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A Comparative Study of Conventional and Nano-NPK on the Growth, Flowering, Bioactive Compounds, and Anatomical Characters of *Solidago virgaurea*

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NANO fertilization helps improve the efficiency of nutrient absorption, thus reducing the harmful effects of overuse of chemical fertilizers and reducing toxicity to plants and soil. *Solidago virgaurea*, a perennial herb, is widely used as an attractive addition to floral arrangements and as a source of valuable compounds. A pot experiment was carried out using a randomized complete block design to investigate the effect of nitrogen, phosphorus, and potassium (NPK) fertilizers on the growth, flowering, bioactive metabolites, and anatomical attributes of *S. virgaurea*. Two sources of NPK, conventional NPK (conv-NPK) and nanoparticle NPK (nano-NPK), were sprayed at three levels (conv-NPK; 1, 1.5, and 2 g/l, nano-NPK; 1, 1.5, and 2 ml/l). The highest significant values for growth parameters (plant height, leaf number, branch number, plant biomass), flowering metrics (flowering start, flower length, full flowering duration, fresh and dry flower weights), photosynthetic pigments (chlorophyll and carotenoids), nutrient (N, P, and K), and carbohydrates contents were attained by the highest level of nano-NPK. Subsequently, the moderate level of nano-NPK followed suit in performance. Furthermore, a moderate level of nano-NPK demonstrated significant superiority or equivalence to a high level of conv-NPK in enhancing various parameters, with the exception of total flavonoid content and total antioxidant capacity. Increasing NPK levels generally increased the accumulation of total phenolics, flavonoids, and antioxidant capacity, with the moderate and highest levels showing the most pronounced effects. Different concentrations of conv-NPK and nano-NPK impacted tissue thickness, vessel dimensions, and midrib zone thickness distinctly. Nano-NPK consistently outperformed conv-NPK in enhancing palisade and spongy tissue thickness, while both fertilizers showed similar improvements in xylem vessel dimensions. Utilizing nano-NPK at moderate to high levels could optimize growth, flowering, and bioactive constituents content in *S. virgaurea*. These findings suggest that nano-fertilizers can be a promising strategy for sustainable agriculture, improving the productivity and quality of high-value plants like *S. virgaurea* while minimizing environmental impact by reducing fertilizer application rates.

Keywords: European goldenrod; Bioactive metabolites; Nano fertilization; Cut flower and Anatomical structure.

1. Introduction

The global ornamental plant market is a significant economic sector with a growing demand for high-quality, sustainably produced plants. One of these important plants is *Solidago virgaurea*, which is very popular as a cut flower and medicinal plant. *S. virgaurea*, commonly known as European goldenrod or woundwort, belongs to the family Asteraceae. The genus *Solidago* comprises approximately 190 species, with around 100 species found in North America and 12 species distributed across South America, Asia, and Europe (Beck et al., 2014; Ahmed et al., 2023). *S. virgaurea* is a perennial herb with an upright stem up to 1 m tall, bearing alternate, slightly hairy leaves. It flowers from July to October, producing yellow racemose inflorescences with distinct ray and disc florets, arranged in racemes or panicles (Fursenco et al., 2020; Calalb et al., 2018). Because of its attractive yellow flowers, it is used in flower arrangements and bouquets as cut flowers, potted plants, flower beds, and borders. It is also resistant to salt and soil turbidity, making it suitable for planting near water features in flower beds. The raw materials from the *Solidago* plants have a lengthy and extensive history of use in traditional medicine across various regions globally. Among these, *S. virgaurea* L. (European goldenrod) is predominantly utilized in Europe and Asia (Woźniak et al., 2018; Fursenco et al., 2020). For centuries, the above-ground

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sections of European goldenrod have been recognized and employed as anti-inflammatory, antimicrobial, spasm-relieving, and diuretic solutions in traditional medicine for addressing various diseases, particularly serving as a urological treatment for kidney and bladder inflammation, urolithiasis, and cystitis (Woźniak et al., 2018; Fursenco et al., 2020; Prêcheur et al., 2020). The European Medicines Agency highlights *S. virgaurea* as one of the extensively used and researched species within the *Solidago* genus in Europe (Fursenco et al., 2020). *S. virgaurea* extracts consist of various compounds including glycosides (virgaureoside, leiocarposide), aglycones (vanillic acid, gallic acid), polyphenolic acids (such as caffeic, chlorogenic, ferulic, synapic, homovanilic acids), flavonoids (mainly quercetin, rutin, and kaempferol glycosides), triterpene saponins, essential oils with monoterpenes (like myrcene, limonene, linalool, camphene, sabinene) and sesquiterpenes (like germacrene D, β-caryophyllene, α-humulene), polysaccharides, and polyacetylenes (Fursenco et al., 2020; Prêcheur et al., 2020; Ahmed et al., 2023). In the European Pharmacopoeia monograph for *Solidaginis virgaureae herba*, flavonoids are considered indicators of quality. The standard mandates a flavonoid content ranging from a minimum of 0.5% to a maximum of 1.5%, expressed in terms of hyperoside (Council of Europe, 2019). There is a close relationship between plant nutrition and its production, quality, and content of bioactive compounds (Abou Elhassan et al., 2023; Awad et al., 2024; Nada et al., 2024).

In this context, fertilizers play a crucial role in the growth, yield, and quality by providing essential nutrients for plant metabolism, increasing biomass, and enhancing flower production and support the regular physiological processes of plant cells when applied in recommended doses or used wisely. Most plants require nitrogen (N), phosphorus (P), and potassium (K), which play a crucial role in supporting the growth and development of plants. N, P, and K are important in the formation and regulation of various vital components within plants, including proteins, hormones, amino acids, enzymes, nucleic acids, and the maintenance of water balance. However, overuse of fertilizers can severely damage soil, disrupting its essential nutrient balance. This imbalance hinders plant root growth and development, making it difficult for plants to absorb vital nutrients and water. Consequently, crop yields decline (Mancy et al., 2020; Liu et al., 2024).

Certain advantageous characteristics of nano-fertilizers compared to traditional fertilizers have been identified (Singh et al., 2024a). These benefits encompass a controlled and gradual delivery of plant nutrients, reduced soil pollution, decreased environmental hazards associated with chemical fertilizers, and enhanced efficacy in stimulating plant growth and increasing yield (Abou Tahoun et al., 2022; MohitRabary et al., 2022; Singh et al., 2024b). Hence, nanotechnology holds significant potential for promoting sustainable agriculture, particularly in developing countries (Singh et al., 2024a). The distinctive characteristics of nanoparticles stem from their elevated surface-to-volume ratio, diminutive size, and unique structural attributes (Ahmed et al., 2023; Sheta et al., 2024). Nanoparticles exert diverse effects on plants, contingent on factors such as chemical structure, surface area, size, reactivity, plant species, and notably, the concentration employed (Ghazaryan et al., 2024). These factors collectively determine whether nanoparticles exhibit efficacy or pose risks of toxicity, including oxidative stress, genotoxicity, and cytotoxic effects (Noohpisheh et al., 2021). Foliar fertilization of crops provides many benefits, such as increased plant response due to improved absorption, lowest fertilizer requirements, and higher efficiency because practically integral uptake occurs due to no amounts being fixed in the soil (Onofrei et al., 2017; Abas et al., 2024).

The primary rationale behind employing nanoparticles in agriculture lies in their dimensions (below 100 nm), enabling penetration into plants, in contrast to conventional fertilizers (GutiéRrez-Ruelas et al., 2021). However, the use of nano-fertilizers raises ethical concerns regarding environmental safety, as nanoparticles may persist in ecosystems, potentially harming non-target organisms and disrupting ecological balance. Human health risks, such as exposure to workers and potential contamination of the food chain, also require careful consideration and regulation. Additionally, issues of social equity arise, as the high cost and limited accessibility of nano-fertilizers may disadvantage small-scale farmers, exacerbating economic disparities in agriculture (Iavicoli et al., 2017; Singh et al., 2024a). Kamel (2025) found that combining nano-NPK with compost and organic manure improved soil fertility and bean productivity, enhancing plant height, branches, pods, pod weight, and seed weight compared to mineral fertilizers alone. It also increased protein and proline levels in seeds, demonstrating superior crop performance and soil health.

The application of N, P, and K fertilizers can significantly influence the production and quality of ornamental plants as well as the phytochemical composition and antioxidant activity of medicinal plants (Nada et al., 2022; 2024; El-Beltagi et al., 2023; Nofal et al., 2024). These nutrients play crucial roles in various plant metabolic processes, including the biosynthesis of secondary metabolites such as phenolics and flavonoids (Wang et al., 2023; Elateeq and Sun, 2024). The combined application of NPK fertilizers can have a synergistic effect on the phytochemical composition of medicinal plants. Studies have shown that the combined application of NPK fertilizers can significantly increase the total phenolic content, total flavonoid content, and antioxidant capacity in various medicinal plants, such as *Vitex negundo* and *Curcuma xanthorrhiza* (Peng and Ng, 2022; Minarni et al., 2023). Nofal et al. (2024) found that nano-NPK foliar application enhanced vegetative and root growth, flowering parameters, and photosynthetic pigments in *Tecoma stans*. However, it is important to note that the optimal NPK fertilization rates can vary depending on the specific plant species, soil type, and environmental conditions. Excessive application of fertilizers can lead to nutrient imbalances and environmental pollution. Moreover, some reports have found inhibition of the biosynthesis of active compounds at high doses of conventional NPK fertilizers (Ibrahim et al., 2012; El-Beltagi et al., 2023). Therefore, it is crucial to optimize fertilizer application rates to maximize the yield and quality of medicinal plants while minimizing environmental impact. On the other hand, studying plant anatomy reveals how fertilization impacts growth. Different treatments alter cell structure, tissue organization, and vascular development. This knowledge helps optimize fertilizer use for improved crop yield and quality, while minimizing environmental impact (El-khawaga, 2018; Al-Dhalimi and Al-ajeel, 2020; Gashash et al., 2022).

This study is novel in its comprehensive investigation of the effects of both conventional and nano-NPK fertilizers on multiple aspects of *S. virgaurea*, including growth, flowering, bioactive compounds, and anatomical characteristics. By comparing these two fertilizer types, we aim to determine the efficacy and potential advantages of using nano-fertilizers in enhancing the cultivation and quality of this valuable plant species. This work aimed to provide insights into optimizing fertilization strategies for *S. virgaurea* while potentially reducing the environmental impact associated with conventional fertilizer use.

2. Materials and Methods

2.1. Experimental site and soil

The experiment was conducted in a private farm at Abu Hammad, Sharkia, Egypt (30°30'45.6"N 31°45'38.3"E), during the two growing seasons of 2022/2023 and 2023/2024. Soil was prepared before planting as a 1:1 (v/v) mixture of sand and peat moss. Soil was analyzed according to Dane and Topp (2020) and Sparks et al. (2020), and the results are shown in Tables 1 and 2. Climatic data for the experimental site are presented in Table 3. A randomized complete block design was used during both seasons.

Table 1. Some physical and chemical properties of the experimental soil (average of both seasons.

Particle size distribution (%)									
Coarse sand	**Fine sand**	**Silt**		**Clay**		**Texture class**			
4.82	45.69	40.25		9.24		Loam			
pH (1:2.5 soil water suspension)	ECe (dS m^{-1}, soil paste extract)	Soluble ions (mmolc L^{-1})							
		Cations				Anions			
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
8.25	1.08	3.29	2.09	3.92	1.54	0.00	2.78	3.90	4.16

Table 2. Chemical analysis of irrigation water used in this experiment

		Soluble ions (mmolc L^{-1})							
pH	EC_W (dS m^{-1})	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
7.11	0.70	1.40	1.92	3.04	0.64	0.00	2.08	3.42	1.50

Table 3. The climatic conditions during the 2022/2023 and 2023/2024 seasons for *S. virgaurea* plant in Abo Hammad, Sharkia, Egypt.

Months	**Mean value of temperature (C)**		**Relative humidity (%)**	
	1st season	**2nd season**	**1st season**	**2nd season**
October	24.49	25.72	57.29	59.75
November	19.64	21.01	60.61	64.74
December	16.89	17.34	65.64	67.20
January	14.03	14.14	69.45	63.07
February	12.56	14.25	69.08	67.05
March	18.07	17.85	53.46	54.98
April	21.70	23.02	47.11	51.69
Average	18.20	19.05	60.38	61.21

Source: Central Laboratory for Agricultural Climate (CLAC) Cairo, Egypt.

2.2. Plant material and cultivation

Cuttings (3-4 cm long) were collected from a healthy and vigorous mother plant of *S. virgaurea* from a private farm in Alqanater El Khayriyah, Qalyubia, Egypt. Cuttings were treated with indole-3-butyric acid (IBA) at a concentration of 2000 mg/l and placed in a 1:1 (v/v) mixture of sand and peat moss under mist propagation conditions for 10-15 days until they had fully established roots. Rooted cuttings were then transplanted into 14-cm diameter pots containing a 1:1 (v/v) mixture of sand and peat moss. Each treatment had three replicates, and each replicate consisted of 5 pots, spaced 30 cm apart. The rooted cuttings were planted in December in both seasons, one plant per pot. Pinching was performed 21 days after planting to promote branching and increase flowering, and drip irrigation was used and maintained the soil moisture content at field capacity.

2.3. Treatments

Ammonium sulphate (20.6% N), calcium superphosphate (15.5% P_2O_5), and potassium sulphate (48% K_2O) were the mineral sources for N, P, and K fertilizers. Conventional NPK (conv-NPK) was applied at 1, 1.5 and 2 g/l while nanoparticle NPK (nano-NPK) was applied at 1, 1.5, and 2 m/l.

Nano fertilization of nitrogen, phosphorus, and potassium fertilizers (nano-NPK) was obtained from National Research Center, Egypt. Nano fertilizers were synthesized using a ball-milling process. In this method, 100 g portions of individual NPK fertilizers were placed in a stainless-steel canister containing metal balls of three different sizes. The canister was then processed in a ball-milling machine at a speed of 1000 rpm for 26 hrs. After milling, samples of the processed fertilizers were collected and analyzed using JEOL Transmission Electron Microscopy (TEM) (JEM-1400 TEM, Japan) to determine the particle size and morphology. For TEM imaging, a drop of well-dispersed nano-particle suspension was placed on an amorphous carbon-coated 200 mesh carbon grid, dried at room temperature, and then examined under the microscope (Wang et al., 2014).

Both fertilizers were sprayed four times, with an interval of 14 days, after 3, 5, 7, and 9 weeks of planting by manually pumping the fertilizer solution onto the plants until it ran out.

2.4. Measurements

2.4.1. Growth and flowering parameters

Plant height (cm) as the main stem from the soil surface to the stem apex, leaf number per plant, and branch number per plant was recorded. Days to flowering onset (flowering starts) were recorded from planting to the opening of the first flower head. The full flowering duration (days) was registered from the first opened flower until the full flowering. Fresh weight (FW) and dry weight (DW) of flowers were recorded per flower (g/flower) at the full bloom stage. Total plant FW and DW (g/plant) were determined at the end of experiment. The DW was recorded after drying in an oven at 60°C for 24 hrs.

Leaf DW was analyzed for nitrogen (N) and phosphorus (P) content (%) using the method described in the AOAC (1995) publication. The potassium (K) content (%) was assessed according to Dewis and Freitas (1970).

2.4.2. Photosynthetic pigments

Chlorophyll a and b and carotenoids contents (mg/g FW) were determined using a method described by Lichtenthaler (1987). Fresh leaf samples (approximately 0.2 g) were extracted with 15 ml of 80% acetone. After filtration, the volume of extract was adjusted to 15 ml with 80% acetone. The absorbance of the extract was then measured at specific wavelengths (663.2 nm, 646.8 nm, and 470 nm) using a UV-Vis spectrophotometer (Jenway 6800, Bibby Scientific Ltd., Staffordshire, UK). The amount of chlorophyll (Chl) and carotenoids (Cart) was determined using the following mathematical formula:

$$Chl.a = 12.25A_{663.2} - 2.79A_{646.8}$$

$$Chl.b = 21.50A_{646.8} - 5.1A_{663.2}$$

$$Total\ Chl = 7.15A_{663.2} + 18.71A_{646.8}$$

$$Cart = (1000A_{470} - 1.8Chl.a - 85.02Chl.b) / 198$$

2.4.3. Determination of bioactive compounds

For the assessment of total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC), leaf powder (100 mg) was immersed in 5 mL of 95% ethanol and stirred for 4 h. Following this, the mixture was incubated for 24 h at room temperature. The mixture was homogenized and centrifuged at 10000 g for 10 min.

2.4.4. Determination of total phenolics

The quantification of TPC in the supernatants was carried out using the Folin-Ciocalteu method (Singleton and Rossi, 1965). One mL of the supernatant was combined with 1 mL of 95% ethanol, 5 mL of distilled water, and 0.5 mL of 50% Folin-Ciocalteu reagent. After a 5-min interval, 1 mL of 5% Na_2CO_3 was introduced and mixed thoroughly. The resultant solution was left to incubate for an hr at 25 °C. Measurement of absorbance occurred at 725 nm using a spectrophotometer against a blank. Gallic acid dilutions were employed in establishing the standard curve. TPC was calculated as mg gallic acid equivalents (GAE)/g DW.

2.4.5. Determination of total flavonoids

TFC was determined using the aluminum chloride ($AlCl_3$) colorimetric method (Chang et al., 2002). Ethanolic extract (0.5 mL) was combined with 1.5 mL of 95% ethanol, 0.1 mL of $AlCl_3$ 10%, 0.1 mL of potassium acetate 1 M, and 2.8 mL of distilled water. After a 30-min incubation period at 25 °C, the absorbance was measured in a spectrophotometer at 415 nm against a blank. The calibration curve was generated using dilutions of quercetin. TFC was expressed as mg quercetin equivalents (QE)/g DW.

2.4.6. Determination of total antioxidant capacity

The determination of TAC was carried out utilizing the phosphomolybdenum method as outlined by Prieto et al. (1999). The standard phosphomolybdate reagent was formulated by combining equal amounts of 4 mM of ammonium molybdate, 28 mM of sodium phosphate, and 0.6 M of H_2SO_4. Subsequently, 3 mL of this reagent solution was mixed with 0.3 mL of the extract. The tubes containing the reaction mixture were then exposed to heat treatment by immersing them in a water bath set at 95 °C for 90 min. Following cooling to room temperature, the absorbance was measured at 695 nm using a spectrophotometer against a blank. The calibration curve was prepared using dilutions of ascorbic acid. The TAC value was denoted as mg ascorbic acid equivalents (AsAE)/ g DW.

2.4.7. Anatomical studies

Data were collected from certain anatomical characteristics of transverse-sections of *S. virgaurea* leaves under different treatments of NPK. The studied anatomical characteristics were thickness of upper epidermal layer (µm), thickness of palisade tissue (µm), thickness of spongy tissue (µm), diameter of xylem vessels (µm), thickness of lower epidermal layer (µm), and thickness of midrib region (µm). Leaf samples were prepared for anatomical studies according to the method described by Nassar and El-Sahhar (1998).

2.5. Statistical analysis

The experiment used a randomized complete block design in both growing seasons. Each treatment included three replicates, and each replicate included five potted plants. The statistical analysis of data was subjected to Analysis of Variance (ANOVA), and means were compared using least significant difference (LSD) test at the 5% level of significance (Snedecor and Cochran, 1980) using COSTAT software (Co Hort software Monterey, USA).

3. Results

3.1. Growth parameters

In this study, the growth parameters for *S. virgaurea* including plant height, leaf number, branch number, and fresh and dry weights, were affected by the spraying of conv-NPK and nano-NPK. Generally, it could be noticed that application of nano-NPK was more favorable than conv-NPK in improving the different growth parameters

(Table 4). Increased fertilizer application rates, regardless of formulation (conv-NPK or nano-NPK), led to significant increases in all measured growth parameters. The highest nano-NPK concentration (2 ml/l) significantly increased plant height (p<0.05) compared to all other treatments, with the highest values recorded at 41.66 and 42.89 cm, in both seasons, respectively, followed by moderate level of nano-NPK. The number of leaves ranged from 35.66 to 54.00 leaves per plant during the growing seasons. The control treatment had the lowest leaf number, followed by the lowest dose of both conv-NPK and nano-NPK. Nano-NPK at the highest level had the maximum number of leaves (50.33 and 54 leaves/plant), followed by 1.5 ml/l nano-NPK (48.66 and 52.66 leaves/plant) in the 1st and 2nd seasons, respectively. In the first season, the highest number of branches (4.33 branch/plant) was observed with 2 ml/l nano-NPK, a 2.67 branch/plant increase compared to the control (1.66 branch/plant). Nano-NPK at 1 and 1.5 ml/l and conv-NPK at 2 g/l also showed higher branch numbers than the control. However, in the second year, no significant differences were noticed in branch numbers between these treatments. The maximum significant values of aboveground biomass FW (27.75 and 26.07 g/plant) and DW (5.51 and 5.24 g/plant) were noticed with the higher rate of nano-NPK (2 ml/l) in the first and second seasons, respectively. This was significantly followed by a moderate level of nano-NPK in the first season. However, during the second growing year, both higher conv-NPK (2 g/l) and moderate nano-NPK (1.5 ml/l) applications demonstrated a significant equivalent effect (p<0.05) on both biomass FW and DW. Nonfertilized control plants recorded the lowest values of all growth parameters.

Table 4. Effect of conventional NPK (conv-NPK) and nano-NPK on growth parameters (plant height, leaves number, branches number, fresh and dry weights) of *S. virgaurea* during 2022/2023 and 2023/2024 seasons.

Treatments	Plant height (cm)	Leaves number/ plant	Branches number/plant	Fresh weight (g/plant)	Dry weight (g/plant)
First season 2022/2023					
Control	36.33 f	35.66 g	1.66 d	18.35 f	3.66 e
Conv-NPK 1 g/l	37.33 e	43.33 f	2.00 de	21.44 e	4.30 d
Conv-NPK 1.5 g/l	38.26 d	44.33 e	2.33 cde	22.39 d	4.48 d
Conv-NPK 2 g/l	39.33 c	45.66 d	2.66 bcd	25.57 c	5.08 c
Nano-NPK 1 ml/l	40.66 b	47.33 c	3.00 bc	25.82 c	5.16 bc
Nano-NPK 1.5 ml/l	41.00 b	48.66 b	3.33 b	26.75 b	5.35 ab
Nano-NPK 2 ml/l	41.66 a	50.33 a	4.33 a	27.75 a	5.51 a
Second season 2023/2024					
Control	38.88 e	39.66 g	1.33 c	19.0 e	3.8 f
Conv-NPK 1 g/l	40.33 d	47.66 f	2.33 b	21.7 d	4.35 e
Conv-NPK 1.5 g/l	41.00 c	49.66 e	2.66 b	22.5 c	4.51 de
Conv-NPK 2 g/l	41.55 c	50.66 d	3.00 ab	24.44 b	4.88 b
Nano-NPK 1 ml/l	41.16 c	51.66 c	2.66 b	22.96 c	4.61 cd
Nano-NPK 1.5 ml/l	42.23 b	52.66 b	3.66 ab	24.07 b	4.82 bc
Nano-NPK 2 ml/l	42.89 a	54.00 a	4.00 a	26.07 a	5.24 a

Values followed by different letters in the same column for each season are significantly different (P<0.05)

3.2. Flowering parameters

Table 5 reveals that both conv-NPK and nano-NPK significantly enhanced flowering characteristics compared to the control plants. Nano-NPK caused early flowering of *S. virgaurea* plants compared to conv-NPK. Nano-NPK concentrations (2, 1.5, and 1 ml/l) consistently exhibited the earliest flowering in both seasons, with the greatest advancement observed in the first season (11.36, 10.33, and 9.34 days earlier, respectively). Conv-NPK treatments also accelerated flowering compared to the control, with 2-1 g/l concentrations resulting in earlier blooms (8.11-6.99 days in the first season and 7.67-6.67 days in the second season). Conversely, nonfertilized plants experienced the latest flowering in both seasons (123.66 and 124.22 days, respectively). Flower length significantly increased with increasing fertilizer levels, with nano-NPK at 2 and 1.5 ml/l and conv-NPK at 2 g/l yielding the largest flowers (12.83, 12.33, and 12.16 cm in the 1st season; 12.66, 12.16, 12.33 cm in the 2nd season) (Fig. 1). Nano-NPK treatments significantly increased flower length compared to the control, with fold changes ranging from 1.62 to 1.89 in the first season and 1.69 to 1.91 in the second season. Conv-NPK treatments also enhanced flower length, but to a lesser extent (1.40-1.79-fold in the first season and 1.41-1.86-fold in the second).

Table 5. Effect of conventional NPK (conv-NPK) and nano-NPK on the flowering parameters (flowering start, flower length, full flowering duration, flower fresh and dry weights) of *S. virgaurea* during 2022/2023 and 2023/2024 seasons.

Treatments	Flowering start (days)	Flower length (cm)	Full flowering duration (days)	Flower fresh weight (g/plant)	Flower dry weight (g/plant)
First season 2022/2023					
Control	123.66 a	6.76 e	6.33 f	3.25 d	0.58 e
Conv-NPK 1 g/l	116.67 b	9.50 d	7.66 e	4.35 c	0.84 d
Conv-NPK 1.5 g/l	116.00 bc	11.50 bc	8.66 d	4.55 c	0.91 c
Conv-NPK 2 g/l	115.55 c	12.16 ab	11.00 bc	5.20 b	1.03 ab
Nano-NPK 1 ml/l	114.32 d	11.00 c	10.33 c	4.44 c	0.89 cd
Nano-NPK 1.5 ml/l	113.33 e	12.33 a	11.33 b	5.14 b	1.02 b
Nano-NPK 2 ml/l	112.30 f	12.83 a	12.33 a	5.53 a	1.09 a
Second season 2023/2024					
Control	124.22 a	6.60 e	7.00 f	3.33 e	0.65 f
Conv-NPK 1 g/l	117.55 b	9.33 d	8.00 e	4.41 cd	0.89 e
Conv-NPK 1.5 g/l	117.00 bc	11.33 c	9.33 d	4.61 c	0.92 d
Conv-NPK 2 g/l	116.55 cd	12.33 ab	10.00 c	5.30 b	1.02 c
Nano-NPK 1 ml/l	115.98 de	11.16 c	9.66 cd	4.41 d	0.89 e
Nano-NPK 1.5 ml/l	115.55 e	12.16 b	11.00 b	5.24 b	1.05 b
Nano-NPK 2 ml/l	114.53 f	12.66 a	11.66 a	5.60 a	1.12 a

Values followed by different letters in the same column for each season are significantly different ($P<0.05$)

Fertilization with both conv-NPK and nano-NPK significantly extended the duration to reach full flowering, ranging from 7.66 to 12.33 days for fertilized plants compared to 6.33-7.00 days for unfertilized controls. Higher fertilization rates led to longer full flowering duration. Nano-NPK at 2 ml/l resulted in the longest full flowering duration, reaching 12.33 and 11.66 days in the first and second seasons, respectively, representing 1.94- and 1.66-fold increases over controls. Similarly, high-rate conv-NPK treatments also significantly increased full flowering duration, by approximately 4.67 and 3 days in the first and second seasons, respectively. Respecting biomass weight of flower, both nano-NPK and conv-NPK fertilizers significantly increased fresh and dry weights of *S. virgaurea* flowers. Nano-NPK at 2 ml/l consistently yielded the highest flower FW in both seasons, reaching 5.53-5.60 g/flower. Conv-NPK at 2 g/l also enhanced flower FW without significant variation when compared with nano-NPK at moderate rate (1.5 ml/l). Flower DW generally followed the trend of flower FW. Thus, nano-NPK at 2 ml/l produced the highest flower DW (1.09-1.12 g/flower), representing a 1.87-1.72-fold increase over the control.

3.3. Photosynthetic pigments

Conv-NPK and nano-NPK significantly increased chlorophyll-a, chlorophyll-b, and total chlorophyll content in *S. virgaurea* plants compared to the control. Nano-NPK generally demonstrated a more pronounced effect. Higher fertilizer doses generally resulted in higher chlorophyll levels. The highest chlorophyll-a content was observed with 2 ml/l nano-NPK in both seasons (3.20 and 3.11 mg/g FW), followed closely by 1.5 ml/l nano-NPK and 2 g/l conv-NPK (Fig. 2A). Similarly, nano-NPK at 1.5 and 2 ml/l showed the highest chlorophyll-b content (0.86-0.89 mg/g FW) in both seasons (Fig. 2B). Total chlorophyll content mirrored the trend observed for chlorophyll-a and chlorophyll-b, with the highest values observed with 1.5 and 2 ml/l nano-NPK (3.88 and 4.09 mg/g FW), representing 1.45-1.57-fold increases over the control (Fig. 2C). In the same way, carotenoid content significantly increased in all fertilized plants compared to the control, as shown in Fig. 2D. All tested concentrations increased carotenoids compared to the control. However, nano-NPK at 2 ml/l recorded the highest carotenoid levels (0.90-0.92 mg/g FW) in both seasons.

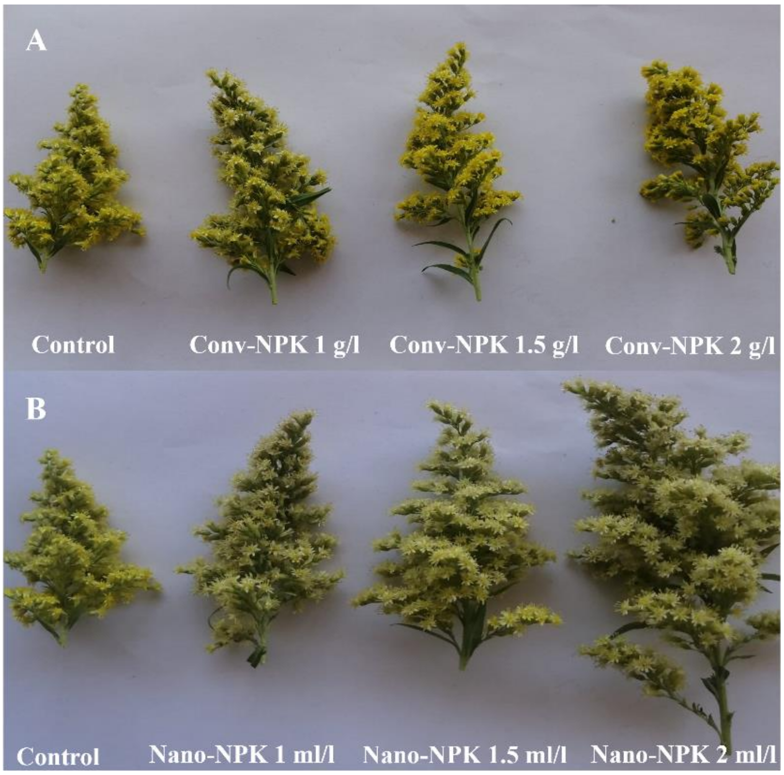

Fig. 1. Effect of conventional NPK (conv-NPK) (A) and nano-NPK (B) at different levels on the flower length of *S. virgaurea*

3.4. Nutrients content

Fertilizing *S. virgaurea* with conv-NPK and nano-NPK significantly increased nitrogen, phosphorus, and potassium levels (p <0.05) compared to the control (Fig. 3). The nutrients accumulation increased with increasing NPK rates. Overall, nano-NPK treatments consistently demonstrated superior nutrient enhancement compared to conv-NPK treatments in both seasons. Nano-NPK at the highest concentration (2 ml/l) resulted in the highest nutrient content. In both growing years, nano-NPK at 2 ml/l resulted in the highest significant content of nitrogen (3.83%-4.23%), phosphorus (0.63%-0.70%), and potassium (2.46-2.56%) followed by 1.5 and 1 ml/l of nano-NPK. The content of phosphorus and potassium was significantly similar (p <0.05) in the high conv-NPK (2 g/l) and low nano-NPK (1 ml/l) concentration treatments during the second season.

The application of NPK fertilizers boosted carbohydrate levels, with nano-NPK showing superior effects over conv-NPK throughout the study (Fig. 3D). Nano-NPK at 2 ml/l demonstrated the highest carbohydrate content (70.53% and 58.87%) followed by nano-NPK at 1.5 ml/l (58.40% and 55.40%) in the first and second seasons, respectively. Interestingly, low nano-NPK application (1 ml/l) resulted in the same significant value for carbohydrate content when compared to high (2 g/l) and moderate (1.5 g/l) conv-NPK applications.

3.5. Bioactive compounds content

The results demonstrated a significant effect (p<0.05) of both fertilization type (conventional vs. nano) and level (1, 1.5 and 2 g/l and 1, 1.5, and 2 ml/l, respectively) on total phenolic content (TPC) total flavonoid content (TFC), and total antioxidant capacity (TAC) in *S. virgaurea* across two growing seasons (Table 6). Increasing the NPK level generally enhanced TPC, TFC, and TAC, with the moderate and highest levels showing the most pronounced effects. Nano-NPK generally led to higher TPC and TAC levels compared to conv-NPK. Nano-NPK at 2 ml/l exhibited the highest significant phenolic content (8.49 and 9.46 mg GAE/g DW in the first and second seasons, respectively), 1.55-2.13-fold increase than control, with nano-NPK 1.5 ml/l and conv-NPK 2 g/l following closely behind.

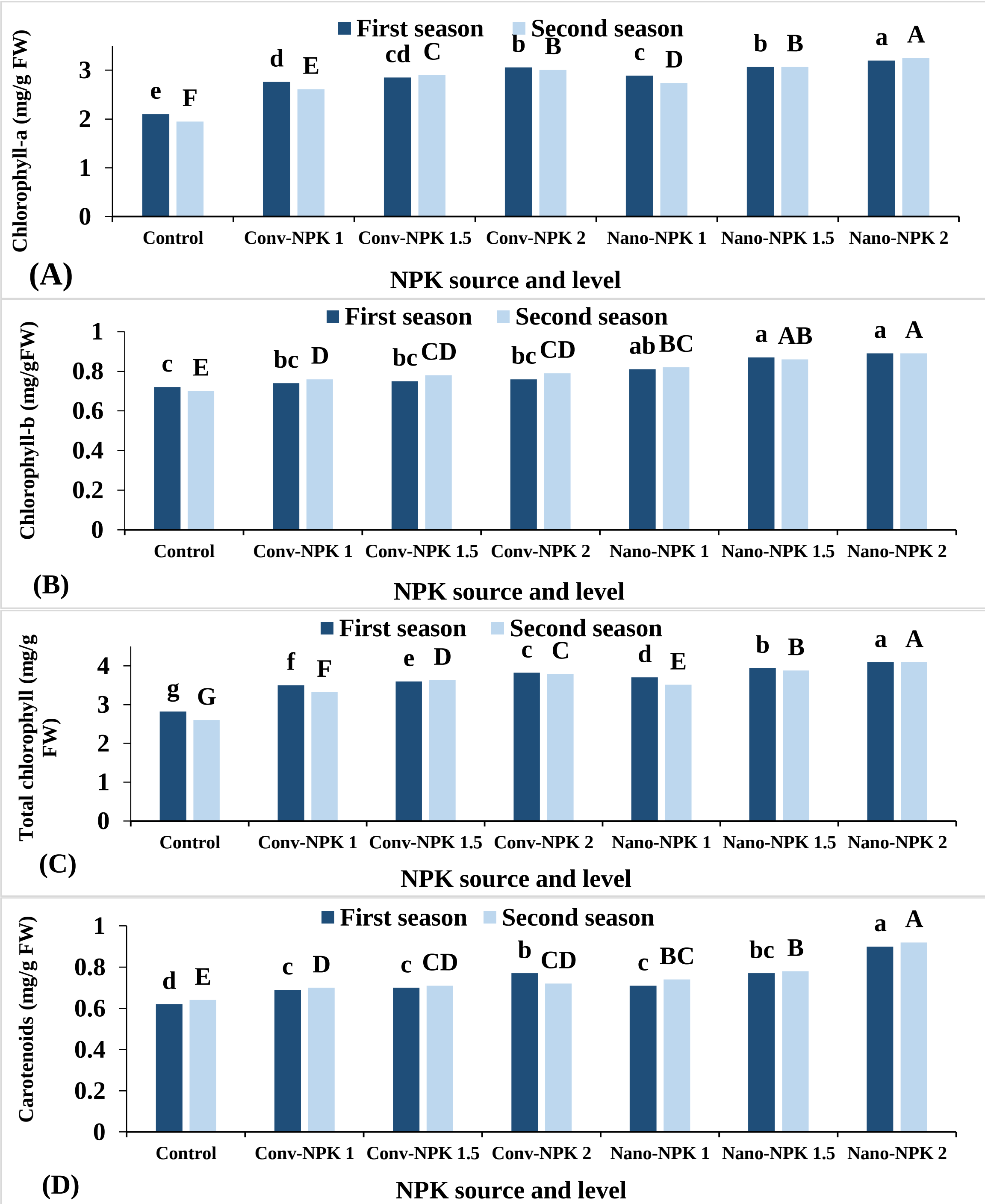

Fig. 2. Effect of conventional NPK (conv-NPK; 1, 1.5, and 2 g/l) and nano-NPK (1, 1.5, and 2 ml/l) on chlorophyll-a, chlorophyll-b (B), total chlorophyll (C), and carotenoids (D) content (mg/g FW) of *S. virgaurea* during 2022/2023 and 2023/2024 seasons. Different letters above the columns indicate statistical differences at the 0.05 level.

Table 6. Effect of conventional NPK (conv-NPK) and nano-NPK on total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) of *S. virgaurea* during 2022/2023 and 2023/2024 seasons.

Treatments	TPC (mg GAE/g DW)		TFC (mg QE/g DW)		TAC (mg AsAE/g DW)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	5.48 c	4.45 e	10.34 c	10.12 d	8.79 d	9.23 c
Conv-NPK 1 g/l	7.27 ab	6.35 cd	12.27 b	13.47 b	9.88 cd	10.13 bc
Conv-NPK 1.5 g/l	6.81 bc	7.65 bcd	12.41 b	13.67 b	10.13 c	10.24 bc
Conv-NPK 2 g/l	7.56 ab	7.98 abc	14.51 a	15.83 a	12.74 a	14.17 a
Nano-NPK 1 ml/l	5.74 c	6.20 d	10.52 c	11.12 cd	10.59 bc	10.37 bc
Nano-NPK 1.5 ml/l	8.07 ab	8.89 ab	10.75 c	12.35 bc	10.67 bc	11.35 b
Nano-NPK 2 ml/l	8.49 a	9.46 a	16.01 a	15.38 a	11.76 ab	13.26 a

Values followed by different letters in the same column for each season are significantly different ($P<0.05$).

Conv-NPK treatments generally resulted in higher TPC with increasing NPK rates, with 2 g/l of conv-NPK and 2 ml/l of nano-NPK exhibiting the highest TFC in the first (14.51 and 16.01 mg QE/g DW) and second seasons (15.83 and 15.38 mg QE/g DW), respectively. These values represent a significant 1.40-1.56-fold increase in TFC compared to the control. Similarly, *S. virgaurea* plants received higher concentration of conv-NPK (2 g/l) or nano-NPK (2 ml/l) exhibited the highest significant TAC. In this respect, a range of 1.34-1.54-fold increase in TAC compared to control was recorded for conv-NPK (12.74 and 14.17 mg AsAE/g DW) and nano-NPK treatments (11.76 and 13.26 mg AsAE/g DW) in the first and second seasons, respectively.

3.6. Anatomical structure

Microscopic measurements of six anatomical characters were performed through transverse sections of *S. virgaurea* leaves to study the effect of different levels of conv-NPK and nano-NPK on these anatomical measurements compared to unfertilized control plants.

Plants treated with conv-NPK at 1 g/l showed an increase in spongy tissue thickness, xylem vessels dimension and midrib zone by +25%, +124.6% and +7.93%, respectively, more than the untreated plants (Table 7). Conversely, the same treatment resulted in a decrease in upper epidermal layer thickness by 16.3% while palisade tissue thickness and lower epidermal layer were constant compared to the control. Conv-NPK applied at 1.5 g/l increased palisade tissue, spongy tissue, xylem vessels dimension, and midrib zone by +60%, +50%, 124%, and +11.8%, respectively, more than the untreated plants. However, lower epidermal layer was reduced by 33.3% under the same treatment. Higher application of conv-NPK (2 g/l) significantly increased palisade tissue thickness by 20%, xylem vessel dimension by 124.6%, and midrib zone thickness by 8.33% (Fig. 4B), compared to the control (Fig. 4A), while measurements of upper epidermal layer, spongy tissue, and lower epidermal layer were equaled with values of untreated plants.

Table 7. Effect of conventional NPK (conv-NPK) and nano-NPK on anatomical structure of *S. virgaurea* leaves.

Characters	Control	Conv-NPK 1 g/l		Conv-NPK 1.5 g/l		Conv-NPK 2 g/l		Nano-NPK 1 ml/l		Nano-NPK 1.5 ml/l		Nano-NPK 2 ml/l	
		AV	±	AV	±	AV	±	AV	±	AV	±	AV	±
Ue	12.3	10.3	-16.3	12.3	0	12.3	0	12.3	0	12.3	0.	12.3	0
P	20.5	20.5	0	32.8	+60	24.6	+20	36.9	+80	32.8	+60	36.9	+80
S	16.4	20.5	+25	24.6	+50	16.4	0	32.8	+100	32.8	+100	20.5	+25
X	67.3	151.2	+124.6	151.2	+124	151.2	+124.6	67.2	-0.14	67.2	+0.14	151.2	+124
L	12.3	12.3	0	8.2	-33.3	12.3	0	8.2	-33.3	12.3	0	8.2	-33.3
M	246	265.5	+7.93	275	+11.8	266.5	+8.33	250	+1.63	307.5	+25	254	+3.25

Ue= upper epidermis thickness, P= palisade tissue thickness, S= spongy tissue thickness, X= xylem vessels dimension, L.=lower epidermis thickness, M= midrib region thickness, AV= Absolute value and ±= ± % of control.

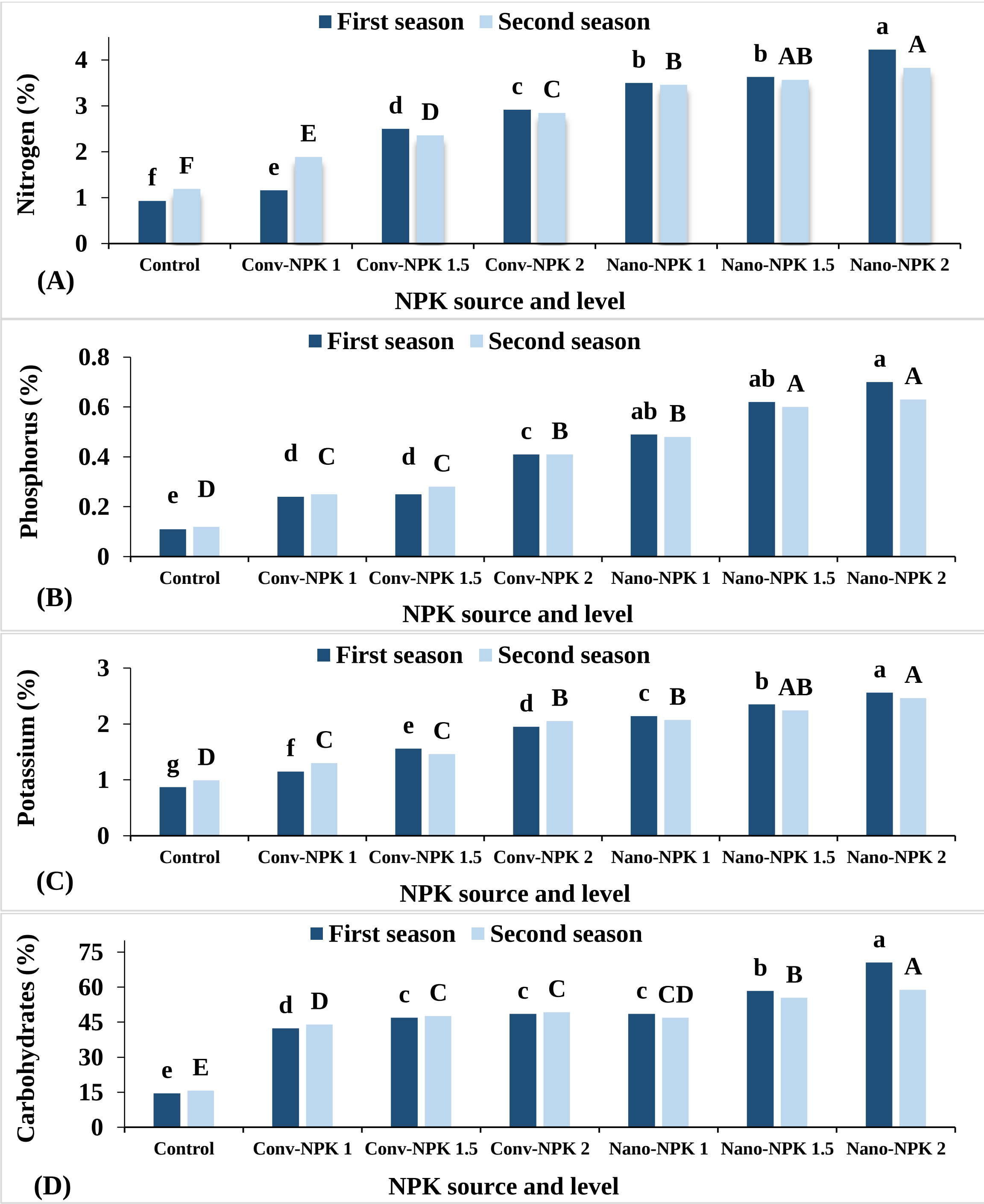

Fig. 3. Effect of conventional NPK (conv-NPK; 1, 1.5, and 2 g/l) and nano-NPK (1, 1.5, and 2 ml/l) on nitrogen (A), phosphorus (B), potassium (C), and carbohydrates (D) content (%) of *S. virgaurea* during 2022/2023 and 2023/2024 seasons. Different letters above the columns indicate statistical differences at the 0.05 level.

The application of nano-NPK at lower concentration (1 ml/l) increased the thickness of palisade and spongy tissues by +80 % and 100 %, respectively, more than the control, while both dimension of xylem vessels and lower epidermal layer were -0.14 % and -33.3%, respectively, less than the control. The moderate application of nano-NPK at 1.5 ml/l resulted in varying degrees of enhancement: from a slight increase, such as a 0.14% increase in xylem vessel dimension, to moderate improvements, like a 25% increase in midrib zone thickness, and substantial increase, with palisade and spongy tissues thickness by 60% and 100% respectively, surpassing the control group. Moreover, the elevated concentration of nano-NPK (2 ml/l) increased the thickness of palisade tissue by 80%, spongy tissue by 25%, xylem vessel dimensions by 124%, and midrib zone thickness by 3.25%

compared to the control. Conversely, the thickness of the lower epidermal layer decreased by 33.3% in comparison to the control (Fig. 4C vs. Fig. 4A).

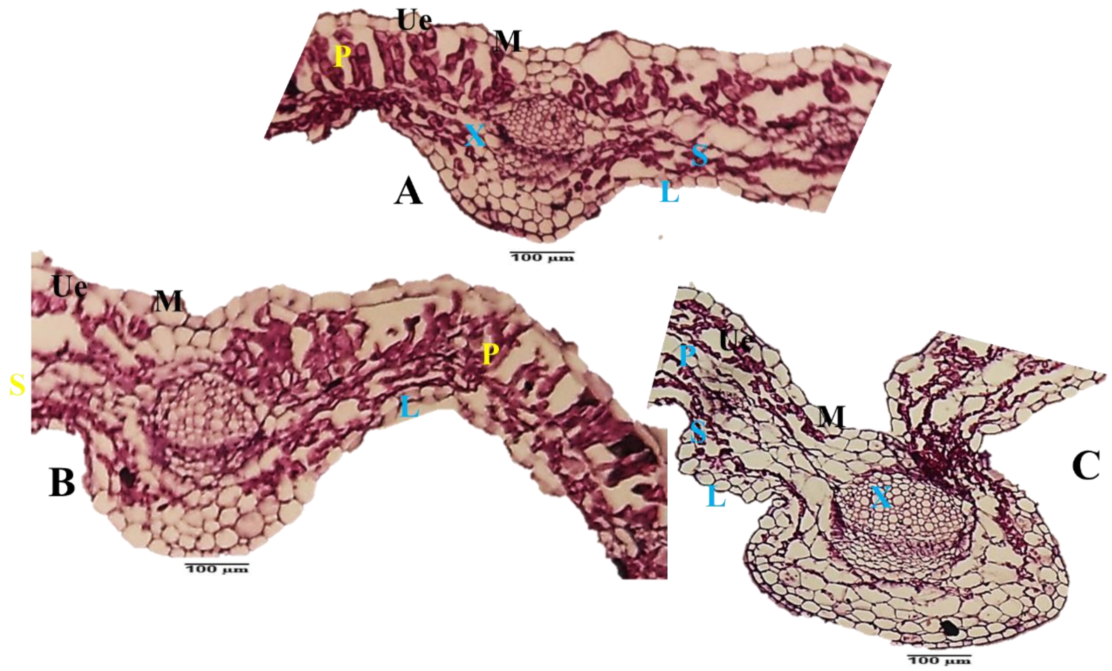

Fig. 4. Microphotographs of Transverse Sections through *S. virgaurea* leaves under the effect of 2 g/l conv-NPK (B) and 2 ml/l nano-NPK (C) compared to unfertilized plants (A). Le = Lower epidermis; M = Midrib zone, P = Palisade tissue; S = Spongy tissue, and Ue = Upper epidermis.

4. Discussion

Fertilization is an essential horticultural practice, crucial for supplying plants with the necessary nutrients depleted from the soil, thus supporting optimal growth and development. Nitrogen, phosphorus, and potassium are essential nutrients for plants. Small-scale farmers often apply high amounts of chemical fertilizers to increase the yield of solidago flowers. There is a need for global action to decrease the harmful effects of excessive chemical fertilization. Implementing novel agricultural practices is crucial to optimize fertilizer utilization, minimize soil toxicity, and ultimately increase agricultural productivity. Nano-fertilizers are engineered nutrient formulations with particle sizes typically in the nanometer range (1-100 nm). This nanoscale dimension significantly enhances their surface area-to-volume ratio, leading to increased solubility, reactivity, and plant uptake efficiency compared to conventional fertilizers (Ghazaryan et al., 2024).

The results of the present study demonstrated that applying a high concentration of nano-NPK (2 ml/l) significantly improved vegetative growth and flower quality in *S. virgaurea* across both seasons compared to untreated plants. This effect was followed by moderate nano-NPK application (1.5 ml/l) and high concentrations of conv-NPK (2 g/l). Nano-fertilizers can offer potential advantages over traditional fertilizers in certain situations due to their distinctive properties, namely their significantly smaller particle size, which can improve nutrient absorption efficiency uptake by plant roots due to increased surface area and potentially improved transport across cell membranes (Singh et al., 2017). Potassium plays a crucial role in various physiological processes, including protein synthesis, by activating enzymes involved in key metabolic pathways. This regulation of protein synthesis ultimately governs all growth processes. Also, nitrogen and phosphorus, crucial for synthesizing nucleic acids (DNA and RNA) and proteins, also contribute to increased cell growth and division (AL-Kaby et al., 2021). Similar to the findings of Le et al. (2022) on *Polyscias fruticosa*, who observed significant increases in plant height and dry matter with nano-fertilizer application, our study demonstrates substantial enhancement in growth and flowering parameters, thereby enhancing the plant's value and economic potential.

Results demonstrate that nano-NPK significantly enhanced flowering characteristics of *S. virgaurea* across both seasons. Plants treated with a relatively high application rate (2 ml/l) exhibited the most pronounced improvements, with significant increases observed in flowering start, flowering length, and both fresh and dry weight of flowers. Our results are consistent with those reported by Amirnia et al. (2014) on *Crocus sativus*, Razavi et al. (2015) on *Echinaceae purpuerea*, Sultana et al. (2015) on *Zinnia elegans*, and Hussein et al. (2017) on *Tagetes erecta*, further supporting the observed trend across different plant species. While lower doses of conventional and nano-NPK fertilizers demonstrated modest improvements in flower parameters compared to the control, these enhancements suggest a positive trend. Notably, foliar application of nano-NPK fertilizer significantly enhanced various plant growth traits, leading to increased vegetative biomass and flower yield compared to traditional or unfertilized treatments. The enhanced performance observed with foliar application of nano-NPK likely results from the rapid uptake of nutrients through leaf stomata and their direct translocation to plant tissues (Singh et al., 2017).

S. virgaurea plants exhibited significantly higher levels of chlorophyll, carotenoids, nutrients, and carbohydrates when treated with nano-fertilizers compared to conventional fertilizers. The observed increase in chlorophyll and carotenoid content in nano-fertilizer-treated plants suggests enhanced photosynthetic efficiency. These pigments play crucial roles in light absorption and energy conversion, ultimately driving plant growth and biomass accumulation. Nano-NPK fertilization elevates leaf nutrient levels, augmenting metabolic processes within the plant by increasing the available surface area for biochemical reactions. Increased leaf nutrient levels, particularly nitrogen and phosphorus, directly influence photosynthetic processes. These nutrients are essential components of chlorophyll and other photosynthetic enzymes, and their availability can significantly enhance carbon assimilation rates, thereby improving the plant's resilience to various physiological stresses (Ahmed and Makki, 2021).

Nano-fertilizers, with their unique properties such as small particle size, high surface area, and potentially enhanced reactivity, can exhibit an enhanced effect on studied characteristics. This allows them to hold numerous ions, facilitating nutrient retention and enabling gradual release to align with crop demands. Moreover, their slow-release mechanism, coupled with super absorbent properties, improves the efficiency of phosphatic and nitrogenous fertilizers by minimizing nutrient loss and enhancing crop productivity (Nofal et al., 2021). This controlled-release system promotes sustainable nutrient management and ensures long-term access to essential elements (Babu et al., 2022). Employing nano-fertilizers in a responsible and optimized manner has the potential to contribute to more sustainable agricultural practices by potentially reducing fertilizer inputs and minimizing environmental impacts. This can enhance crop yields while potentially reducing production costs. Ongoing research into optimizing nano fertilizer formulations and application techniques promises to further unlock the potential for increased crop productivity and heightened environmental sustainability (Babu et al., 2022).

Application of nitrogen, phosphorus, and potassium fertilizers can substantially alter the phytochemical profile and enhance the antioxidant capacity of medicinal plants (Nada et al., 2022; 2024; El-Beltagi et al., 2023). The present study demonstrated that increasing NPK levels generally stimulated the accumulation of total flavonoid content (TFC), total phenolic content (TPC), and total antioxidant capacity (TAC) in *S. virgaurea* plants. The moderate and highest NPK levels exhibited the most significant enhancement of these phytochemicals. These compounds possess valuable antioxidant and anti-inflammatory properties, enhancing the medicinal value of *S. virgaurea*. Nitrogen is a crucial component of amino acids, which serve as the building blocks of proteins, including enzymes involved in the biosynthesis of phenolic compounds. Adequate nitrogen supply is essential for the synthesis and activity of these enzymes, ultimately influencing the production of phenolic compounds (Ma et al., 2023). Studies have shown that increasing nitrogen levels can lead to a significant increase in the total phenolic content and antioxidant activity, however, higher concentrations may cause an inhibitory effect (Ma et al., 2023). Phosphorus plays a vital role in various cellular processes, including energy transfer (as ATP), the synthesis of nucleic acids (DNA and RNA), and the formation of phospholipids. Adequate phosphorus supply is essential for optimal plant growth and development and can enhance the activity of enzymes involved in the biosynthesis of different secondary metabolites (Villamarin-Raad et al., 2023). Potassium plays a vital role in maintaining cell turgor, regulating stomatal opening, and activating various enzymes involved in metabolic processes. Potassium fertilization can enhance the overall growth and development of medicinal plants, leading to increased biomass and, consequently, higher yields of secondary metabolites including phenolic and flavonoid compounds (Gaaliche et al., 2019).

While this study focuses on the effects of NPK fertilizers, it's worth noting that other studies, such as that by Ahmed et al. (2023), have demonstrated the positive impact of iron oxide nanoparticles on the growth and phytochemical profile of *S. virgaurea*. They found that applying iron oxide nanoparticles to *S. virgaurea* plants four times at a concentration of 1 mg/l significantly enhanced plant growth and the levels of essential nutrients like N, P, K, Cu, and Zn compared to untreated plants and those treated with a lower concentration (0.5 mg/l). Furthermore, the study revealed that applying the higher nanoparticle concentration (1 mg/l) five times notably increased the production of beneficial compounds in the plants, such as flavonoids (rutin and quercetin) and essential oils. The flavonoid content obtained in our study ranged from 10.12 to 16.01 mg QE/g DW, which corresponds to 1.01% – 1.60% (w/w). The flavonoid content observed in this study falls within the range specified by the European Pharmacopoeia for *Solidaginis virgaureae* herba products (0.5% to 1.5% expressed as hyperoside), indicating acceptable quality (Council of Europe, 2019).

Previous reports have shown that the types of fertilizers and nutrients significantly affect the anatomical and ultrastructure characters of different plant organs (El-khawaga, 2018; Al-Dhalimi and Al-ajeel, 2020; Gashash et al., 2022). In the current study, all NPK levels applied to *S. virgaurea* increased the thickness of the palisade tissue, xylem vessel dimensions, and the midrib zone, with the most pronounced effect observed at higher levels. This aligns with the findings of Gashash et al. (2022), who reported that application of varying P and Zn fertilizer levels enhanced anatomical characteristics in *Beta vulgaris*. Sarker et al. (2010) observed that phosphorus deficiency in maize plants led to significant reductions in plant growth and anatomical modifications, including thinner leaves, smaller vascular bundles with reduced size of the metaxylem vessel cavity in the phosphorus-deficient leaves. Nano-NPK at varied levels prompted substantial enhancements in tissue characteristics of *S. virgaurea*, suggesting nuanced responses to fertilization. Nano-NPK seems to be superior for promoting palisade and spongy tissue growth, which are crucial for photosynthesis and overall leaf function as well as tolerance to various stresses (Yang et al., 2025). While conv-NPK and nano-NPK both affect xylem vessels and the midrib zone, the effects of nano-NPK on xylem vessels seem more dependent on concentration. The observed increases in the thickness of the palisade tissue and xylem vessel dimensions may be attributed to enhanced cell division and expansion, which are influenced by nutrient availability and hormonal regulation (Yang et al., 2025). The increase in xylem vessel dimensions observed in NPK-treated plants may enhance water and nutrient transport, resulting in improved plant vigor, flower production, and bioactive compounds accumulations. According to Al-Dhalimi and Al-ajeel (2020), the application of 100 ppm Zn nanoparticles on *Helianthus annuus* can significantly influence various anatomical parameters, including the thickness of the epidermis, length and width of vascular bundles, diameter of vessels, and thickness of the cortex. Considering the significant positive effects of nano-NPK on photosynthetic tissues in *S. virgaurea*, and similar effects on other tissues, it emerges as a better choice. The ability of nano-NPK to deliver substantial improvements at lower concentrations (1 and 1.5 ml/l) suggests it is more efficient and potentially cost-effective.

Conclusions

Fertilization plays a pivotal role in modern horticulture, ensuring adequate nutrient supply for optimal plant growth and development. However, excessive use of conventional chemical fertilizers can have detrimental environmental impacts. Through a detailed pot experiment comparing conventional NPK (conv-NPK) and nano-NPK applications at various levels, it was evident that nano-NPK treatments, particularly at elevated concentrations, substantially improved growth parameters, flowering characteristics, photosynthetic pigments, nutrient content, and anatomical features of *S. virgaurea*. Notably, moderate level of nano-NPK exhibited similar or superior performance compared to high levels of conv-NPK in most aspects, except for specific bioactive components like total flavonoid content and total antioxidant capacity. The study underscores the potential of nano-NPK to optimize plant growth, flowering, and bioactive compound accumulation, offering a promising alternative that enhances plant quality and potentially increases economic returns for farmers while addressing environmental concerns associated with traditional chemical fertilizers. Further study is needed to investigate the optimal NPK fertilization strategies for maximizing the production of specific bioactive compounds *in S. virgaurea* while minimizing environmental impact. Further research into the long-term effects and cost-effectiveness of nano-NPK applications can provide valuable insights for sustainable plant cultivation practices.

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