

Soil and Leaf Nutrient Responses in Strawberry to Nano-urea and *Azotobacter* Applications

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Nanofertilizers release nutrients slowly and in a controlled manner, matching the plants' growth needs. By reducing the need for chemical inputs such as fertilizers and pesticides, nanotechnology contributes to more sustainable agricultural practices. The present study used *nano-urea* with *Azotobacter* on strawberry cv. 'Winter Dawn' under protected cultivation to evaluate its impact on soil fertility status and leaf nutrient content. Formulations as per the treatment requirements were sprayed onto the strawberry plants. The plants provided with nano-urea formulations exhibited enhanced levels of nitrogen (2.49%), phosphorus (0.37%), and potassium (2.60%) compared to the plants treated with conventional urea. Soil nutrient analysis showed enhanced levels of NPK in soil samples from traditional application treatments. A higher concentration of microorganisms in the soil was observed when urea was applied at the nano level. Nano-urea combined with *Azotobacter* can significantly impact the soil's NPK levels. This innovative blend enhances nutrient availability in the soil and promotes sustainable agricultural practices. By leveraging the nanotechnology-infused urea alongside the nitrogen-fixing process of *Azotobacter* bacteria, the soil experiences a synergistic boost in nitrogen, phosphorus, and potassium content. This dynamic duo fosters a conducive environment for plant growth while minimizing nutrient leaching and environmental degradation.

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INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is a high-value fruit crop renowned for its flavour, nutritional benefits, and economic importance (Barbey *et al.* 2021). Achieving optimal growth and yield in strawberry cultivation is contingent upon a complex interplay of factors, including soil fertility, and nutrient availability (Mukherjee 2022). Traditional fertilization practices, while effective, often face challenges related to nutrient use efficiency and environmental sustainability (Swify *et al.* 2023). Introducing innovative agronomic technologies, such as nano-fertilizers and beneficial microorganisms, presents a promising alternative to enhance plant nutrition and growth (Goyal *et al.* 2023).

Nano-urea, a novel form of nitrogen fertilizer, has emerged as a potential game-changer in nutrient management due to its enhanced delivery mechanisms and improved

nutrient use efficiency (Salama *et al.* 2024). The nanoscale formulation of urea allows for controlled release and targeted delivery, which could potentially mitigate nutrient losses and reduce the frequency of application (Sales *et al.* 2024). This technology aligns with the growing emphasis on precision agriculture and sustainable farming practices (Singh *et al.* 2024; Pratama and Rahayu 2024).

Simultaneously, *Azotobacter*, a free-living nitrogen-fixing bacterium (Yilihamu *et al.* 2020), has been recognized for its capacity to improve soil fertility and promote plant growth (Ibrahim *et al.* 2022). *Azotobacter* not only contributes to the nitrogen economy of the soil, but it also enhances the bioavailability of essential nutrients and supports plant health through the production of growth-promoting substances (Basu *et al.* 2021; Ibrahim *et al.* 2022). Its integration into crop management strategies could complement the effects of nano-urea, offering a synergistic approach to optimizing soil fertility and plant nutrition (Jayara *et al.* 2023; Abd-Elsalam 2024).

Additionally, increased dependence on conventional chemical fertilizers threatens the soil microbial community (Iqbal *et al.* 2022). Chemical fertilizers are reported to be expensive (Abebe *et al.* 2022) and have adverse effects on soil microbial population. In such aspects, biofertilizers can stand as the best alternative for enhancing the fertility of the soil as these are environment-friendly (Kour *et al.* 2020), economical, and can provide a better yield to the crop (Nagananda *et al.* 2010; Nosheen *et al.* 2021). These produce growth regulators auxin, cytokinins, and gibberellins (Mourya and Singh 2022) and can fix between 2 to 15 mg N/g carbon sources (Gurikar *et al.* 2022). Exopolysaccharide production by *Azotobacter* has been proven (Ali *et al.* 2023) to play an important role in improving soil porosity and transport of heavy metal pollutants (Hindersah *et al.* 2018).

These bacterial cells (*Azotobacter* spp.) are highly sensitive to acidic pH, temperature, and high salt conditions in soil (Mikić 2023). The beneficial effect of *Azotobacter* has been reported on crop growth and yield (Zhou *et al.* 2023), stimulation of rhizospheric microbes (Azzawi and Kamal 2023), biosynthesis of biologically active substances (Kaur and Sharma 2024) and producing phytopathogenic inhibitors (Pattaeva *et al.* 2023). After the death of the *Azotobacter* cell, the protein of the cell is mineralized in the soil (Wang *et al.* 2023). It also helps modify nutrient uptake, ultimately boosting the biological nitrogen fixation in the soil and crop (Jehan *et al.* 2023).

Despite the promising potential of these interventions, there has been limited research on their combined effects on soil fertility and leaf nutritional composition in strawberry cultivation. Understanding how nano-urea and *Azotobacter* interact to influence soil nutrient dynamics, plant growth parameters, and leaf nutrient profiles is critical for advancing sustainable agricultural practices and improving strawberry production systems.

This study aims to evaluate the effects of nano-urea and *Azotobacter* interventions on soil fertility and leaf nutritional composition in strawberry plants. This research seeks to provide valuable insights for optimizing strawberry cultivation practices and contributing to the broader goal of sustainable agriculture.

MATERIALS AND METHODS

The research study was performed at Horticulture farm, Lovely Professional University, Punjab, India, from 2022 to 23 and 2023 to 24. Planting material for strawberries was sourced from the ICAR regional centre, Shimla. All intercultural operations and fertility management practices were carried out as per the standard in the

Package of Practices of Cultivation of Fruit (Thind and Mahal 2021). The experiment was laid out in Randomized Block Design (RBD) under protected conditions, having three replications per treatment and sixteen treatments in total (as given under the treatment details section). Each replication consisted of ten plant units spaced at 45 cm x 30 cm. Only the dosage of applied traditional urea was varied (25%, 50%, and 75% urea) in RDF (Recommended Dose of Fertilizer); the rest of the dosages of phosphorus and potassium were applied as recommended without any variation. Nano-urea was used as a foliar application to the strawberry plants as per the treatment specifications along with *Azotobacter* inoculation (@ 2mL per litre soil drenching) and the results were compared to the conventional (basal) application of the chemical fertilizers.

Initial soil sampling was done at depths of 15 and 30 cm using a composite sample of the experimental soil. Specimens were analyzed for soil organic carbon and soil N, P, and K. Post-harvest data regarding soil fertility status at 120 DAP was collected from 15 and 30 cm depth within each replicated plot. Soil organic carbon (SOC) was determined by the method as described by Walkley and Black (1934). Soil NPK was estimated by the method described by Tel (1984). *Azotobacter* colonies were cultured using Jensen's media (Allen 1953). For leaf sampling, fully matured leaves were selected to determine the leaf nutrient analysis for NPK. Total nitrogen (N) was analyzed using a nitrogen analyzer (Kjello Plus, Pelican, Chennai). The phosphorus (P) was estimated by using the phosphovanadomolybdate method (Jackson 1973), and potassium (K) by using a flame photometer (Labtronics, Panchkula, India).

Treatment Details

A total of 16 treatments were used, designated by symbol **T** as per the details: **T₁**: RDF (PAU recommendation), **T₂**: 25% RDF + N₁, **T₃**: 25% RDF + N₂, **T₄**: 50% RDF + N₁, **T₅**: 50% RDF + N₂, **T₆**: 75% RDF + N₁, **T₇**: 75% RDF + N₂, **T₈**: 25% RDF + N₁+ *Azotobacter*, **T₉**: 25% RDF + N₂+ *Azotobacter*, **T₁₀**: 50% RDF + N₁+ *Azotobacter*, **T₁₁**: 50% RDF + N₂+ *Azotobacter*, **T₁₂**: 75% RDF + N₁+ *Azotobacter*, **T₁₃**: 75% RDF + N₂+ *Azotobacter*, **T₁₄**: 25% RDF + *Azotobacter*, **T₁₅**: 50% RDF + *Azotobacter*, **T₁₆**: 75% RDF + *Azotobacter*. Note: N₁: 300 ppm nano-urea and N₂: 400 ppm nano-urea

Statistical Analysis

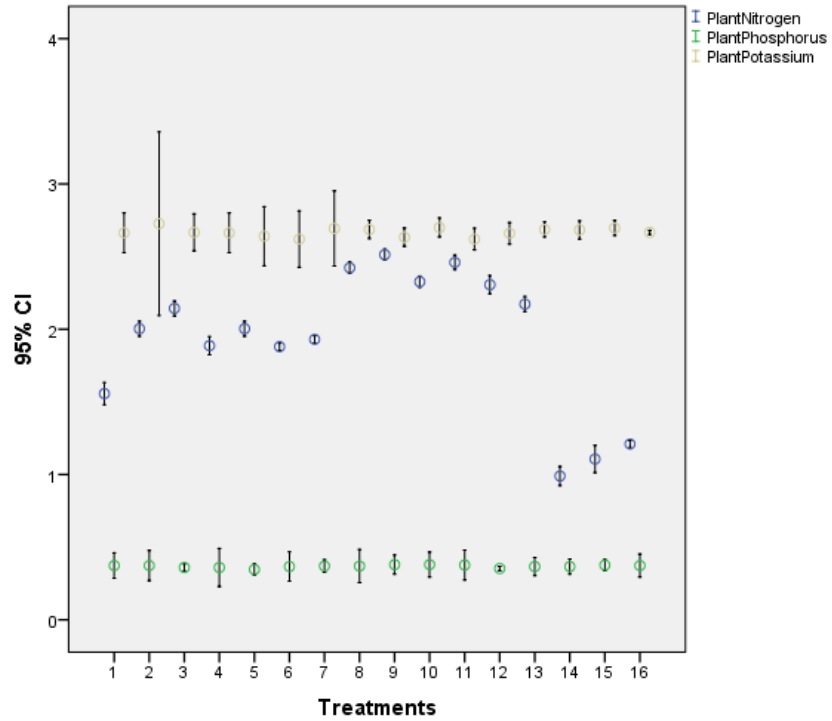
The data generated were statistically analyzed using IBM SPSS Statistics (SPSS Inc., IBM Corp., Armonk, NY, USA) software at a 5% significance level to arrive at the homogenous subsets. Variables having non-significant differences are superscripted with the same alphabet.

RESULTS AND DISCUSSION

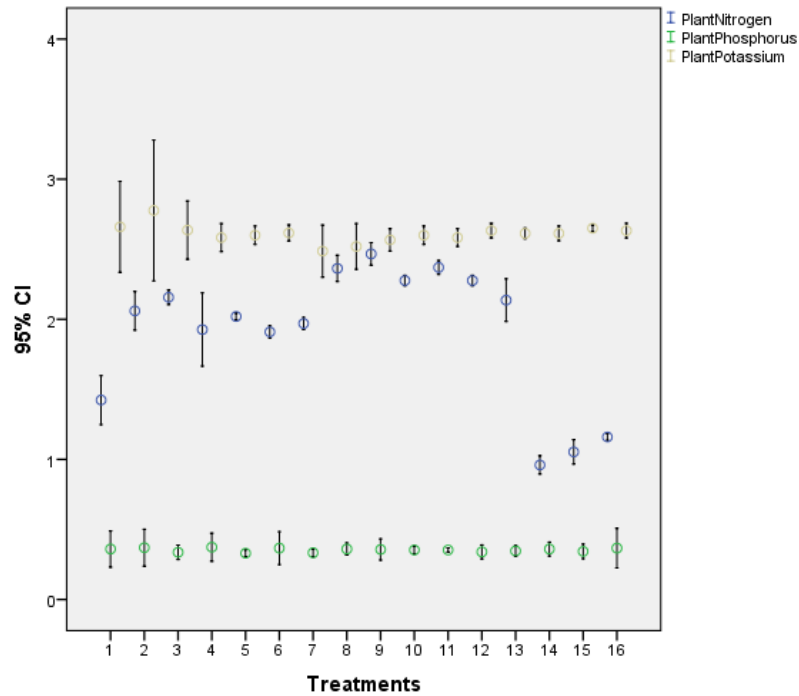
Plant Nutrient Analysis

Underapplication of nano-urea resulted in significant variations in the leaf nitrogen content; however, the variable treatments failed to produce any considerable change in the phosphorus and potassium levels in the strawberry plants (Table 1 and Fig. 1 a-c). A thorough examination of the data indicated a noteworthy impact of nano-urea on the nitrogen levels of the strawberry cultivar 'Winter Dawn' throughout the two years of the research experiment. The nitrogen percentage in the leaves ranged from 1.42% to 2.51% during the two years. Application of 25% of the recommended dose of fertilizers along

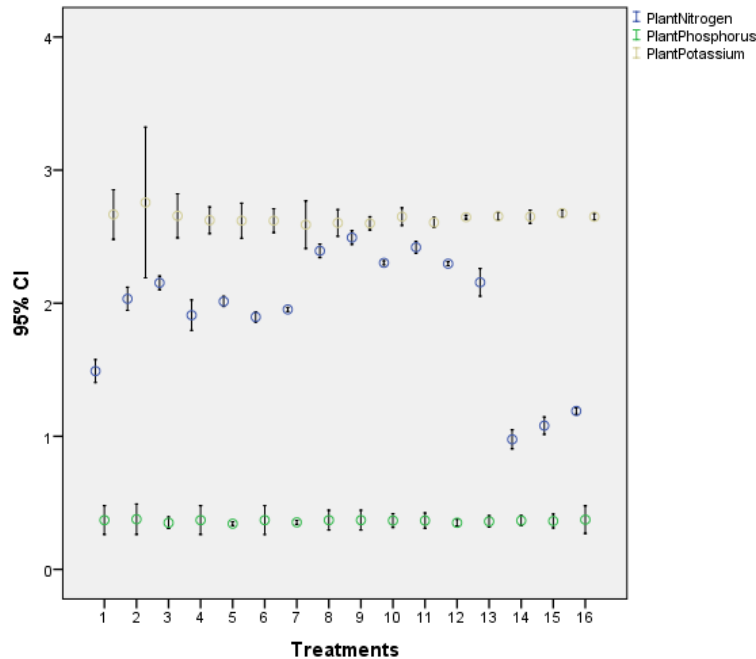
with 400 ppm nano-urea and azotobacter inoculation resulted in a maximum nitrogen content of 2.51% in the strawberry leaves.



(a) Plant nutrient status 2022



(b) Plant nutrient status 2023



(c) Plant nutrient status (Pooled)

Fig. 1 (a-c). Impact of nano-urea and *Azotobacter* application on plant nutrient content

However, the application of varying doses of nitrogen failed to make any considerable impact on the leaf phosphorus and potassium content of strawberry plants. Phosphorus in leaves ranged from 0.33 to 0.37%, whereas potassium levels in leaves ranged from 2.66 to 2.78%.

Since nitrogen dosage and source were varied among the treatments, a significant impact was observed in the leaf nitrogen content. In contrast, there was statistically non-significant variation among other treatments for phosphorus and potassium levels. Treatments having higher doses of nano-urea applications outperformed conventional nitrogen applications to the strawberry plants. Nano-urea, characterized by its nano-scale particles, provides a distinct advantage over conventional urea through more efficient and rapid absorption by the leaf surface (Iqbal *et al.* 2019; Dimkpa *et al.* 2022). This method bypasses the soil-plant interface, reducing nitrogen losses that are commonly associated with leaching, volatilization, or immobilization in the soil (Yadav *et al.* 2023).

The direct availability of nitrogen to the photosynthetic tissues ensures immediate utilization for amino acid synthesis and other nitrogen-demanding metabolic processes, which are crucial for plant growth and productivity (Ji *et al.* 2023). The results of the current study align with the findings of Abdel-Aziz and Abdel-Hakim (2021), who observed that the levels of primary nutrients, as well as micronutrients like Fe and Mn in *Capsicum annuum* and lettuce, were much more remarkable when treated with nano fertilizer compared to plants fertilized with conventional fertilizers. Furthermore, Sharaf-Eldin (2022) was of the view that the utilization of nano fertilizers (N) enhanced the effectiveness of fertilizer usage at a dosage lower than the amount that is recommended.

Table 1. Synergistic Impact of Nano-urea and *Azotobacter* in Enhancing Plant NPK Uptake

Treatments	Plant Nutrient Analysis (NPK)								
	Nitrogen			Phosphorus			Potassium		
	2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
T ₁	1.56 ^d	1.42 ^d	1.49 ^d	0.37 ^a	0.36 ^a	0.37 ^a	2.66 ^a	2.66 ^{bc}	2.66 ^{ab}
T ₂	2.00 ^g	2.06 ^g	2.03 ^g	0.37 ^a	0.37 ^a	0.37 ^a	2.73 ^a	2.78 ^c	2.75 ^b
T ₃	2.14 ^h	2.16 ^h	2.15 ^h	0.36 ^a	0.34 ^a	0.35 ^a	2.67 ^a	2.64 ^b	2.65 ^{ab}
T ₄	1.89 ^e	1.93 ^e	1.91 ^e	0.36 ^a	0.37 ^a	0.37 ^a	2.66 ^a	2.58 ^{ab}	2.62 ^{ab}
T ₅	2.00 ^g	2.02 ^{fg}	2.01 ^{fg}	0.35 ^a	0.33 ^a	0.34 ^a	2.64 ^a	2.60 ^{ab}	2.62 ^{ab}
T ₆	1.88 ^e	1.91 ^e	1.90 ^e	0.37 ^a	0.37 ^a	0.37 ^a	2.62 ^a	2.62 ^{ab}	2.62 ^{ab}
T ₇	1.93 ^f	1.97 ^{ef}	1.95 ^f	0.37 ^a	0.33 ^a	0.35 ^a	2.69 ^a	2.49 ^a	2.59 ^a
T ₈	2.42 ^j	2.36 ^j	2.39 ^j	0.37 ^a	0.36 ^a	0.37 ^a	2.69 ^a	2.52 ^{ab}	2.60 ^a
T ₉	2.51 ^l	2.47 ^k	2.49 ^k	0.38 ^a	0.36 ^a	0.37 ^a	2.63 ^a	2.57 ^{ab}	2.60 ^{ab}
T ₁₀	2.33 ^j	2.28 ^j	2.30 ^j	0.38 ^a	0.35 ^a	0.37 ^a	2.70 ^a	2.60 ^{ab}	2.65 ^{ab}
T ₁₁	2.46 ^k	2.37 ^j	2.42 ^j	0.38 ^a	0.35 ^a	0.37 ^a	2.62 ^a	2.58 ^{ab}	2.60 ^a
T ₁₂	2.31 ⁱ	2.28 ⁱ	2.29 ^j	0.35 ^a	0.34 ^a	0.35 ^a	2.66 ^a	2.63 ^b	2.65 ^{ab}
T ₁₃	2.17 ^h	2.14 ^h	2.16 ^h	0.37 ^a	0.35 ^a	0.36 ^a	2.69 ^a	2.61 ^{ab}	2.65 ^{ab}
T ₁₄	0.99 ^a	0.96 ^a	0.98 ^a	0.37 ^a	0.36 ^a	0.36 ^a	2.68 ^a	2.61 ^{ab}	2.65 ^{ab}
T ₁₅	1.11 ^b	1.05 ^b	1.08 ^b	0.38 ^a	0.34 ^a	0.36 ^a	2.70 ^a	2.65 ^b	2.67 ^{ab}
T ₁₆	1.21 ^c	1.16 ^c	1.19 ^c	0.37 ^a	0.37 ^a	0.37 ^a	2.67 ^a	2.63 ^b	2.65 ^{ab}

T₁: RDF (PAU recommendation), T₂: 25% RDF + N₁, T₃: 25% RDF + N₂, T₄: 50% RDF + N₁, T₅: 50% RDF + N₂, T₆: 75% RDF + N₁, T₇: 75% RDF + N₂, T₈: 25% RDF + N₁+ *Azotobacter*, T₉: 25% RDF + N₂+ *Azotobacter*, T₁₀: 50% RDF + N₁+ *Azotobacter*, T₁₁: 50% RDF + N₂+ *Azotobacter*, T₁₂: 75% RDF + N₁+ *Azotobacter*, T₁₃: 75% RDF + N₂+ *Azotobacter*, T₁₄: 25% RDF + *Azotobacter*, T₁₅: 50% RDF + *Azotobacter*, T₁₆: 75% RDF + *Azotobacter*. Note: N₁: 300 ppm Nano-urea, N₂: 400 ppm Nano-urea

Application of acetobacter in the treatments where reduced dosage of conventional nitrogen fertilizer was applied were compensated with sufficient availability, efficient absorption and uptake in strawberry plants. Multiple studies suggest that applying biofertilizers such as *Azotobacter* can impact the mineral composition of plants by enhancing the nutrient content in plants (Kolega *et al.* 2020). *Azotobacter* applied to the soil enhances nitrogen levels through biological fixation while promoting root health and nutrient uptake (Aasfar *et al.* 2021). The present result aligns with other research showing a positive correlation between nitrogen supply in the nutrient solution and the nitrogen content in leaves when *Azotobacter* inoculation is used (Razmjooei *et al.* 2022). This dual approach ensures sustained nitrogen availability in leaves, which is vital for photosynthesis and growth, potentially explaining increased nitrogen levels without significant variation in phosphorus and potassium, given the consistent dosage.

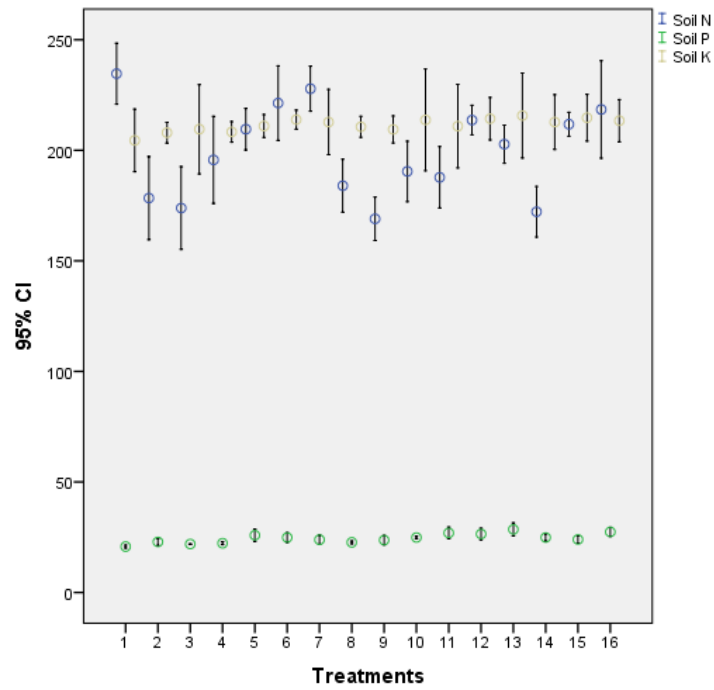
Soil Nutrient Analysis

A significant impact of nano-urea was observed on the final soil nutrient status (NPK) in the Winter Dawn strawberry cultivar, especially for soil nitrogen, across two experimental years. Soil nitrogen levels ranged from 151 to 235 kg per ha. Treatments where the recommended dosage of nitrogen through the soil was applied recorded maximum soil nitrogen content. Phosphorus levels in the soil ranged from 20.8 kg per ha to 28.5 kg per ha. Similarly soil potassium levels ranged from 200 to 216 kg per ha.

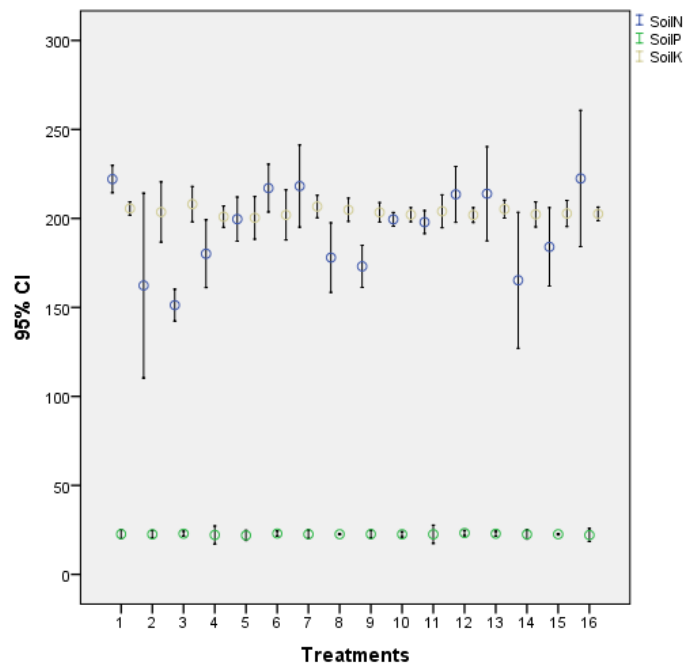
Table 2. Synergistic Impact of Nano-urea and *Azotobacter* in Enhancing Soil NPK Uptake

Treat-ments	Soil Nutrient Analysis (NPK)								
	Nitrogen			Phosphorus			Potassium		
	2022	2023	Pooled	2022	2023	Pooled	2022	2023	Pooled
T ₁	234.65 ^j	222.10 ^h	228.38 ^k	20.76 ^a	22.61 ^a	21.69 ^a	204.45 ^a	205.56 ^{ab}	205.01 ^a
T ₂	178.39 ^{ab}	162.32 ^{ab}	170.36 ^{ab}	22.89 ^{bc}	22.52 ^a	22.70 ^{abcd}	207.96 ^{ab}	203.61 ^{ab}	205.79 ^a
T ₃	173.87 ^a	151.28 ^a	162.58 ^a	21.90 ^{ab}	22.89 ^a	22.40 ^{ef}	209.54 ^{ab}	208.01 ^b	208.77 ^a
T ₄	195.65 ^{de}	180.16 ^{cd}	187.90 ^{cd}	22.28 ^b	22.15 ^a	22.22 ^{ab}	208.32 ^{ab}	200.97 ^a	204.65 ^a
T ₅	209.56 ^{fg}	199.61 ^{efg}	204.59 ^{fg}	25.87 ^{ef}	21.91 ^a	23.89 ^{de}	210.98 ^{ab}	200.34 ^a	205.66 ^a
T ₆	221.34 ^{hi}	217.04 ^{gh}	219.19 ^{ij}	24.87 ^{de}	22.93 ^a	23.90 ^{de}	213.87 ^{ab}	201.95 ^{ab}	207.91 ^a
T ₇	227.86 ^{ij}	218.18 ^h	223.02 ^{jk}	23.87 ^{cd}	22.54 ^a	23.21 ^{bcd}	212.76 ^{ab}	206.67 ^{ab}	209.72 ^a
T ₈	183.98 ^{bc}	178.00 ^{bcd}	180.99 ^c	22.67 ^{bc}	22.58 ^a	22.62 ^{abc}	210.65 ^{ab}	204.85 ^{ab}	207.75 ^a
T ₉	169.00 ^a	173.08 ^{bcd}	171.04 ^b	23.67 ^{cd}	22.63 ^a	23.15 ^{bcd}	209.45 ^{ab}	203.42 ^{ab}	206.44 ^a
T ₁₀	190.45 ^{cd}	199.48 ^{efg}	194.97 ^{de}	24.87 ^{de}	22.53 ^a	23.70 ^{cde}	213.75 ^{ab}	202.09 ^{ab}	207.92 ^a
T ₁₁	187.76 ^{bcd}	197.91 ^{ef}	192.84 ^{de}	26.90 ^{fg}	22.53 ^a	24.72 ^{ef}	210.91 ^{ab}	204.00 ^{ab}	207.46 ^a
T ₁₂	213.67 ^{gh}	213.52 ^{fgh}	213.60 ^{hi}	26.45 ^{fg}	23.25 ^a	24.85 ^{ef}	214.30 ^{ab}	201.91 ^{ab}	208.11 ^a
T ₁₃	202.75 ^{ef}	213.87 ^{fgh}	208.31 ^{gh}	28.53 ^h	22.88 ^a	25.71 ^f	215.71 ^b	205.26 ^{ab}	210.49 ^a
T ₁₄	172.21 ^a	165.26 ^{abc}	168.74 ^{ab}	24.87 ^{de}	22.48 ^a	23.68 ^{cde}	212.76 ^{ab}	202.28 ^{ab}	207.52 ^a
T ₁₅	211.78 ^{fgh}	184.00 ^{de}	197.89 ^{ef}	23.98 ^{cd}	22.55 ^a	23.27 ^{bcd}	214.72 ^{ab}	202.79 ^{ab}	208.75 ^a
T ₁₆	218.45 ^{ghi}	222.43 ^h	220.44 ^{ijk}	27.39 ^{gh}	22.09 ^a	24.74 ^{bcd}	213.36 ^{ab}	202.55 ^{ab}	207.96 ^a

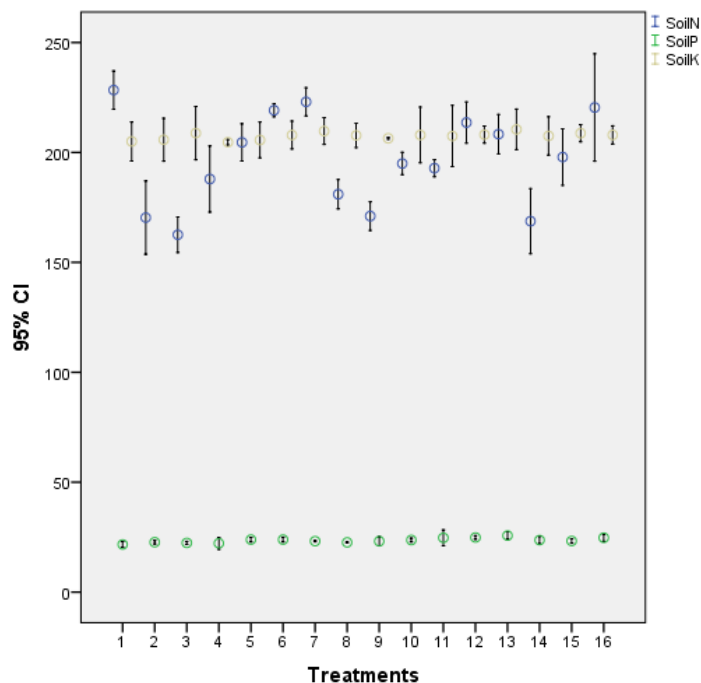
T1: RDF (PAU recommendation), T2: 25% RDF + N1, T3: 25% RDF + N2, T4: 50% RDF + N1, T5: 50% RDF + N2, T6: 75% RDF + N1, T7: 75% RDF + N2, T8: 25% RDF + N1+ *Azotobacter*, T9: 25% RDF + N2+ *Azotobacter*, T10: 50% RDF + N1+ *Azotobacter*, T11: 50% RDF + N2+ *Azotobacter*, T12: 75% RDF + N1+ *Azotobacter*, T13: 75% RDF + N2+ *Azotobacter*, T14: 25% RDF + *Azotobacter*, T15: 50% RDF + *Azotobacter*, T16: 75% RDF + *Azotobacter*. Note: N1: 300 ppm nano-urea, N2: 400 ppm nano-urea



(a) Soil nutrient status (2022)



(b) Soil nutrient status (2023)



(c) Soil nutrient status (pooled)

Fig. 2 (a-c). The impact of nano-urea and *Azotobacter* in enhancing soil NPK levels

The basal application of nitrogen (N) is critical for enhancing the availability of nitrogen in the soil (Yadav *et al.* 2017), thereby optimizing crop growth and yield (Hammad *et al.* 2018). Nitrogen, being volatile and prone to leaching, may sometimes be unavailable to the plant for more prolonged durations after application. Foliar application of nano-urea targets the leaf tissues and proves to be more economical than soil application of the chemical nitrogen.

Overall, the basal application of nitrogen is crucial in enhancing nitrogen availability in soil.

Soil Organic Carbon

As indicated by Table 3 and Fig. 3 (a, b, c), nano-urea significantly influenced soil nutrient analysis (available organic carbon) of the Winter Dawn strawberry cultivar over the two experimental years (2022-23 and 2023-24). Soil organic carbon ranged from 3.90 kg per ha to 3.38 kg per ha.

As per the observations, a higher level of soil organic carbon was recorded in the treatments where reduced soil application of chemical nitrogen fertilizer was done. Treatments involving nano-urea and azotobacter application had a higher level of soil organic carbon.

Table 3. Synergistic Impact of Nano-urea and *Azotobacter* in Enhancing Soil Organic Carbon and *Azotobacter* Count

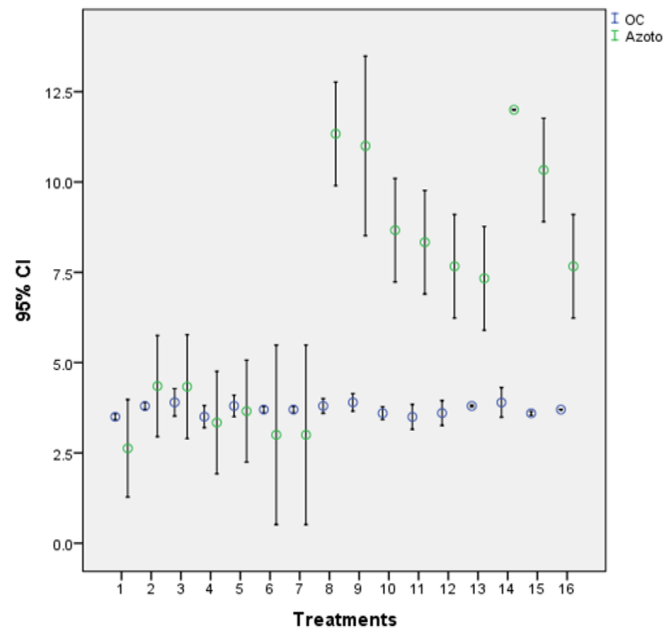
Treatments	Soil (Organic Carbon)			<i>Azotobacter</i> Count		
	2022-23	2023-24	Pooled	2022-23	2023-24	Pooled
T ₁	3.50 ^a	3.38 ^a	3.44 ^a	2.63 ^a	2.67 ^a	2.65 ^a
T ₂	3.80 ^{cd}	3.69 ^{ghi}	3.74 ^{ef}	4.35 ^b	4.83 ^c	4.59 ^c
T ₃	3.90 ^d	3.75 ⁱ	3.82 ^f	4.33 ^b	4.67 ^{bc}	4.50 ^c
T ₄	3.70 ^{bc}	3.62 ^{efg}	3.66 ^{cde}	3.34 ^{ab}	4.00 ^{bc}	3.67 ^b
T ₅	3.60 ^{sb}	3.53 ^{cd}	3.56 ^{bc}	3.66 ^{ab}	4.00 ^{bc}	3.83 ^{bc}
T ₆	3.60 ^{ab}	3.47 ^{bc}	3.53 ^{ab}	3.00 ^a	3.67 ^b	3.33 ^{ab}
T ₇	3.50 ^a	3.39 ^{ab}	3.45 ^a	3.00 ^a	3.67 ^b	3.33 ^{ab}
T ₈	3.80 ^{cd}	3.76 ⁱ	3.78 ^f	11.33 ^{ef}	11.83 ^f	11.58 ^g
T ₉	3.90 ^d	3.74 ^{hi}	3.82 ^f	11.00 ^{ef}	11.17 ^f	11.08 ^g
T ₁₀	3.80 ^{cd}	3.66 ^{fgh}	3.73 ^{def}	8.67 ^d	9.83 ^e	9.25 ^e
T ₁₁	3.80 ^{cd}	3.66 ^{gh}	3.73 ^{def}	8.33 ^{cd}	9.17 ^e	8.75 ^e
T ₁₂	3.70 ^{bc}	3.58 ^{def}	3.64 ^{cd}	7.67 ^{cd}	8.17 ^d	7.92 ^d
T ₁₃	3.60 ^{ab}	3.54 ^{cde}	3.57 ^{bc}	7.33 ^c	8.00 ^d	7.67 ^d
T ₁₄	3.90 ^d	3.69 ^{ghi}	3.79 ^f	12.00 ^f	11.50 ^f	11.75 ^g
T ₁₅	3.70 ^{bc}	3.54 ^{cde}	3.62 ^{bc}	10.33 ^e	10.00 ^e	10.17 ^f
T ₁₆	3.50 ^a	3.40 ^{ab}	3.45 ^a	7.67 ^{cd}	8.17 ^d	7.92 ^d

T1: RDF (PAU recommendation), T2: 25% RDF + N1, T3: 25% RDF + N2, T4: 50% RDF + N1, T5: 50% RDF + N2, T6: 75% RDF + N1, T7: 75% RDF + N2, T8: 25% RDF + N1+ *Azotobacter*, T9: 25% RDF + N2+ *Azotobacter*, T10: 50% RDF + N1+ *Azotobacter*, T11: 50% RDF + N2+ *Azotobacter*, T12: 75% RDF + N1+ *Azotobacter*, T13: 75% RDF + N2+ *Azotobacter*, T14: 25% RDF + *Azotobacter*, T15: 50% RDF + *Azotobacter*, T16: 75% RDF + *Azotobacter*. Note: N1: 300 ppm nano-urea, N2: 400 ppm nano-urea

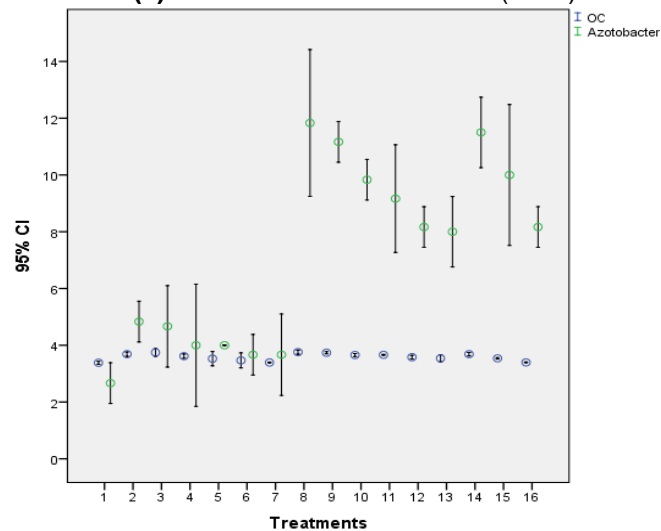
The basal application of conventional nitrogen fertilizers can decrease available organic carbon in soil (Li *et al.* 2017). This is attributed to heightened microbial activity stimulated by nitrogen influx, which accelerates microbial metabolic processes (Ge *et al.* 2010; Hutchins *et al.* 2022). Consequently, microbial communities utilize organic carbon for energy and growth, depleting the available pool (Garcia-Pausas and Paterson 2011). Chemical fertilizers can enhance microbial activity and hasten the breakdown of soil organic materials (Arunrat *et al.* 2020). Specific microbial taxa, favoured by the elevated nitrogen availability (Fierer *et al.* 2012), may exhibit enhanced competitiveness over others, leading to shifts in community composition and function (Herren and McMahon 2018). This alteration in microbial diversity and activity can result in the preferential decomposition of organic carbon compounds, further reducing their availability in the soil matrix. The current findings align with the research conducted by Gong *et al.* (2009), who proposed that using chemical fertilizers has led to significant negative impacts on the

physical and chemical characteristics of soil, such as the decline in soil organic carbon levels.

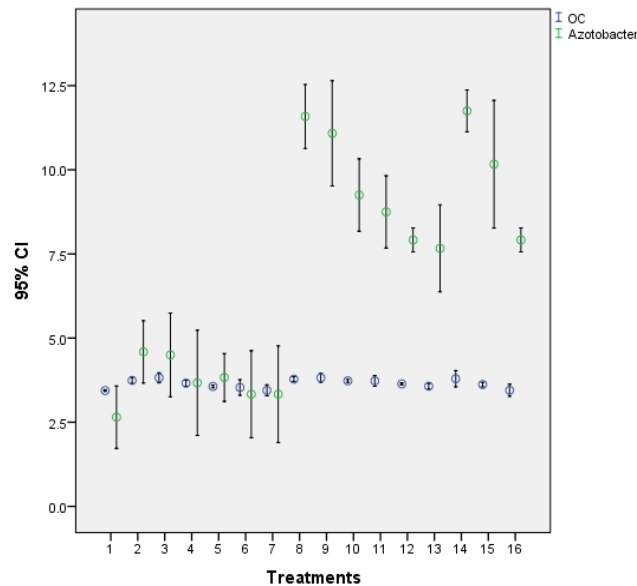
Additionally, elevated nitrogen levels stimulate plant growth and alter root exudation patterns, affecting organic carbon inputs to the soil (Yin *et al.* 2013; Murphy *et al.* 2017; Meng *et al.* 2024). Changes in plant physiology may decrease below ground carbon allocation, reducing available carbon for microbial use (Savage *et al.* 2016; Gross and Harrison 2019). Understanding these dynamics is crucial for devising sustainable nutrient management strategies (Lei *et al.* 2023).



(a) OC and azotobacter count (2022)



(b) OC and azotobacter count (2023)



(c) OC and azotobacter count (pooled)

Fig. 3(a through c). The impact of nano-urea and *Azotobacter* in enhancing organic carbon and *Azotobacter* counts

Azotobacter Count

Table 3 and Fig. 3 (a, b, c) depict *Azotobacter* count (CFU $\times 10^6$) variation over the experimental years (2022-23 and 2023-24) for the Winter Dawn strawberry cultivar. Nano-urea significantly impacted *Azotobacter* count throughout both years, highlighting its influence on soil microbial dynamics.

During the initial year of trial (2023-24), T14 exhibited the highest *Azotobacter* count (11.33×10^6 cfu (Fig. 3a). In the second-year trial (2023-24), T8 displayed the highest *Azotobacter* count (11.83×10^6 cfu (Fig. 3b). Pooled data also revealed maximum *Azotobacter* count (11.75×10^6 cfu) under treatment T14. Minimum azotobacter count was recorded in the soil samples under control treatment as the chemical nitrogen dosage was higher compared to other treatments.

In the study of soil microbiology and fertility, the application of conventional nitrogenous fertilizers has been noted to impact various microbial populations, including the diazotrophic (nitrogen-fixing) bacteria such as *Azotobacter* (Aasfar *et al.* 2021). Notably, basal application of nitrogen fertilizers appears to decrease the population of *Azotobacter* in soil, a phenomenon that can be attributed to several interconnected factors (Jnawali *et al.* 2015). Higher concentrations of nitrogen fertilizer suppress the activities of nitrogen-fixing microorganisms. The results are in line with the above findings. Bacterial proliferation is frequently constrained by the scarcity of easily accessible carbon substrate, even in soils with a high carbon-to-nitrogen ratio (Demoling *et al.* 2007; Singh *et al.* 2015).

When synthetic nitrogen fertilizers are applied to the soil, particularly as a basal dose, they provide plants with readily available inorganic nitrogen. This abundant nitrogen supply reduces the ecological niche and the competitive advantage for *Azotobacter*, whose nitrogen-fixing capability becomes redundant in the presence of high nitrogen levels. The decrease in *Azotobacter* count following nitrogen application is also linked to the metabolic burden that nitrogen fixation imposes on bacteria (Han *et al.* 2024); when nitrogen is readily available, the energy-intensive process of nitrogen fixation is unnecessary, leading to a competitive disadvantage for nitrogen-fixers. Research findings have shown that bio-

fertilization has led to a higher microbial population than mineral fertilization (El-Sawah *et al.* 2018; Gao *et al.* 2020).

CONCLUSIONS

A reasonable conclusion came from the current study regarding applying nitrogen to strawberry plants. Foliar application of nano-urea at 400 ppm along with a 25% recommended dose of nitrogen through conventional nitrogenous fertilizers as soil application and augmentation with azotobacter led to enhanced leaf nutrient status of the strawberry plants concerning nitrogen content. Similarly, soil nutrient analysis recorded the highest nitrogen in treatments where the recommended dose of fertilizer (urea) was used. To have better nutrient availability and soil microbial load, it is recommended that nitrogen should be applied in nano form, as soil microfauna is sensitive to higher soil acidity caused by the application of chemical fertilizers.

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