

synergistic Effects of Shading, Integrated Fertilization, and Salicylic Acid Application on Growth, Phytochemical Composition, and Antioxidant Activity of Red Beet (*Beta vulgaris* L.)

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Abstract

The objective of this study was to evaluate the effects of green netting (Saran), fertilization (organic and chemical), and spraying with salicylic acid on the growth and active ingredient content of red beet (*Beta vulgaris* L.). The experiment was carried out during the winter season of 2024-2025 in the fields of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Tikrit, using a completely randomized block design (RCBD) as a factorial experiment with three factors and three replications. The treatments comprised three shading levels (none, one month, two months), three fertilization types (chemical, organic, chemical plus organic), and two salicylic acid concentrations (0 and 50 mg/L). The results showed that all factors and their interactions had a significant effect on the traits studied. The triple interaction (application of 50 mg/L salicylic acid, organic fertilization, and shade for two months) produced the maximum levels of chlorophyll content, stem diameter, total soluble solids (TSS), Betalain concentration, and antioxidant activity (DPPH). Meanwhile, the lowest values were recorded when these transactions were absent. According to the findings, the utilization of salicylic acid, organic fertilization, and the application of proper shading techniques results in boosted growth, heightened efficiency of physiological processes, and heightened generation of active compounds, all of which have a favorable impact on the quality of the crop.

Keywords : Red beets, salicylic acid, chemical fertilization, organic fertilization, Betalain, antioxidants

Introduction:

Red beetroot (*Beta vulgaris*) is an important root crop in many countries worldwide, as it contains a range of bioactive compounds such as Betalains, phenolic compounds, and flavonoids, in addition to various vitamins and minerals. [1] Antioxidants are molecules that are important in preserving cells and

tissues from harm and in decreasing oxidative stress, which is associated with the development of many diseases such as heart disease, diabetes and cancer [2] The productivity and quality of the red beetroot crop are significantly influenced by the agricultural practices implemented in the field, especially fertilization. Chemical

fertilization significantly enhances plant growth and output; yet, its overuse results in detrimental consequences on soil and the environment, including soil fertility deterioration and groundwater contamination, as well as financial implications[3] Thus, in recent studies, efforts have been directed towards the search for alternative or complementary strategies to chemical fertilization such as the use of organic fertilisers that improve the physical, chemical and biological qualities of the soil [4].

Shading is one of the agricultural practices that can have an effect on the growth, yield, and quality of beetroots. This is because shading reduces the intensity of solar radiation and lowers the ambient temperature around the plant. This, in turn, helps to reduce environmental stress and improves certain physiological processes, such as photosynthesis and the efficiency with which water is used .This has a positive effect on the plant [5]. The application of plant growth regulators is also recognized as one of the contemporary approaches which aid to increase the plant performance under varied environmental situations , Salicylic acid is a crucial physiological component that regulates numerous essential activities in plants, including the enhancement of photosynthesis, stimulation of nutrient uptake, and augmentation of the plant's resilience to diverse environmental challenges .[6]

Therefore, this study aims to examine these factors in relation to the growth, productivity, and increased concentration of the active ingredient in red beets, with a focus on reducing reliance on chemical fertilizers through the use of organic fertilizers, with the goal of achieving a more sustainable agricultural system. The triple interaction of shading, organic fertilization, and salicylic acid has not been studied before in red beet under Iraqi conditions.

Materials and Methods:

The experiment was carried out in the fields of the Department of Horticulture and Landscape Architecture, College of Agriculture, University of Tikrit, during the winter season of 2024-2025. 10 plants in the experimental unit The experiment was conducted to determine the effects of green netting (Saran), fertilization (chemical and organic) and spraying with salicylic acid on the growth and active ingredient content of red beetroot. The red beetroot seeds were sown at proper spacing in the prepared soil for cultivation .The experiment was conducted in three replicates with a completely randomized block design (RCBD) with three variables: shade with green netting (no shading, shading for one month post-planting, shading for two months post-planting). The second factor involved the chemical and organic fertilization of red beetroots, utilizing NPK 20-20-20 compound fertilizer applied at the

recommended rate of 200 kg per hectare, equating to 2 kg for the experimental area of 67.5 m², resulting in an application of 37 g of fertilizer per experimental unit. The fertilizer was administered in two applications: the initial application occurred post-germination, followed by a second application one month later [7]. Organic fertilizer, namely palm waste, was sprayed at a rate of 20 tons per hectare before planting, with 200 kg of palm waste added to each experimental plot, equating to 3.7 kg per unit [8]. Fertilization treatments were administered, with one half utilizing chemical fertilizers and the other half employing organic fertilizers. The third variable was the application of salicylic acid (absence of spraying, spraying at 50 mg/L). Application was performed in three phases: the initial at the appearance of true leaves, the subsequent at the commencement of stem swelling, and the final at maturity, utilizing a 2-liter hand sprayer. Surfactant was utilized as a replacement for the dispersion to diminish the surface tension of water molecules [9].

Table (1) Some physical and chemical properties of soil

Attribute	Unity	Value
Soil texture	-	clay mixture
Sand	%	25
Silkenage		40
Clay		35
PH	----	6.7
Electrical conductivity	ds m-1	4.3

Ca ²⁺	mmol L-1	9
Mg ²⁺		9.8
Na ⁺		1.3
K ⁺		0.7
SO ₄ ⁻		23
Cl ⁻		17.3
HCO ₃ ⁻		2.3
Available nitrogen	mg kg-1	24.2
Available phosphorus		0.311
Available potassium		3.2
Lime	g kg-1	229.6
Organic matter		1.58

The subsequent characteristics were assessed:

1 **Total chlorophyll concentration in leaves (mg/100 g fresh weight):**

This was estimated using a spectrophotometer at two wavelengths (663 and 645 nm), and the total chlorophyll content (mg pigment per 100 g fresh leaf tissue) was calculated [10]

2 **Root circumference (cm):** The root circumference was measured by wrapping a string around the broadest section of the root and measuring the length of the thread with a ruler for each experimental unit and computing the average for five roots.

3 **The betalain content in the roots (mg/g fresh weight):** was quantified using the method of [11] with a UV spectrophotometer, measuring the absorption spectra at a wavelength of 540 nm.

4 **Total sugar content in roots (%):** The sugar content was

determined using the sulfuric acid–phenol method, and absorbance was measured in a microplate reader at a wavelength of 490 nm[12].

. 5DPPH Antioxidant Activity (%) : Antioxidant activity was

determined using the 2,2-diphenyl-1-picrylhydrazine (DPPH) radical scavenging assay as a detection method [13].

Table (2) Temperatures, Relative Humidity, Rainfall Rate, and Solar Radiation

Month	Maximum temperature (°C)	Minimum temperature (°C)	Temperatures	Relative humidity	Solar irradiance Mj/m ²	Rainfall rate
September	39.62	20.41	29.90	28.52	18.98	0.0
October	35.17	17.00	25.75	38.55	14.26	0.0
November	26.82	8.77	16.83	47.00	9.95	0.0

Results and Discussion :

.1Total chlorophyll in leaves: The statistical analysis results presented in Table 3 illustrate the impact of the experimental treatments on the total chlorophyll content in red beetroot plants. The treatment utilizing a spray concentration of 50 mg/L (S1) yielded a significant result, achieving the maximum measurement of 69.40 mg, in contrast to the control treatment (S0), which recorded the lowest measurement of 66.88 mg. The shade treatment notably influenced the chlorophyll content of the plant; the shading treatment 3N exhibited the highest quantity at 85.50 mg, whilst the control treatment N1 displayed the lowest content at 40.78 mg. The organic fertilization treatment F3 surpassed the chemical fertilization treatment F1, attaining a maximum level of 74.10 mg, whilst

F1 recorded the minimum content of 63.29 mg.

The results of the dual interaction between salicylic acid spraying and fertilization showed a significant difference in chlorophyll content, with the treatment S1F3 recording the highest content at 75.38 mg, while the treatment S0F1 recorded the lowest content at 61.98 mg. The results of the interaction between salicylic acid spraying and shading also showed a significant difference in chlorophyll content, with the treatment S1N3 recording the highest percentage at 87.29 mg, while the treatment S0N1 recorded the lowest percentage at 39.72 mg , The interaction between shading and fertilization revealed a notable disparity between the two treatments: the N3F3 treatment produced the highest content at 92.81 mg, whilst the N1F1 treatment resulted in the lowest content at

37.52 mg .Regarding the effect of the three-way interaction among the study treatments, there were significant differences between them. The S1F3N3 interaction treatment outperformed with the highest percentage of 94.45 mg, significantly different from the S0F1N1 treatment, which recorded the lowest percentage of 35.92 mg . In addition to its involvement in preventing chlorophyll degradation

and delaying the ageing process, salicylic acid is responsible for the high chlorophyll concentration. Salicylic acid also plays a part in enhancing the role of chloroplasts and stimulating the activity of enzymes involved in photosynthesis [8] Fertilizing is very important to supply the necessary nutrients for the development of chlorophyll and to boost the effectiveness of photosynthesis [14].

Table 3: The effects of shading, fertilization (chemical and organic), and spraying with salicylic acid, as well as their interactions, on total chlorophyll content (mg/100g fresh weight) in red beetroot plants.

Salicylic acid content (mg/100g fresh weight) in red beet root plants.					
Salicylic acid S	Fertilization F	Shading N			S × F
		N1	N2	N3	
S0	F1	35.92	70.73	79.30	61.98
	F2	40.52	76.34	80.62	65.83
	F3	42.72	84.57	91.18	72.82
S1 50 mg. L ⁻¹	F1	39.13	73.41	81.26	64.60
	F2	40.80	77.64	86.18	68.20
	F3	45.59	86.12	94.45	75.38
LSD0.05		0.8243			0.4759
S × N					Average S
S0		39.72	77.21	83.70	66.88
S1		41.84	79.06	87.29	69.40
LSD 0.05		0.4759			0.2748
N × F					Average F
F1		37.52	72.07	80.28	63.29
F2		40.66	79.99	83.40	67.02
F3		44.16	85.34	92.81	74.10
LSD 0.05		0.5829			0.3365
Average N		40.78	78.13	85.50	
LSD 0.05		0.3365			

. 2 Total root circumference (cm):

The results of the statistical analysis in Table 4 showed the effect of the study treatments on root

circumference in red beetroot plants. The treatment involving spraying with 50 mg/L salicylic acid (S1) was statistically superior and recorded

the highest root circumference of 21.26 cm compared to the control treatment S0, which recorded the lowest root circumference of 20.94 cm. The shading treatment also significantly affected root circumference, with treatment N3 recording the highest circumference of 24.97 cm and the control treatment N1 recording the lowest root circumference of 15.58 cm. The organic fertilization treatment F3 also outperformed the chemical fertilization treatment F1, achieving the highest root circumference of 22.59 cm compared to the chemical fertilization treatment F1, which achieved the lowest circumference of 19.47 cm. The results of the two-way interaction between salicylic acid spraying and fertilization showed a significant difference in root circumference, with treatment S1F3 recording the highest circumference of 22.41 cm, while treatment S0F1 recorded the lowest circumference of 18.91 cm. The results of the interaction between salicylic acid spraying and shading were significant in terms of root circumference, with treatment S1N3 recording the highest root

circumference of 25.31 cm, while treatment S0N1 recorded the lowest circumference of 15.73 cm. As for the interaction between shading and fertilization, a significant difference was observed between the two treatments: the N3F3 treatment outperformed the others, recording the largest root circumference of 25.92 cm, while the N1F1 treatment yielded the smallest root circumference of 12.52 cm. The investigation of the three-way interaction among the research variables indicated significant differences among them. The S1F3N3 treatment outperformed the others, yielding the largest root circumference of 26.25 cm, a statistically significant difference from the S0F1N1 treatment, which recorded the smallest root circumference of 12.06 cm. In addition to the role that salicylic acid has in regulating development and boosting physiological processes, the increase in root circumference can be linked to better vegetative growth, which is the result of higher cell division and elongation. This is due to the availability of nutrients that are a result of fertilization [15].

Table 4: The effects of shading, fertilization (chemical and organic), and salicylic acid spraying, as well as their interactions, on the root circumference (cm) of red beets

Salicylic acid S	Fertilization F	Shading N			S × F
		N1	N2	N3	
S0	F1	12.06	21.51	23.17	18.91
	F2	15.04	23.24	25.143	21.14
	F3	20.11	22.63	25.60	22.78
S1	F1	12.99	22.58	24.51	20.03
	F2	16.25	22.59	25.18	21.34
	F3	17.04	23.95	26.25	22.41
LSD 0.05		0.4973			0.2871
S × N					Average S
S0		15.73	22.46	24.64	20.94
S1		15.42	23.04	25.31	21.26
LSD 0.05		0.2871			
N × F					Average F
F1		12.52	22.04	25.16	19.47
F2		15.64	22.91	25.16	21.24
F3		18.58	23.29	25.92	22.59
LSD 0.05		0.3517			0.2030
Average N		15.58	22.75	24.97	
LSD 0.05		0.2030			

-3Total Dissolved Solids (TSS)

The statistical analysis results presented in Table 5 illustrate the influence of the study variables on the TSS content in red beetroot plants. The treatment with 50 mg/L salicylic acid (S1) significantly outperformed the control treatment (S0), recording the highest content at 7.483% compared to the control treatment (S0), which recorded the lowest content at 6.748%. The shading treatment had a significant effect on the plant's soluble sugars, with the 3N shading treatment recording the highest content at

8.720% and the control treatment N1 recording the lowest content at 5.536%. Similarly, the organic fertilization treatment F3 outperformed the chemical fertilization treatment F1, achieving the highest content of 8.410% compared to the chemical fertilization treatment F1, which achieved the lowest content of 5.617%. The outcomes of the two-way interaction between salicylic acid application and fertilization revealed a substantial disparity in soluble solids, with treatment S1F3 exhibiting the highest content at

8.479%, whereas treatment S0F1 had the lowest amount at 5.384%. The interaction between salicylic acid spraying and shade shown notable variations in total soluble sugars, with treatment S1N3 exhibiting the highest percentage at 9.214%, whereas treatment S0N1 displayed the lowest percentage at 5.195% .As for the interaction between shading and fertilization, a significant difference was observed between them, with treatment N3F3 yielding the highest content at 10.669%, while treatment N1F1 yielded the lowest content at 4.462%. The results of the three-way interaction among the study treatments indicated significant differences among them, with the S1F3N3 interaction treatment yielding the highest percentage of 10.830%, a significant difference from the S0F1N1 treatment, which recorded the lowest percentage of 4.122% .The increase in soluble

solids is due to better carbohydrate accumulation due to increased photosynthetic efficiency as shading decreases light and heat stress thus improving light use efficiency and promoting sugar production [16]. Fertilization also promotes increased carbohydrate metabolism and storage in the roots.

The increase in TSS is attributed to the increased accumulation and translocation of photosynthetic products to the roots, particularly soluble sugars. Amino acids, organic acids, and other soluble compounds also contribute to raising TSS values. Furthermore, improved nutritional status is a result of organic fertilization and spraying with salicylic acid. TSS values are an important indicator of crop quality, as they are associated with increased concentrations of active compounds[4].

Table 5: The effect of shading, fertilization (chemical and organic), and salicylic acid spraying, as well as their interactions, on total Melting solids (TSS) in the roots (%) of red beets.

Salicylic acid S	Fertilization F	Shading N			Average S × F
		N1	N2	N3	
S0	F1	4.122	5.656	6.373	5.384
	F2	5.094	6.668	7.796	6.520
	F3	6.369	8.148	10.508	8.342
S1	F1	4.802	5.871	6.880	5.851
	F2	6.477	7.951	9.932	8.120
	F3	6.350	8.256	10.830	8.479
LSD 0.05		0.2655			0.1533
S × N					Average S
S0		5.195	6.824	8.226	6.748

S1	5.876	7.360	9.214	7.483
LSD 0.05	0.1533			0.0885
N × F				Average N
F1	4.462	5.764	6.626	5.617
F2	5.786	7.310	8.864	7.320
F3	6.359	8.202	10.669	8.410
LSD 0.05	0.1877			0.1084
Average N	5.536	7.092	8.720	
LSD 0.05	0.1084			

-4DPPH Antioxidants: The results of the statistical analysis in Table 6 illustrate the effect of the study variables on the DPPH antioxidant content (%) in red beetroot. The treatment with 50 mg/L salicylic acid (S1) greatly surpassed the control treatment (S0), achieving the maximum percentage at 43.78%, while the control treatment (S0) recorded the lowest percentage at 41.79%. The shade treatment significantly influenced the antioxidant content of the plants, with the 3N shading treatment exhibiting the maximum concentration at 55.00%, whilst the control treatment N1 displayed the lowest content at 29.43%. The organic fertilization treatment F3 surpassed the chemical fertilization treatment F1, attaining a maximum percentage of 47.26%, while F1 recorded the minimum percentage of 38.30%. The results of the two-way interaction between salicylic acid application and fertilization revealed a considerable disparity in antioxidant content, with treatment S1F3 exhibiting the maximum level

at 48.88%, whereas treatment S0F1 had the lowest level at 39.57%. The interaction results between salicylic acid application and shading revealed a notable disparity in antioxidant content, with treatment S1N3 achieving the maximum percentage at 56.74%, whereas treatment S0N1 exhibited the lowest percentage at 39.57%. The interaction between shading and fertilization revealed a notable disparity, with treatment N3F3 exhibiting the highest content at 58.60%, whereas treatment N1F1 had the lowest content at 21.06%. The three-way interaction among the study treatments exhibited significant differences, with the S1F3N3 interaction treatment achieving the highest percentage of 60.82%, markedly surpassing the S0F1N1 treatment, which recorded the lowest percentage of 20.88%. The rise in DPPH antioxidants is due to the higher amount of active chemicals such as betalains and phenolics that are significant in the inhibition of free radicals and reduction of oxidative stress [17].

This effect is due to the interplay especially in shadowing and high between the analyzed parameters, fertilization level.

Table (6) Effect of shading, fertilization (chemical, organic), and salicylic acid spraying, and their interaction on DPPH antioxidant content (%) in the roots of red beet plants.

Salicylic acid S	Fertilization F	Shading N			S × F
		N1	N2	N3	
S0	F1	20.88	42.37	52.47	38.57
	F2	31.22	41.37	50.92	41.17
	F3	34.79	45.75	56.38	45.64
S1	F1	21.25	40.12	52.71	38.02
	F2	32.75	43.85	56.70	44.43
	F3	35.73	50.08	60.82	48.88
LSD 0.05		0.3885			0.2243
S × N					Average S
S0		28.96	43.16	53.26	41.79
S1		29.91	44.68	56.74	43.78
LSD 0.05		0.2243			0.1295
N × F					Average F
N1		21.06	41.24	52.59	38.30
N2		31.98	42.61	53.81	42.80
N3		35.26	47.91	58.60	47.26
LSD 0.05		0.2747			0.1586
Average N		29.43	43.92	55.00	
LSD 0.05		0.1586			

-5 Betalain content in roots: The results of the statistical analysis in Table 7 confirmed the effect of the study factors on the Betalain content in red beetroot plants. The treatment with salicylic acid spray at 50 mg/L (S1) significantly surpassed the control treatment S0, achieving the highest percentage of 16.104 mg. g-1, while S0 recorded the lowest at 15.024 mg. g-1. The shading treatment notably influenced the chlorophyll content, with the 3N shading treatment yielding the highest content of 18.372 mg. g-1,

whereas the control treatment N1 exhibited the lowest at 12.412 mg. g-1. Additionally, the organic fertilization treatment F3 excelled with a percentage of 16.571 mg. g-1, in contrast to the chemical fertilization treatment F1, which attained the lowest percentage of 14.452 mg. g-1. The two-way interaction between salicylic acid application and fertilization revealed a significant disparity in betaine content, with the S1F3 treatment yielding the highest level of 17.249 mg/g, whereas the S0F1 treatment

exhibited the lowest at 13.949 mg/g. Furthermore, the interaction between salicylic acid application and shading significantly influenced betaine content, with the S1N3 treatment recording the highest percentage of 18.807 mg/g, while the S0N1 treatment showed the lowest at 11.792 mg/g. Additionally, the interaction between shading and fertilization demonstrated a significant difference, with the N3F3 treatment achieving the highest content of 19.053 mg/g, while the N1F1 treatment resulted in the lowest content of 11.206 mg/g. The three-way interaction among the study treatments was significant, with the S1F3N3 interaction

yielding the greatest value of 19.576 mg g⁻¹, significantly differing from the S0F1N1 treatment, which recorded the lowest value of 10.670 mg m⁻³. According to [18], the rise in Betalain content is indicative of the stimulation of secondary metabolite compounds. This stimulation is connected to the role that salicylic acid plays in activating the biosynthesis pathways of phenolic compounds and plant colors. Furthermore, organic fertilization creates a vital environment in the soil, which has a favorable impact on the compounds that are being taken into consideration. [18].

Table 7: Effect of shading, fertilization (chemical and organic), and salicylic acid spraying, as well as their interactions, on the Betalain content in the roots (mg/g fresh weight) of red beetroot

Salicylic acid S	Fertilization F	Shading N			F × S
		N1	N2	N3	
S0 Without spray	F1	10.670	13.797	17.379	13.949
	F2	11.904	15.885	17.899	15.230
	F3	12.802	16.345	18.531	15.893
S1 Spray (50 mg. L ⁻¹)	F1	11.742	15.217	17.921	14.960
	F2	12.706	16.679	18.926	16.104
	F3	14.646	17.524	19.576	17.249
LSD 0.05		0.1906			0.1100
S × N					Average S
S0		11.792	15.342	17.936	15.024
S1		13.031	16.473	18.807	16.104
LSD 0.05		0.1100			0.0635
N × F					Average F
F1		11.206	14.507	17.650	14.454
F2		12.305	16.282	18.412	15.667
F3		13.724	16.934	19.053	16.571
LSD 0.05		0.1348			0.0778
Average N		12.412	15.908	18.372	
LSD 0.05		0.0778			

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