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Impact of Chitosan and Folic Acid on Growth, Leaf Qualities, and Antioxidant Compounds of Purslane (*Portulaca oleracea* L.)

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Abstract

Purslane (*Portulaca oleracea* L.) is a nutrient-rich leafy vegetable valued for its nutritional and medicinal properties. Improving its quality and bioactive compounds through eco-friendly inputs is essential for sustainable production. This study evaluated the effects of foliar-applied chitosan and folic acid on growth, leaf quality, and antioxidant compounds of purslane. A field experiment was conducted in a randomized complete block design with chitosan (0, 50, and 100 mg l^{-1}) and folic acid (0, 25, and 50 mg l^{-1}), applied singly or in combination. The combined treatment of 100 mg l^{-1} chitosan and 50 mg l^{-1} folic acid was the most effective, producing plant height of 36.7 cm, leaf area of 17.8 cm^2, and 92 leaves $plant^{-1}$, representing 27 to 30% increases over the control. Fresh weight reached 91.2 g $plant^{-1}$, a 26% improvement. Leaf quality improved as total chlorophyll (33.2 mg 100 g^{-1} FW) and carotenoids (5.46 mg 100 g^{-1} FW) rose by 13% and 10%, respectively. Antioxidant levels were also enhanced: phenols (41.12 mg GAE g^{-1} DW), flavonoids (15.91 mg RE g^{-1} DW), tannins (20.11 mg TAE g^{-1} DW), saponins (40.65 mg g^{-1} DW), and ascorbic acid (55.82 mg 100 g^{-1} FW), with 8 to 22% increases over single treatments and 12 to 31% over the control. DPPH radical scavenging activity reached 77.32%, 54% higher than the control (50.11%) and greater than single applications of chitosan (62.33%) or folic acid (69.58%), confirming a synergistic effect. These results suggest that chitosan and folic acid can serve as cost-effective and eco-friendly biostimulants to enhance purslane production and nutritional value under sustainable agriculture.

Keywords: DPPH; flavonoids; phenols; secondary metabolites; synergistic

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INTRODUCTION

Purslane (*Portulaca oleracea* L.) is an annual herbaceous, succulent plant belonging to the Portulacaceous family (Ercisli et al., 2008). It is widely spread in tropical and subtropical regions (Pereira et al., 2009). It is used in traditional medicine to treat many diseases due to its antioxidant properties. It also contains anti-inflammatory, anti-tumor, anti-fungal, and antibacterial compounds, including those effective against intestinal bacteria (Okuda et al., 2021). It has a protective effect on cells, nerves, and the liver due to its content of phenolic alkaloids such as oleracein A, B, and E, as well as flavonoid compounds, polysaccharides, fatty acids, terpenoids, and vitamins (Miraj, 2016). The aqueous extract of the plant is essential in reducing oxidative damage caused by a high-fat diet, treating hyperinsulinemia, reducing weight, preventing type II diabetes, and enhancing the body's immunity by eliminating the accumulation of free radicals, thus fighting cancer (Kamel et al., 2024). Purslane seeds are used to treat insomnia, hepatitis, gallbladder inflammation, and joint pain. While its juice is used to treat kidney disease

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and bladder (Farkhondeh et al., 2019). The polysaccharide extract of the plant reduces the risk of stomach cancer and DNA damage caused by hydrogen peroxide by improving immunity and oxidative status (Khodadadi et al., 2018).

Chitosan is a natural mucopolysaccharide and a polymer of 1,4-glycosidically linked glucosamine (2-amino-2-deoxy-D-glucopyranose), which is derived from the deacetylation of chitin (Román-Doval et al., 2023). It is a natural, bioavailable, environmentally safe, and inexpensive carbon source after lignocellulosic biomass (Malerba and Cerana, 2016). It is produced from the exoskeleton of arthropods and the cell walls of fungi. It is a large ketone polysaccharide with anti-fungal, antibacterial, and antiviral properties. It is a plant biostimulant that enhances the morphological and physiological parameters (Khalil and Badr-Eldin, 2021). It is added to medicinal plants to increase the production of secondary metabolites, increase biomass, absorb nutrients, and increase the size of chloroplasts and chlorophyll. Therefore, it is used as a foliar spray, seed treatment (seed coating), or nutritional supplement in tissue culture media (Jacob et al., 2023).

Folic acid is one of the forms of vitamin B9 that is soluble in water and is considered a donor and acceptor in the carbon monoxide one-carbon (C1) transport reactions. These reactions are essential for synthesizing amino and nucleic acids (Al-Elwany et al., 2022). Folic acid plays a crucial role in plant growth, influencing its response to light, nitrogen, and carbon metabolism and stress resistance (Gorelova et al., 2017). The deficiency of folic acid in plants is linked to the weak production of nucleotides in plants, as two of its derivatives participate in the construction of DNA, namely 10-formyl-THF and 5,10-methenyl THF (Bailey and Ayling, 2009). Folic acid also scavenges free radicals or reactive oxygen species generated during photosynthesis and respiration. Its auxin-like activity contributes to cell division (Hassan et al., 2016). It has a role in converting glycine to serine and participates in the biosynthesis of methionine, lignin, and choline, in addition to its role in the photorespiration cycle (Al-Maliky et al., 2019).

Globally, sustainable agriculture greatly emphasizes providing healthy food and preventative benefits that enhance community health and reduce pollution. Environmentally friendly natural stimulants are a suitable technology for increasing production in quantity and quality. However, little is known about how chitosan and folic acid, especially in combination, affect the growth, leaf quality, and antioxidant compounds of purslane. This study aims to evaluate the effect of foliar-applied chitosan and folic acid, both alone and in combination, on improving the morphological parameters, qualitative properties, and antioxidant compounds in purslane.

MATERIALS AND METHOD

Experimental site and soil analysis

A field experiment was conducted on a plot of land owned by a farmer in the Babylon Governorate of Iraq during the 2024 agricultural season. The field was located at a longitude of 32.501° E and a latitude of 44.333° N. The soil was tested for its chemical and physical properties in the Soil and Water Resources Department Laboratories of Agriculture College, Al-Qasim Green University (Table 1). Seeds of local variety were sown on March 15, 2024 (Al Wase and Zahwan, 2022), on experimental units measuring 1.2 m × 1.4 m, with an agricultural spacing of 20 cm × 20 cm. Triple superphosphate fertilizer was added during the soil preparation for planting at a ratio of 100:100:75 (N:P:K) according to the recommendation proposed by El-Sherbeny et al. (2015). Irrigation was contingent on the need, and hand weeding was utilized for weed control. The experimental units were sprayed with stimulating compounds: chitosan at concentrations of 0, 50 (Ch^{50}), and 100 (Ch^{100}) mg l^{-1} (Jacob et al., 2023), folic acid at concentrations of 0, 25 (FA^{25}), and 50 (FA^{50}) mg l^{-1} (Bakhoum et al., 2022), and their mixtures. Foliar spraying was used two times, 20 and 40 days after sowing, while the control treatment was spread with distilled water only.

Measurement parameters

The first flower appeared on May 15, mowing 10 plants randomly from each experimental unit (excluding the guard line plants) and measuring

Table 1. Chemical and physical properties of soil

pH	OM	Ec	mg kg^{-1}			cmol kg^{-1}			Sand	Silt	Clay	Soil
	(%)	(dS m^{-1})	N	P	K	Ca	Mg	Na	(%)	(%)	(%)	
7.17	1.22	3.92	28.30	11.90	249.80	11.46	2.98	0.59	43.34	34.83	21.83	Loam

Note: OM = Organic matter, Ec = Electrical conductivity

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all parameters. The parameters including the growth parameters (plant height, number of branches, number of leaves, leaf area, and the fresh and dry weight of the plant), the total amount of leaf quality indicators (chlorophyll, carotenoids, soluble solids, carbohydrates, oxalic acid, and nitrate value), and antioxidant compounds in total (phenols, flavonoids, tannins, saponins, ascorbic acid, and antioxidant activity).

Purslane leaves' quality was estimated by some essential parameters, including the content of total chlorophyll and carotenoids. These pigments were measured through an extraction with acetone and spectrophotometry according to the method by Wellburn (1994). The total soluble solids in the leaf juice were measured using a digital refractometer at room temperature. The total carbohydrate concentration was determined by phenol-sulphuric acid assay (Sablania et al., 2019). To measure the accumulation of oxalic acid, dry samples were digested with hydrochloric acid and octal, then incubated with sulphuric acid and potassium and manganese chromate for 60 minutes, and then the optical density was equilibrated by the method of Popoola et al. (2014). The nitrate value in the leaf aqueous extract was measured using a spectrophotometer according to the procedure followed by Kurniawati et al. (2017). Various analytical methods have been reported for determining antioxidant compounds in food. These include high-performance liquid chromatography (HPLC) and Fourier-transform infrared spectroscopy (FTIR). It is characterized by its high accuracy in determining compound concentrations.

Nevertheless, these methodologies are costly, intricate, onerous, and protracted, requiring skilled personnel. In contrast, the UV method requires minimal skill, is available at a modest cost, and is highly sensitive and reliable. In addition, this experiment involves estimating the total antioxidant compounds without the need to quantify the compounds of each group, which requires additional time, effort, and cost. It may be a suitable topic for future research. Total phenol was measured using the Folin-Ciocalteu method at 755 nm, then calibrated with gallic acid equivalents (Chrysargyris et al., 2019). Whereas the aluminum chloride method (Shraim et al., 2021) was used to determine the total flavonoids at an absorbance of 510 nm, the optical density was expressed as rutin equivalents. The method of Adegbusi et al. (2022) was used to calculate the total tannins using the Folin-Ciocalteu reagent, and the results were then converted to tannic acid equivalents. Leaf extracts were tested for their ability to scavenge free radicals (DPPH) at 517 nm as Baliyan et al. (2022) described. The total saponin content was determined using a methanol and aescin calibration curve at 560 nm, based on the method of Senguttuvan et al. (2014) and modified by Vittaya et al. (2022). However, a starch-iodide complex was used for redox titration to determine the concentration of ascorbic acid in leaf extracts. UV spectroscopy at 265 nm was then used to assess the sensitivity of the reaction endpoint. It enabled the acid concentration calculation to be adjusted according to the approved, modified method (Hagos et al., 2022).

Data analysis

The experimental treatments were dispensed in a randomized complete block design with three replicates. After collecting data, they were analyzed using ANOVA, means were contrasted with the least significant difference (LSD) test ($p \leq 0.05$), and trend analysis ($p \leq 0.01$) was used to examine the direction of response of antioxidant compounds with experimental treatments.

RESULTS AND DISCUSSION

Growth parameters

The ANOVA in Table 2 demonstrates the significant impact of treating purslane with chitosan alone. The Ch^{50} treatment produced the highest averages for plant height, number of branches, number of leaves, leaf area, and fresh and dry weight, compared with Ch^{100} and control treatments. This improvement in growth parameters is consistent with the findings of Bakhoum et al. (2022), who reported similar enhancements when chitosan was applied to the lupine plant at a concentration of 50 mg l^{-1}. Chitosan promotes plant growth by enhancing photosynthetic activity, inducing photosynthetic constituents, regulating major photobiochemical processes, and promoting carbohydrate production (Ahmed et al., 2020). In addition, it also works to increase the production of amino acids and sugars (Hidangmayum et al., 2019), and it improves metabolic processes such as nutrient absorption, protein creation, and cell division (Chakraborty et al., 2020). Ultimately, these processes reflect in the plant's growth and development. The effect of Ch^{100} on growth parameters decreased due to accumulation on the surface of the leaves. It triggered an abiotic stress response in the signaling system, prompting the

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plant to produce antioxidant compounds as a defense mechanism. It is evident from the superiority of this treatment in terms of antioxidant compounds (Table 4). These results are consistent with those obtained by Raut et al. (2022) in their experiment on chickpea plants, which showed a decline in growth characteristics and yield at concentrations of chitosan above 60 mg l^{-1}.

As shown in Table 2, spraying with folic acid significantly affected the purslane plant. The FA^{50} treatment was significantly superior as it produced the highest averages for plant height, number of branches, number of leaves, leaf area, and fresh and dry weight, compared with the FA^{25} and control treatments. Folic acid plays a positive role in enhancing leaf enzymatic activity, as well as nucleic acid and amino acid synthesis (El-Moghazy and Al-Azzony, 2019), which is also necessary for synthesizing lipids, proteins, chlorophyll, and lignin, and for catalyzing and regulating gene expression (Gorelova et al., 2017). In addition, increases cell division and elongation rates by contributing to the production of natural hormones (Al-Maliky et al., 2019) and increasing vegetative plant growth and dry matter accumulation. Similar improvements were reported by Bakhoum et al. (2022) following the application of folic acid to flax and geranium plants.

The data in Table 2 also indicate that the combined treatment $Ch^{100} \times FA^{50}$ was significantly superior in all growth indicators, indicating that the combined treatments produced synergistic effects on plant growth. The relationship between chitosan and folic acid is powerful, complementary, and synergistic. While folic acid acts as a supportive and essential nutrient, providing these processes with fuel and building blocks (DNA, proteins, chlorophyll), chitosan is a regulator and catalyst for the physiological and hormonal processes responsible for growth. Combining the two amplifies their effects, significantly improving vegetative growth indicators (Sathiyabama and Manikandan, 2021). A modern key finding in this research is that combined treatments are not limited to nutrition, as with traditional fertilizers. Still, they are a form of plant biotechnology that interferes with the plant's physiological and biochemical processes to comprehensively stimulate and improve its performance, resulting in a purslane plant of high quality in terms of yield, nutrition, and medicinal properties.

Leaf qualities

The data in Table 3 indicate a significant effect of chitosan treatment on the qualitative characteristics of purslane leaves. The Ch^{50} treatment significantly outperformed, recording the highest means of total chlorophyll pigments, carotenes, soluble solids, and carbohydrates. Chitosan activates photosynthesis, enhances nutrient uptake (Sharif et al., 2018), and stimulates the biosynthesis of plant hormones that contribute to cell division and elongation. Consequently, increased pigments, larger chloroplasts, and greater carbohydrate accumulation result (Godase et al., 2023). These results are consistent with those of Raut et al. (2022) and Deotale et al. (2019), who conducted experiments on chickpea and pigeonpea plants exhibiting enhanced quality parameters at a 50 mg l^{-1} chitosan concentration. The Ch^{100} treatment recorded the highest mean of oxalic acid and nitrates compared to the Ch^{50} and control treatments. These results are consistent with those of Elsheery et al. (2020), who found that foliar spraying with chitosan increases calcium absorption and accumulation in plant tissues

Table 2. Impact of chitosan and folic acid on growth parameters of purslane

Treatment	PH	BN	LN	LA	FW	DW
Control	17.5	8.14	165.2	322.5	74.6	6.21
Ch^{50}	20.3	9.16	184.2	369.2	81.2	6.51
Ch^{100}	18.8	8.53	173.3	345.2	78.5	6.33
FA^{25}	18.3	8.36	169.4	325.1	79.5	6.44
FA^{50}	19.6	8.91	177.6	357.6	80.6	6.48
$Ch^{50} \times FA^{25}$	20.6	9.11	181.9	372.9	85.3	6.64
$Ch^{50} \times FA^{50}$	21.3	9.43	195.4	390.5	88.5	6.73
$Ch^{100} \times FA^{25}$	21.8	9.70	206.3	402.8	91.2	6.81
$Ch^{100} \times FA^{50}$	22.3	9.95	213.8	418.6	93.8	6.88
LSD ($p = 0.05$)	0.08	0.02	15.2	22.3	0.05	0.01

Note: PH = Plant height (cm), BN = Number of branch, LN = Number of leaf, LA = Leaf area (cm^2), FW = Fresh weight (g), DW = Dry weight (g)

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through specific physiological and cellular mechanisms. These mechanisms include activating ATPase pumps and improving cell membrane permeability, thereby helping to increase oxalic acid accumulation.

The data in Table 3 also show that folic acid at a concentration of 50 mg l^{-1} significantly affected all qualitative leaf traits. Folic acid stimulates glycine biosynthesis, contributing to the synthesis of porphyrins and chlorophyll in chloroplasts (Al-Maliky et al., 2019). It plays an active role in sugar metabolism and improves physiological parameters (Badr ElSayed et al., 2025). These results are consistent with those of Khan et al. (2022).

As shown in Table 3, the Ch^{100} × FA^{50} treatment performed significantly better than all the other treatments regarding leaf quality indicators. Al-Saadi et al. (2025) reported that the combination of chitosan with many other compounds has a synergistic effect. Chitosan activates the plant's defence and growth systems. Meanwhile, folic acid provides these systems with the essential building blocks (such as carbon and nitrogen) needed to operate at maximum efficiency. This makes it the perfect combination of a stimulant and a supplier. Together, they multiply their effect, improving the qualitative and quantitative characteristics of leaves. Generally, there is a lack of documented scientific studies on the combination treatments between chitosan and folic acid and testing of the response of purslane. Still, many studies exist on the effect of each compound individually.

Antioxidant compounds

The statistical data in Table 4 indicate a significant effect of spraying with chitosan on the antioxidant compounds of purslane leaves. Compared with the treatments Ch^{50} and the control, Ch^{100} significantly impacted all these compounds: total phenols, flavonoids, tannins, saponins, ascorbic acid, and antioxidant activity. These increases resulted from sensitizing the cells' defense systems to the Ch^{100} concentration. It increased the efficiency of producing secondary

Table 3. Impact of chitosan and folic acid on leaf qualities of purslane

Treatment	Ch	CA	TSS	CHO	OA	NV
Control	29.3	4.95	1.82	15.40	5.12	16.54
Ch^{50}	31.6	5.18	1.92	16.49	5.32	16.77
Ch^{100}	29.8	5.02	1.83	15.85	5.49	17.32
FA^{25}	30.3	5.09	1.84	15.91	5.36	16.91
FA^{50}	30.8	5.11	1.89	16.33	5.46	17.11
Ch^{50} × FA^{25}	31.4	5.31	1.95	16.62	5.57	17.66
Ch^{50} × FA^{50}	31.8	5.36	1.97	17.05	5.69	17.89
Ch^{100} × FA^{25}	32.1	5.41	2.01	17.46	5.81	18.44
Ch^{100} × FA^{50}	33.2	5.46	2.02	17.79	5.75	18.13
LSD (p = 0.05)	0.11	0.06	0.01	0.08	0.03	0.09

Note: Ch = Total chlorophyll (mg 100 g^{-1} FW), CA = Total carotenoids (mg 100 g^{-1} FW), TSS = Total soluble solid (%), CHO = Total carbohydrates (mg glucose g^{-1} DW), OA = Oxalic acid (mg g^{-1} DW), NV = Nitrate value (%)

Table 4. Impact of chitosan and folic acid on antioxidant compounds of purslane

Treatment	TP	TF	TT	TS	AS	DPPH
Control	36.55	13.21	15.68	34.91	45.60	50.11
Ch^{50}	37.91	13.93	16.33	36.44	49.23	61.42
Ch^{100}	38.88	14.21	16.65	38.62	50.59	62.33
FA^{25}	37.58	13.44	15.93	35.58	49.09	54.81
FA^{50}	39.26	14.24	17.71	39.53	51.68	69.58
Ch^{50} × FA^{25}	39.86	14.82	17.58	41.29	52.61	65.47
Ch^{50} × FA^{50}	40.27	15.46	18.71	42.87	53.59	69.48
Ch^{100} × FA^{25}	40.81	15.63	19.39	44.11	54.76	73.62
Ch^{100} × FA^{50}	41.12	15.91	20.12	45.65	55.82	77.32
LSD (p = 0.05)	1.33	0.11	0.58	0.82	0.13	2.67

Note: TP = Total phenols (mg Gallic g^{-1} DW), TF = Total flavonoids (mg Rutine g^{-1} DW), TT = Total tannins (mg Tannic g^{-1} DW), TS = Total saponins (mg g^{-1} DW), AS = Ascorbic acid (mg 100 g^{-1} FW), DPPH = Antioxidant activity (%)

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Table 5. Trend analysis of antioxidant compounds in purslane

Antioxidant compounds	Means	ANOVA	Linear trend	Quadratic tread
TP	39.14±1.55	0.01	0.01	0.059
TF	14.54±0.97	0.01	0.01	0.001
TT	17.57±1.57	0.01	0.01	0.001
TS	39.89±3.85	0.01	0.01	0.001
AS	51.44±3.19	0.01	0.01	0.012
DPPH	64.90±8.75	0.01	0.01	0.009

Note: Values are presented as mean±SEM (n = 9), TP = Total phenols, TF = Total flavonoids, TT = Total tannins, TS = Total saponins, AS = Ascorbic acid, DPPH = Antioxidant activity

metabolites via the shikimic acid pathway, thereby increasing the production of phenolic compounds, flavonoids, and other secondary metabolites (Baviskar et al., 2025). These results are consistent with those of Bakhoum et al. (2022).

As shown in Table 4, folic acid at a concentration of 50 mg l^{-1} significantly affects the antioxidant compound content in leaves. This increase is attributed to folic acid's important role in carbohydrate and nitrogen metabolism and subsequent involvement in amino and nucleic acid synthesis (Alsamadany et al., 2022). Consequently, this promotes the production of secondary metabolites with antioxidant properties (Al-Elwany et al., 2022).

The data also indicate that the combination treatment $Ch^{100} \times FA^{50}$ is superior, as it produced the highest levels of antioxidant compounds in purslane plants. The synergistic relationship between them, chitosan acts as a natural resistance inducer when sprayed on plants, activating intracellular signaling pathways and increasing the production of reactive oxygen species as a defense mechanism. It prepares the plant to cope with abiotic stresses. As an antioxidant, folic acid helps manage and regulate these oxidative signals, preventing them from reaching toxic levels and allowing the plant to build up its defenses by increasing the antioxidant compounds (Abdiazar et al., 2024).

Trend analysis (Table 5) was conducted on antioxidant compound data and experimental treatments in order to determine the nature of the relationship and its responsiveness. Despite varying response magnitudes, a significant linear relationship was found at a probability level of 0.01 for all antioxidant compound indices. It was an essential objective of the experiment. However, ascorbic acid and DPPH exhibited a clear curved pattern (quadratic), suggesting a limit beyond which production decreases. Phenols exhibited only a linear response. A linear relationship was observed between concentration and single treatments for chitosan and folic acid, while combined treatments exhibited a synergistic effect. The effect increased with increasing concentrations of the treatment. At the same time, a comparative reference relationship was observed, demonstrating the superiority of all treatments compared to the control treatment. The achievement of a synergistic relationship between the experimental substances suggests that experiments should be conducted with concentrations higher than those used in this study. This relationship may indicate the influence of the identified antioxidant compounds and other compounds affected by the treatments that led to the activation of DPPH, such as enzymes and unsaturated fatty acids (Zaman et al., 2019).

CONCLUSIONS

Foliar-applied chitosan and folic acid enhanced purslane performance, with the combined application being consistently superior to single treatments. In particular, the $Ch^{100} \times FA^{50}$ treatment produced the highest vegetative growth, leaf chlorophyll and carotenoids, and elevated antioxidant compounds, confirming an apparent synergistic effect. These results indicate that integrating chitosan and folic acid is more efficient than applying either stimulant alone, providing a cost-effective and environmentally friendly strategy to improve purslane yield and nutritional quality under sustainable agriculture systems. Future studies should explore different concentrations or other natural stimulants.

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