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Olive leaf extract application enhances wheat productivity through improving growth, anatomical traits, and source-sink relationships

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Abstract: Wheat (*Triticum aestivum* L.) is the most important food crop for human consumption, underlining the need for increased production to satisfy rising population demands. This study was conducted to evaluate the effect of olive leaf extract (OLE) as an organic biostimulant on the growth and productivity of wheat plant, focusing on physio-biochemical and anatomical traits during the 2019/20 and 2020/21 seasons. OLE was applied at three concentrations (0.5, 1.0, and 2.0%), along with distilled water as a control, first as a grain soak and then as a foliar spray on 45-day-old plants. Results indicated that all OLE concentrations significantly enhanced root and shoot growth parameters, except for root/shoot and leaf area ratios, which decreased. OLE treatments also increased photosynthetic pigments in the flag leaf, as well as NPK, crude protein, and total carbohydrates in shoots compared to untreated plants. Anatomically, OLE treatments positively enhanced different studied anatomical features of the main stem and the flag leaf. The most important enhancements were the increased cross-sectional area of vascular bundles and the mesophyll tissue. These physio-anatomical alterations are consistent with the higher grain yield and quality. Among the treatments, 2.0% OLE proved most effective in both seasons. The study recommends using 2.0% OLE as an organic biostimulant to improve wheat growth and productivity while reducing reliance on synthetic growth regulators and inorganic fertilizers.

Keywords: OLE, growth, physiology, anatomy, wheat

1.Introduction

Wheat (Triticum aestivum L.) is the most vital food crop for human consumption, making the improvement of wheat production essential to meet the demands of a growing population. The diverse qualities and uses of wheat grains underscore their significance as a staple food for most of humanity (Irada & Samira, 2010). In Egypt, local wheat production and consumption are vastly imbalanced. Efforts to achieve self-sufficiency focus on increasing grain yield in line with ensuring food safety, reducing production costs, and adopting eco-friendly strategies. Recently, there has been a rising interest in using plant extracts as biostimulants to reduce reliance on synthetic growth regulators and inorganic fertilizers, thereby promoting eco-friendly, sustainable practices (Desoky *et al*., 2019; Wanas & Khamis, 2021; Wanas & Bazeed, 2023; Motawea, 2024; Wanas & Shabka, 2025).

Olive (Olea europaea L.) leaves are a valuable source of bioactive compounds, particularly phenols, with oleuropein being the most prevalent. Oleuropein, mainly composed of oleanolic acid and hydroxytyrosol, derives its biological activities from the hydroxytyrosol moiety, a potent catechol group (Kourti *et al*., 2024). Other phenolic compounds in olive leaves include tyrosol, caffeic acid, gallic acid, syringic acid, coumaric acid, and luteolin (Korukluoğlu *et al*., 2004). They also contain phytohormones such as GA3, GA4, and zeatin, along with nutrients like Ca and Fe (Ulgar *et al*., 2004). Olive leaves have traditionally been used as a remedy against various diseases, and olive leaf extract exhibits antibacterial, anti-HIV, vasodilator, and hypoglycemic qualities (De Leonardis *et al*., 2008; Fares *et al*., 2011) and

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antioxidant properties (Bouaziz *et al*., 2008). The demand for olive leaves has increased recently for use as food additives and functional food ingredients, either as liquid extracts or powders. Despite their well-documented medicinal and nutritional benefits, to our knowledge, the use of OLE for improving plant growth and productivity is still limited. Motawea (2024) reported that OLE at 2 g L^{-1} effectively improved the growth, physiological performance, and yield characteristics of faba bean plants under both normal and salt stress conditions, owing to its antioxidant and growth-promoting properties. The aim of this study was to assess the effects of OLE on the growth, physio-biochemical, anatomical, and yield characteristics of wheat plants.

2. Materials and Methods

In today's modern agricultural systems, which are called eco-friendly agri-technology or green technology, natural supplements and substitutes have been utilized to improve plant performance and minimize the contamination of edible plant parts. At the Experimental Station of the Faculty of Agriculture, Damietta University, Damietta (31° 25 56 N, 31° 48 09 E), Egypt, two separate pot experiments were performed over the seasons of 2019/20 and 2020/21. The wheat cultivar Misr1 (Triticum aestivum L.) was used as a botanical material in this study. Wheat grains were purchased from the Directorate of Agriculture in Damietta, Egypt.

2.1. Experimental design

This study examined the effects of olive leaf extract (OLE) at concentrations of 0.5%, 1.0%, and 2.0% on wheat growth, physio-biochemical, anatomical, and yield parameters. The experiment included four treatments: distilled water (T1, control), 0.5% OLE (T2), 1.0% OLE (T3), and 2.0% OLE (T4). The assigned concentrations of OLE and distilled water were applied as grain-soaking materials for 8 hours and later as foliar sprays on 45-day-old plants. Pre-planting treated grains were planted on November 20th in 30 cm diameter pots filled with a 10 kg mixture of clay and sand (1:1 v/v) during the 2019/20 and 2020/21 seasons. Three weeks after planting, seedlings were thinned to one seedling per pot. The experimental design was a randomized complete-block design with four replicates, each containing six pots per treatment. Tap water irrigation and other recommended agricultural practices for wheat cultivation were applied across both seasons.

2.2. Preparation of olive leaf extract (OLE)

Olive (Olea europaea L.) leaves were collected from the olive trees cv. Picual located in Kalabsho, Dakahlia, Egypt, in the middle of November. Stock olive leaf extract was made using the method described by Bisher (2014) with some modifications. The leaf samples were cleaned and air-dried at a laboratory temperature for ten days before being ground into a fine powder and extracted with distilled water. 200 g of the dried leaf powder were macerated with 400 ml of distilled water at 40 °C for 24 h. After that, Buchner funnel suppression was used to filter the extract. It required a few repetitions of this procedure using distilled water to obtain the proper amount. Then, the crude extract (200 g L^{-1} or 20%) was stored in the refrigerator at 5°C until the preparation of the concentrations utilized in this investigation, namely 0.5%, 1.0%, and 2.0%. Ulger *et al.* (2004) determined the concentrations of sugars, mineral nutrients, and phytohormones in "Memecik" as summarized in Table 1.

Table 1: Concentrations of total sugars, nutrients, and phytohormones in 'Memecik' olive leaves (Ulger *et al*., 2004)

Total sugars (mg g^{-1} DW)					40.18			
Mineral nutrients								
mg g^{-1} DW					µg g^{-1} DW			
N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
10.40	1.10	5.90	20.60	1.70	93.77	33.82	21.61	29.13
Phytohormones (µg g^{-1} DW)								
IAA		GA_3	GA_4		Zeatin		ABA	
1.29		3.27	33.38		11.76		0.31	

2.3. Sampling and collecting data

2.3.1. Vegetative growth parameters

At 100 days after planting (DAP), eight plants were randomly selected from each treatment in both seasons to measure growth characteristics, including root size (cm^3), root dry weight (g), plant height (cm), number of tillers, shoot dry weight (g), and number of leaves per plant.

To accurately measure root system size, the plant pot is tilted while a gentle stream of water is carefully poured over the soil surface until completely eliminated from the roots. The roots were dried with paper towels, submerged in a known volume of water in a volumetric flask, and the increase in water volume was recorded to determine root system size (Wanas, 1996). The disc method of Waidyanatha & Goonasekera (1975) was used to measure total leaf area (cm^2) plant-1. The data of the plant dry matter and the total leaf area were used for computing the following growth indices:

a) **Root/shoot ratio**: It compares the dry matter accumulation in roots to that in shoots, as follows:

$$Root\ Shoot\ ratio = \frac{Root\ dry\ weight Plant^{-1}}{Shoot\ dry\ weight Plant^{-1}}$$

b) Leaf area ratio (LAR) plant^{-1}: It indicates the leaf area (cm2) needed to produce one gram of plant dry biomass. It was calculated using the formula of Radford (1967):

$$LAR\ (cm^2\ g^{-1}) = \frac{Total\ leaf\ area\ (cm^2)\ Plant^{-1}}{Plant\ dry\ weight}$$

2.3.2. Photosynthetic pigments

The concentrations of chlorophylls "a" & "b" and carotenoids were determined in the flag leaf at 100 DAP in both seasons. The method involved extracting the pigments using dimethylformamide (DMF) and then measuring their optical densities with a spectrophotometer at 664, 647, and 480 nm according to Wellburn (1994). The concentrations were presented as mg g^{-1} fresh weight (FW).

2.3.3. Anatomical study

Based on the observed differences in the morphological measurements of wheat plants due to OLE treatments in the first season, a comparative anatomical study using microscopy was conducted on the main stem and flag leaf of treated plants compared to the control during the second season. At 100 DAP, 1 cm long specimens were collected from the middle portion of the flag leaf blade and the terminal internode of the main stem. These were immediately fixed in FAA (10 ml formalin, 5 ml glacial acetic acid, 85 ml 70% ethyl alcohol) for at least 48 hours. After rinsing with 50% ethyl alcohol, the specimens were dehydrated in a butyl alcohol series and embedded in paraffin wax with a melting point of 56–58°C. Sections were cut at 20 µ thickness using a rotary microtome, double-stained with crystal violet-erythrosine, clarified in xylene, and mounted in Canada balsam (Nassar & El-Sahhar, 1998). The sections were then examined under a microscope to record measurements and counts using a micrometer eyepiece.

2.3.4. Chemical analysis of shoots and grains

Samples of shoots at 100 DAP and of mature grains at harvest date (165 DAP) were taken from 4 replicates per treatment. These samples were dried at 70°C until constant weight was achieved, then ground into a fine powder, and stored in paper sachets at 25°C. The concentrations of total nitrogen, phosphorus, potassium, and carbohydrates were determined using methodologies outlined by Horneck & Miller (1998), Jackson (1973), Horneck & Hanson (1998), Dubois *et al*. (1956), Crude protein content was calculated by multiplying the total nitrogen concentration in shoots and grains by a conversion factor of 5.75 as recommended by A.O.A.C. (2005).

2.3.5. Yield characteristics

Eight plants from each treatment were randomly taken at the harvest time to record the number of spikes plant-١, main spike length, grain number and weight of the main spike, grain^{-100} weight (g), total grain yield (g) plant-1 and straw yield (g) plant-١. The relative grain yield of treated plants was calculated as a percentage of the control yield.

2.4. Statistical analyses

Morphological, chemical, and yield data were analyzed using one-way ANOVA for a randomized complete block design in the IBM SPSS Statistics program (version 29.0.1.0). The least significant difference (LSD) test ($P \leq 0.05$) was used to compare treatment means with the control control, following Snedecor & Cochran (1989).

3. Results

3.1. Growth characteristics

Table 2 demonstrates that key growth parameters of wheat plants, including root system size, plant height, root and shoot dry weights, number of tillers and leaves plant^{-1}, and total plant leaf area, were significantly increased with all applied OLE treatments. In contrast, OLE treatments significantly reduced both the root/shoot ratio and leaf area ratio (LAR) compared to the control during both growing seasons. The magnitude of these changes was proportional to the OLE concentration, with 2.0% OLE being the most effective treatment.

3.2. Photosynthetic pigments

Table 3 presents the mean values of chlorophyll a, b, and carotenoids, along with their sum and percentage change relative to control values. At 100 DAP, the three OLE concentrations significantly enhanced photosynthetic pigments in the flag leaves of treated wheat plants compared to untreated ones. The increase in chlorophyll a, b, carotenoids, and their sum was proportional to the applied OLE concentrations in both seasons. The 2.0% OLE treatment showed the highest values for these pigments and their sum, with percentage increases of 143.54, 103.68, and 128.80% in the first season and 113.89, 102.63, and 119.88% in the second season for chlorophyll a, b, and carotenoids, respectively.

3.3. Chemical composition of wheat shoots

According to Table 4, the three applied concentrations of OLE significantly enhanced the levels of N, P, K, crude protein, and total carbohydrates in wheat shoots during both seasons compared to the control. The highest increases were consistently achieved with the 2.0% OLE treatment. In the first season, the percentage increases for N, P, K, crude protein, and total carbohydrates with 2.0% OLE were 32.43%, 109.09%, 36.15%, 32.34%, and 39.09%, respectively. In the second season, these increases were 31.58%, 50.00%, 26.09%, 31.58%, and 44.42%.

Table 2: Effect of OLE on certain growth parameters of wheat plants at 100 DAP during 2019/20 and 2020/21 seasons.

Parameters / Treatments		Roots size plant^{-1} (cm^3)	Roots DW (g) plant^{-1}	Plant height (cm)	Tillers No plant^{-1}	Leaves No. plant^{-1}	Shoots DW (g) plant^{-1}	Root / Shoot ratio	Total DW plant^{-1}	Total leaf area (cm^2) plant^{-1}	LAR cm^2 g^{-1} DW
Season 2019/2020											
Control		44.50	6.88	77.75	4.25	29.00	29.90	0.23	36.78	828.30	22.52
OLE	0.5 %	49.80	8.43	91.50	6.25	35.25	43.55	0.20	51.95	1040.04	20.02
	1.0 %	53.40	8.94	98.13	6.75	36.50	47.05	0.19	55.99	1077.80	19.25
	2.0 %	60.88	9.51	100.63.	7.75	40.13	55.97	0.17	65.48	1183. 74	18.08
LSD at 0.05		2.23	0.39	2.78	0.56	1.63	1.18	0.01	1.36	56.36	0.48
Season 2020/2021											
Control		39.65	5.35	71.25	4.00	26.٧5	26.73	0.21	32.08	786.56	18.86
OLE	0.5 %	45.50	7.38	86.50	6.25	32.50	40.87	0.18	48.25	868.20	17.99
	1.0 %	49.75	7.75	90.00	6.50	35.00	45.15	0.17	52.90	916.22	17.31
	2.0 %	53.63	8.72	94. 25	8.00	38.75	54.65	0.16	63.37	1006.21	15.88
LSD at 0.05		1.47	0.21	1.87	0.48	1.18	0.68	0.01	1.06	42.16	0.39

Abbreviations: OLE= Olive leaf extract, DAP= Days after planting, No. = Number, LAR= Leaf area ratio, DW= Dry weight.

Table 3: Effect of OLE on photosynthetic pigment concentrations (mg g-1 FW) in wheat flag leaves at 100 DAP during 2019/20 and 2020/21seasons.

Pigments / Treatments		Chlorophyll				Carotenoids		Total determined pigments	
		a		b					
		$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Season 2019/20									
Control		2.71	0.00	1.63	0.00	1.84	0.00	6.18	0.00
OLE	0.5 %	4.98	+83.76	2.33	+42.94	2.82	+53.26	10.13	+63.92
	1.0 %	5.63	+107.75	2.80	+71.78	3.25	+76.63	11.68	+89.00
	2.0 %	6.60	+143.54	3.32	+103.68	4.21	+128.80	14.13	+128.64
LSD at 0.05		0.30	-	0.18	-	0.23	-	0.51	-
Season 2020/21									
Control		2.٥2	0.00	1.52	0.00	1.71	0.00	5.75	0.00
OLE	0.5 %	3.75	+48.81	1.88	+23.68	2.63	+53.80	8.26	+43.65
	1.0 %	3.99	+58.33	2..2	+44.74	2.90	+69.59	8.91	+54.96
	2.0 %	5.39	+113.89	3.08	+102.63	3.76	+119.88	12.23	+112.70
LSD at 0.05		0.25	-	0.15	-	0.30	-	0.43	-

Abbreviations: OLE = Olive leaf extract, DAP = Days after planting, FW= Fresh weight, ± % = ± % relative to the control

Table 4: Effect of OLE on the concentration of NPK and crude protein and total carbohydrates (mg g^{-1} DW) in wheat shoots at 100 DAP during 2019/20 and 2020/21 seasons.

Estimations / Treatments		N		P		K		Crude protein		Total Carbs.	
		$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Season 2019/20											
Control		27.60	0.00	1.10	0.00	٢٨.44	0.00	158.70	0.00	378.17	0.00
OLE	0.5 %	32.64	+18.26	1.60	+45.45	32.66	+14.84	187.68	+18.26	440.32	+16.43
	1.0 %	36.04	+30.58	2.20	+100.0	٣6.36	+27.84	207.23	+30.58	458.20	+21.16
	2.0 %	36.55	+32.43	2.30	+109.0 9	38.72	+36.15	210.16	+32.43	526.01	+39.09
LSD at 0.05		0.60	-	0.1	-	0.33	-	1.79	-	4.20	-
Season 2020/21											
Control		26.60	0.00	1.00	0.00	26.52	0.00	152.95	0.00	359.14	0.00
OLE	0.5 %	31.40	+18.05	1.30	+30.00	30.84	+16.29	180.55	+18.05	446.95	+24.45
	1.0 %	35.00	+31.58	1.50	+50.00	32.07	+20.93	201.25	+31.58	433.05	+20.58
	2.0 %	35.00	+31.58	1.50	+50.00	33.44	+26.09	201.25	+31.58	518.67	+44.42
LSD at 0.05		0.68	-	0.09	-	0.28	-	2.13	-	5.15	-

Abbreviations: OLE = Olive leaf extract, DAP = Days after planting, DW= Dry weight, Carbs. = Carbohydrates, ± % = ± % relative to the control

3.4. Anatomical studies

3.4.1. Anatomy of the main stem

As shown in Table 5, the three applied treatments of OLE positively influenced various anatomical features of the main stem terminal internode. OLE at 0.5%, 1.0%, and 2.0% increased the stem diameter by 25.43%, 27.44%, and 24.98%, respectively. The hollow stem diameter and the stem wall thickness both increased, contributing to the overall stem diameter enhancement. For instance, stem wall thickness rose by 20.80%, 26.91%, and 47.17% compared to the control. The results indicate that OLE was most effective at 2.0% (Figure 1). Additionally, the stem wall thickness increased alongside the thickness of its constituent tissues, including the epidermis, chlorenchyma tissue beneath the epidermis, peripheral sclerenchyma, and parenchymatous ground tissue. The number and dimensions of vascular bundles also increased, with the thickness of xylem and phloem tissues contributing to the increased vascular bundle thickness.

3.4.2. Anatomy of the flag leaf blade

As shown in Table 6, the thickness of the midvein was increased over the control value by 6.44, 21.31, and 15.83% with OLE treatments of 0.5, 1.0, and 2.0%, respectively. This increase in the midvein thickness was correlated with increases in the thickness of the uppermost and lowermost sclerenchyma tissues and the main vascular bundle. The main vascular bundle reached its maximum thickness (130.93 µm, 123.58% of the control) with the 2.0% OLE treatment (Figure 2), which also produced the widest bundle (136.91% of the control). The vascular bundle's increased thickness was primarily due to increased thickness of the bundle's phloem, xylem, and sheath. Additionally, the lamina thickness was also increased with all applied OLE treatments and reached its highest percentage increase with 2.0% OLE (26.40% over the control value). This increase was mainly due to thicker upper and lower epidermises and mesophyll tissue, with mesophyll tissue showing the greatest percentage increase (26.25% over the control) at the 2.0% OLE concentration.

3.5. Yield characteristics

The results in Table 7 indicate that OLE application at concentrations of 0.5, 1.0, and 2.0% significantly enhanced total grain yield per plant compared to the control by 27.78, 42.31, and 155.13% in the first season and by 36.56, 51.65, and 147.64% in the second season. This improvement was accompanied by increases in the number of spikes plant-1, spike length, grain number and weight spike-1, and overall grain weight. Additionally, straw yield per plant increased significantly in both seasons, primarily due to higher tiller numbers and shoot dry weight (Table 2).

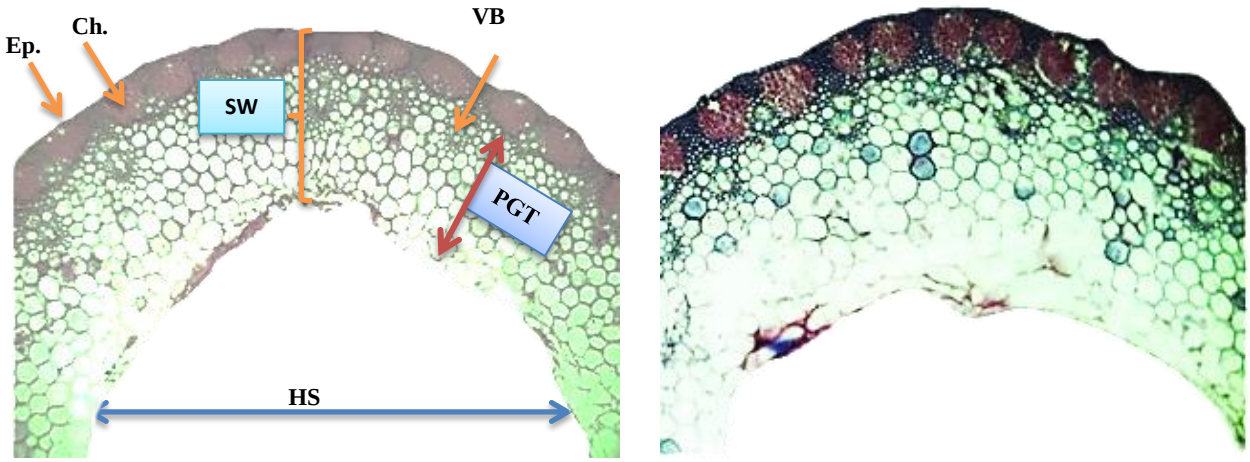
3.6. Grain chemical components

Table 8 indicates that OLE treatments at 0.5%, 1.0%, and 2.0% significantly enhanced the concentrations of N, P, K, crude protein, and total carbohydrates in wheat grains compared to untreated plants across both growing seasons. The increases aligned with the applied OLE concentrations, with the highest percentage gains observed at 2.0% OLE: 25.46% (N), 68.42% (P), 27.51% (K), 25.46% (crude protein), and 20.10% (total carbohydrates) in the first season, with similar trends in the second season.

Table 5: Effect of OLE on the anatomical features of the main stem terminal internode of wheat at 100 DAP during the 2020/21 season.

Treatments / Measurements & counts	Control	OLE					
		0.5 %		1.0 %		2.0 %	
	$\bar{X}$	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Diameter of whole section	2234.79	2763.72	+23.67	2843.54	+27.24	2981.55	+33.42
Diameter of hollow stem	1384.95	1737.14	+25.43	1765.00	+27.44	1730.87	+24.98
Thick. of stem wall	424.92	513.29	+20.80	539.27	+26.91	625.34	+47.17
Thick. of epidermis	12.84	13.61	+6.00	14.66	+14.17	16.23	+26.40
Thick. of ChBE	78.47	87.59	+11.62	90.48	+15.31	113.86	+45.10
Thick. of PS	11.93	14.82	+24.22	15.13	+26.82	16.00	+34.12
Thick. of PGT	321.68	415.27	+29.09	419.00	+30.25	479.25	+48.98
No of VB	49.00	52.00	+6.12	51.50	+5.10	58.00	+18.37
Thick. of the largest VB	146.20	158.93	+8.71	159.85	+9.34	165.21	+13.00
Width of the largest VB	112.88	114.16	+1.13	115.36	+2.20	116.12	+2.87
Thick. of bundle sheath	12.77	14.18	+11.04	15.02	+17.62	15.66	+22.63
Thick .of phloem tissue	43.15	46.01	+6.63	46.90	+8.69	48.06	+11.38
Thick .of xylem tissue	77.51	84.56	+9.10	82.91	+6.97	85.83	+10.73

Abbreviations: OLE = Olive leaf extract, DAP = Days after planting, Thick. = Thickness, No = Number, ChBE = Chlorenchyma beneath the epidermis, PS= Peripheral sclerenchyma, PGT= Parenchymatous ground tissue, VB= Vascular bundle, ±% = ±% Relative to the control values.



Control **OLE at 2.0%**

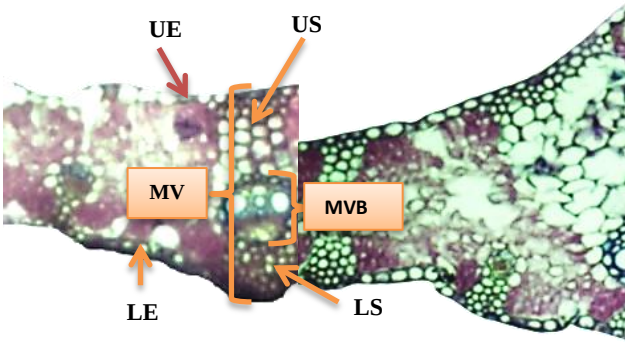
Figure 1: Transverse sections (100 X) through the middle part of the main stem terminal internode of wheat plant.

Abbreviations: Ep. = Epidermis, Ch. = Chlorenchyma, HS = Hollow stem = PGT= Parenchymatous ground tissue, VB = Vascular bundle.

Table 6: Effect of OLE on the anatomical features of the wheat flag leaf at 100 DAP during the 2020/21 season

Treatments / Measurements & counts	Control	OLE					
		0.5%		1.0%		2.0%	
	$\bar{X}$	$\bar{X}$	±%	$\bar{X}$	±%	$\bar{X}$	±%
Thick. of mid-vein	298.93	328.36	+9.84	350.66	+17.31	443.65	+48.41
Thick .of US	116.78	128.82	+10.31	147.07	+25.94	217.07	+85.88
Thick. of LS	47.90	52.67	+9.96	49.19	+2.69	59.15	+23.49
Thick. of the main VB	105.59	116.29	+10.13	120.63	+14.24	130.93	+23.58
Width of the main VB	106.07	118.92	+12.11	120.73	+13.82	145.22	+36.91
Thick. of bundle sheath	10.83	11.25	+3.88	11.93	+10.16	12.80	+18.19
Thick. of phloem tissue	35.42	37.52	+5.93	36.52	+3.11	40.04	+13.04
Thick. of xylem tissue	48.51	56.27	+16.00	60.25	+24.20	65.29	+34.59
Thick. of lamina	218.97	230.00	+5.04	235.59	+7.59	276.77	+26.40
Thick. of upper epidermis	15.65	16.12	+3.00	17.00	+8.63	18.66	+19.23
Thick .of lower epidermis	13.01	13.88	+6.69	16.77	+28.90	17.84	+37.13
Thick .of mesophyll tissue	190.31	200.00	+10.01	201.82	+6.05	240.27	+26.25

Abbreviations: OLE = Olive leaf extract, DAP = Days after planting, Thick. = Thickness, No = Number, US = Uppermost sclerenchyma, LS = Lowermost sclerenchyma, VB = Vascular bundle, ±% = ±% relative to the control values.



Control **OLE at 2.0%**

Figure 2: Transverse sections (150 X) through the middle part of the wheat flag leaf blade.

Abbreviations: MV= Mid-vein, MVB= Main vascular bundle, LP = Lower epidermis, UE = Upper epidermis, US = Uppermost sclerenchyma, LS = Lowermost sclerenchyma.

Table 7: Yield characteristics of wheat plants as affected by OLE treatments during 2019/20 and 2020/21 seasons.

Parameters / Treatments		N0.of spikes Plant^{-1}	Length of main spike (cm)	No .of grains main spike^{-1}	Grain weight (g) main spike^{-1}	Grain yield (g) plant^{-1}	Straw yield (g) plant^{-1}	1000-grain weight (g)	*Relative grain yield (%)
Season 201920									
Control		3.75	14.66	34.00	1.64	4.68	7.90	50.33	100.00
OLE	0.5%	5.50	15.40	36.63	1.99	5.98	13.23	66.24	127.78
	1.0 %	6.00	16.10	37.50	2.04	6.66	13.68	71.05	142.31
	2.0 %	8.37	16.30	50.75	2.42	11.94	19.59	70.66	255.13
LSD at 0.05		0.72	0.21	1.12	0.18	0. 69	1.33	0.75	-
Season 2020/21									
Control		3.50	14.50	33.25	1.47	4.24	8.50	50.26	100.00
OLE	0.5%	5.25	15.25	35.50	1.79	5.79	13.02	60.10	136.56
	1.0 %	6.00	16.58	37.13	1.95	6.43	13.45	62.95	151.65
	2.0 %	8.13	15.90	48.25	2.25	10.50	19.40	68.50	247.64
LSD at 0.05		0.61	0.27	1.32	0.15	0.71	1.18	1.08	-

Abbreviations: OLE = Olive leaf extract, DAP = Days after planting.
*Relative grain yield was calculated as a percentage of the control grain yield.

Table 8: Effect of OLE on some chemical constituents (mg g-1 DW) in wheat grains during the 2019/20 and 2020/21 seasons.

Determinations / Treatments		N		P		K		Crude protein		Total Carbs.	
		X̄	±%	X̄	±%	X̄	±%	X̄	±%	X̄	±%
Season 2019/20											
Control		32.60	0.00	1.90	0.00	38.10	0.00	187.45	0.00	579.96	0.00
OLE	0.5%	36.28	+11.29	2.50	+31.56	41.10	+7.87	208.61	+11.29	612.98	+5.69
	1.0 %	40.31	+23.65	3.10	+63.16	46.48	+21.99	231.78	+23.65	653.84	+12.74
	2.0 %	40.90	+25.46	3.20	+68.42	48.58	+27.51	235.18	+25.46	696.56	+20.10
LSD at 0.05		1.15	-	0.18	-	0.36	-	4.20	-	13.32	-
Season 2020/21											
Control		31.60	0.00	1.55	0.00	36.8٠	0.00	181.70	0.00	568.93	0.00
OLE	0.5%	35.05	+10.92	2.10	+35.48	38.89	+5.68	201.54	+10.92	602.56	+5.91
	1.0 %	40.20	+27.29	2.40	+54.84	42.37	+15.14	231.15	+27.22	650.42	+14.32
	2.0 %	40.75	+28.92	2.40	+54.84	43.99	+19.54	234.31	+28.95	685.28	+20.45
LSD at 0.05		1.37	-	0.20	-	0.42	-	4.56	-	11.18	-

Abbreviations: OLE = Olive leaf extract, DW+ Dry weight, Carbs. = Carbohydrates, ± % = ± % relative to the control values.

4. Discussion

The present investigation revealed that OLE treatments significantly enhanced various growth parameters in wheat plants. Notably, these treatments increased the dry weights of both roots and shoots while reducing the root/shoot ratio, indicating more assimilates are being allocated to tiller production, which contributed to a greater number of leaves and total leaf area plant^{-1} (Table 2), higher formed spike numbers, and improved grain and straw yields (Table 7). Additionally, OLE treatments reduced the leaf area required to produce one gram of plant dry biomass (LAR), which, accompanied by increased photosynthetic pigment concentrations (Table 3), suggests enhanced photosynthetic efficiency, greater assimilate synthesis, and faster translocation rates to sink sites such as developing grains.

The robust growth in OLE-treated plants may be due to the extract's richness in bioactive compounds, such as flavonoids and phenolic acids (M'rabet *et al*., 2023) which neutralize free radicals and protect cells from oxidative damage (Parkash *et al*., 2007). OLE also contains phytohormones like GA_3, GA_4, and zeatin, along with essential nutrients such as calcium and iron (Ulger *et al*., 2004). These components stimulate cell division and enlargement, enhance chlorophyll formation, and delay leaf senescence. Zeatin, in particular, promotes the development of lateral roots and branches (Hwang *et al*., 2012; Taize *et al*., 2014), consistent with our findings (Table 2).

Calcium, a key macronutrient in olive leaves, plays structural and signalling roles. It acts as an osmotic agent in vacuoles, stabilizes membranes, strengthens cell walls, and serves as a secondary messenger in signaling pathways (Dodd *et al*., 2010; Wanas *et al*., 2018). Calcium also supports essential growth processes, such as cell division and assimilates synthesis, particularly under stress (Pereira and Mello, 2002). It helps maintain a balanced hormonal status

with higher IAA and GAs levels and lower ABA and ethylene levels in various plant organs (Ferguson, 1988).

Our results also showed that OLE significantly increased concentrations of key nutrients and bioconstituents in wheat shoots, which can be attributed to the organic extract's potential stimulation of nutrient absorption through the well-developed root system of treated plants (Table 2). Furthermore, the enhanced leaf area (Table 2), increased photosynthetic pigments (Table 3), lowered LAR, and dry matter accumulation in the shoots all indicate improved photosynthetic efficiency. This improvement led to greater production of photosynthates, such as sugars, and more efficient mineral transport from roots to shoots, resulting in greater export of minerals and assimilates to sink sites, including developing spikes.

The obtained results revealed that all examined anatomical traits of the stem terminal internode and flag leaf at the heading stage were positively influenced by OLE treatments. These changes included increased thickness of mesophyll, phloem, and xylem tissues, which are involved in photosynthates production, transport, and raw material transport, respectively. The improvements in these tissues suggest that OLE enhanced the translocation of raw materials, leading to greater nutrient absorption and more efficient allocation of photosynthates to sink sites, such as developing spikes and grains. This enhanced nutrient and photosynthates partitioning contributed to better heading and higher grain yield with improved quality (Tables 7 & 8).

Other studies have also emphasized the importance of increasing phloem and xylem cross-sectional areas, along with enhanced photosynthates production and nutrient uptake, to improve the growth and productivity of various crops (Khamis, 2021; Taha *et al*., 2021; Wanas and Bazeed, 2023, Metowea, 2024; Wanas and Shabka, 2025).

The anatomical improvements in the stem and leaf blade induced by OLE may be attributed to its high content of natural growth stimulants, including nutrients, antioxidants, and growth-promoting hormones, particularly zeatin, a cytokinin. Cytokinins are known to promote cell division and enlargement, thereby increasing the extension growth of plant organs (Hwang *et al*., 2012; Taize *et al*., 2014).

The improvement in grain yield of plant-1 by OLE treatments can be attributed to OLE's ability to enhance tiller number, leaf area, photosynthetic pigments, dry matter accumulation, and concentrations of NPK, protein, and carbohydrates. Anatomical enhancements in the stem terminal internode and flag leaf blade likely improved nutritional supply to developing spikes. The rise in grain weight may result from enhanced assimilate partitioning or mobilization of soluble stem reserves toward developing grains, potentially due to elevated cytokinin levels (Dietrich, *et al*., 1999) Cytokinin-rich OLE may also boost sink capacity during heading, leading to improved wheat yield. Wanas (2007) reported that higher endogenous cytokinin levels increase grain weight by augmenting assimilate allocation to developing grains. Furthermore, OLE's richness in mineral nutrients, antioxidants, and growth-promoting hormones contributes to its growth-enhancing effects, enabling wheat plants to grow and yield more effectively.

5. Conclusion

In conclusion, olive leaf extract (OLE) significantly enhanced wheat grain and straw yields when used as a grain soaking agent followed by foliar spraying on 45-day-old plants. This improvement is due to OLE's ability to promote robust early growth, favorable physio-biochemical performance, and anatomical changes in the stem terminal internode and flag leaf blade, improving nutrient and assimilate supply to developing grains (sink site), which contributes to higher grain yield and quality. Based on these findings, we strongly recommend using OLE at a 2.0% concentration as an organic biostimulant for wheat and other food crops to achieve high yields with good quality while reducing reliance on synthetic growth regulators and mineral fertilizers, aligning with eco-friendly sustainable agriculture practices.

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