Acidity (%)	TSS: Acid
T_1 (control, 100 % RDF)	6.93^{cd}	0.55^{e}	12.65^{bc}
T_2 (25 % RDF + N_1)	6.60^{bc}	0.47^{bc}	13.94^{cd}
T_3 (25 % RDF + N_2)	8.43^{fg}	0.51^{d}	16.54^{cd}
T_4 (50 % RDF + N_1)	7.67^{def}	0.55^{e}	13.98^{cde}
T_5 (50 % RDF + N_2)	7.97^{ef}	0.54^{e}	14.66^{cde}
T_6 (75 % RDF + N_1)	7.40^{cde}	0.57^{e}	13.07^{bc}
T_7 (75 % RDF + N_2)	8.00^{ef}	0.57^{e}	13.96^{cd}
T_8 (25 % RDF + N_1+ *Azotobacter)*	7.23^{cde}	0.47^{bc}	15.29^{cde}
T_9 (25 % RDF + N_2+ *Azotobacter)*	9.10^{g}	0.42^{a}	21.73^{g}
T_{10} (50 % RDF + N_1+ *Azotobacter)*	8.20^{efg}	0.46^{b}	17.83^{def}
T_{11} (50 % RDF + N_2+ *Azotobacter)*	8.47^{fg}	0.48^{bc}	17.76^{fgh}
T_{12} (75 % RDF + N_1+ *Azotobacter)*	7.80^{def}	0.49^{cd}	15.81^{cd}
T_{13} (75 % RDF + N_2+ *Azotobacter)*	7.93^{ef}	0.48^{bcd}	16.41^{cd}
T_{14} (25 % RDF + *Azotobacter)*	5.07^{a}	0.56^{e}	9.00^{a}
T_{15} (50 % RDF + *Azotobacter)*	5.80^{ab}	0.56^{e}	10.39^{ab}
T_{16} (75 % RDF + *Azotobacter)*	5.93^{ab}	0.57^{e}	10.42^{ab}

Where,

* **N_1:** 300 ppm Nano Urea **N_2:** 400 ppm Nano Urea

Table 2. Effect of Nano Urea and *Azotobacter* inoculation on sugars (Total, Reducing and Non-Reducing) in strawberry fruit.

Treatments	Total Sugars	Reducing Sugars	Non-reducing Sugars
T_1 (control, 100 % RDF)	6.83^{b}	5.33^{a}	1.43^{ab}
T_2 (25 % RDF + N_1)	7.43^{c}	5.90^{b}	1.46^{b}
T_3 (25 % RDF + N_2)	8.17^{d}	6.60^{cdefg}	1.49^{ab}
T_4 (50 % RDF + N_1)	7.57^{b}	6.33^{bcde}	1.17^{ab}
T_5 (50 % RDF + N_2)	7.60^{b}	6.10^{bc}	1.43^{ab}
T_6 (75 % RDF + N_1)	7.57^{b}	6.13^{bcd}	1.36^{ab}
T_7 (75 % RDF + N_2)	7.60^{b}	6.47^{cdef}	1.08^{a}
T_8 (25 % RDF + N_1+ *Azotobacter)*	8.50^{de}	7.03^{fg}	1.39^{ab}
T_9 (25 % RDF + N_2+ *Azotobacter)*	8.77^{e}	7.17^{g}	1.52^{b}
T_{10} (50 % RDF + N_1+ *Azotobacter)*	8.37^{de}	6.87^{efg}	1.43^{ab}
T_{11} (50 % RDF + N_2+ *Azotobacter)*	8.40^{de}	6.93^{fg}	1.39^{ab}
T_{12} (75 % RDF + N_1+ *Azotobacter)*	8.07^{d}	6.73^{efg}	1.27^{ab}
T_{13} (75 % RDF + N_2+ *Azotobacter)*	8.13^{d}	6.67^{defg}	1.39^{ab}
T_{14} (25 % RDF + *Azotobacter)*	6.30^{a}	5.20^{a}	1.05^{a}
T_{15} (50 % RDF + *Azotobacter)*	6.44^{ab}	5.24^{a}	1.14^{ab}
T_{16} (75 % RDF + *Azotobacter)*	6.50^{ab}	5.17^{a}	1.27^{ab}

(Table 2). The higher nitrogen use efficiency due to applying nano fertilizer in combination with *Azotobacter might* significantly increase the total sugar content. Nano-fertilizers release nutrients gradually and continuously, increasing the efficiency with which plants absorb nutrients (16). It was also reported that by making leaf nutrient content and specific growth promoters more readily available, it seemed that the application of bacteria reduced the over-application of nitrogen (17).

Antioxidants, anthocyanin and ascorbic acid content

There were significant variations in the biochemical attributes among various treatments. Maximum antioxidants [1.90 µmol Trolox Equivalent (TE) /g Fresh Weight (FW)] were recorded in T_{11} (50 % RDF + N_2 *Azotobacter*) and T_{10} (50 % RDF +N_1 + *Azotobacter*) (Table 3) followed by T_9 (25 % RDF +N_2 + *Azotobacter)* and T_8 (25 % RDF +N_1 + *Azotobacter*) having 70.33 µmol TE/g FW and 67.33 µmol TE/g FW respectively. Treatments 50 % RDF + *Azotobacter* (T_{15}) and 75 % RDF + *Azotobacter* (T_{16}) received almost similar antioxidant values and the lowest antioxidant was obtained with 25 % RDF + *Azotobacter* (T_{14}) treatment. Maximum anthocyanin (Table 3) content (0.274 mg/100 g) was also observed in treatment T_9 (25 % RDF + N_2 + *Azotobacter*) followed by T_3 (25 % RDF + N_2) with antioxidant content of 0.269 mg 100 g^{-1}. Additionally, the highest ascorbic acid (Table 3) was recorded under treatment T_{11} (50 % RDF + N_2 + *Azotobacter*), having a count of 55.7 mg 100 g^{-1}, followed by T_9 (25 % RDF + N_2 + *Azotobacter*) with the value of 55.0 mg per 100 g. The microbial inoculant's enhanced ability to fix atmospheric nitrogen and excrete growth-promoting substances may have contributed to the corresponding increase in ascorbic acid content. This would have sped up physiological processes like synthesizing carbohydrates in strawberries (18). Similar findings were also reported (14). Researchers also confirmed that applying nano fertilizer on strawberry improved the enzymatic antioxidants with increasing nano fertilizer doses (19).

The synergistic application of nano urea with *Azotobacter* can enhance the quality attributes of strawberries through several mechanisms. Nano urea facilitates efficient nutrient delivery, ensuring plants' optimal nitrogen uptake. Concurrently, *Azotobacter,* a nitrogen-fixing bacterium, further supplements nitrogen availability in the soil, promoting sustained plant growth and development. This combined approach increases nutrient assimilation and enhances plant vigour, improving fruit quality attributes such as size, colour, flavour and nutritional content. Overall, the synergistic action of nano urea and *Azotobacter* contributes to the enhanced quality of strawberries, thus offering a sustainable solution for modern agricultural practices.

Conclusion

Balancing nitrogen application in strawberries is critical as it affects the growth phase of the crop as well as the quality and shelf life. The present investigation reveals that urea application in nano form exhibited greater receptiveness by the strawberry cv. Winter Dawn. However, using the conventional form of urea was effective only compared to treatments where graded doses of conventional urea were applied in conjugation with *Azotobacter* applications. An optimum dose of 400 ppm nano urea, along with 25 % RDF and *Azotobacter* application, proved to be the best treatment among all the treatments. However, further investigations involving multiple varieties and multi-locational trials are needed to evaluate the effects of nitrogen fertilization on various fruit quality attributes over numerous seasons.

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Table 3. Effect of Nano Urea and *Azotobacter* inoculation on Antioxidants, Anthocyanins and Ascorbic acid content in strawberry fruit.

Treatments	Antioxidants	Anthocyanins	Ascorbic acid
T_1 (control, 100 % RDF)	1.48^{c}	0.235^{d}	46.7^{cd}
T_2 (25 % RDF + N_1)	1.61^{d}	0.248^{e}	47.7^{de}
T_3 (25 % RDF + N_2)	1.72^{ef}	0.269^{ij}	51.0^{fg}
T_4 (50 % RDF + N_1)	1.78^{fg}	0.252^{ef}	48.0^{ef}
T_5 (50 % RDF + N_2)	1.81^{gh}	0.258^{fgh}	48.7^{def}
T_6 (75 % RDF + N_1)	1.80^{gh}	0.254^{fg}	50.0^{efg}
T_7 (75 % RDF + N_2)	1.84^{ghi}	0.257^{fgh}	46.3^{bcd}
T_8 (25 % RDF + N_1+ *Azotobacter)*	1.69^{e}	0.268^{i}	50.7^{efg}
T_9 (25 % RDF + N_2+ *Azotobacter)*	1.80^{gh}	0.274^{j}	55.0^{h}
T_{10} (50 % RDF + N_1+ *Azotobacter)*	1.87^{hi}	0.268^{i}	53.0^{gh}
T_{11} (50 % RDF + N_2+ *Azotobacter)*	1.90^{i}	0.263^{hi}	55.7^{h}
T_{12} (75 % RDF + N_1+ *Azotobacter)*	1.85^{ghi}	0.259^{gh}	53.0^{gh}
T_{13} (75 % RDF + N_2+ *Azotobacter)*	1.87^{hi}	0.261^{h}	50.0^{efg}
T_{14} (25 % RDF + *Azotobacter)*	1.22^{a}	0.203^{a}	42.0^{a}
T_{15} (50 % RDF + *Azotobacter)*	1.38^{b}	0.209^{b}	43.7^{ab}
T_{16} (75 % RDF + *Azotobacter)*	1.40^{b}	0.216^{c}	44.3^{abc}

infrastructure and resources.

Authors' contributions

SMB, MR, RKG prepared the research plan and conducted the research work and analysis. NC, JSD and SS helped with the writing and proofreading of the manuscript. All authors approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The Authors declare that there is no conflict of interest.

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