YIELD AND PHYSIO-BIOCHEMICAL PROPERTIES OF WHEAT GRAIN BENEATH ORGANIC AND INORGANIC FERTILIZATION AND APPLICATION OF BIOSTIMULATOR

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Abstract. Four particular bread wheat assortments were included within the display to examine their yield and the physiological and biochemical properties underneath the effects of different organic and inorganic fertilization as well as biostimulator applications. The study was conducted at the Field Crop Research Center-Sulaimani \ KRG – IRAQ. In addition to mineral fertilizer, farmyard manure, poultry manure, and biofertilizer were utilized in a field experiment. The treatments were repeated in another field experiment with a biostimulator application. The biostimulator application in three diverse development stages of the studied wheat varieties was arranged to diminish the insufficiency coming about from the impact of organic fertilizers compared to the impact of synthesized fertilizer. The criteria related to the yield and yield components and some physiological and biochemical properties (Soluble Sugar, Phenols, Flavonoids, and Carotenoids Content) of wheat grains were assessed. The influence of organic sources increased the flag leaf area and chlorophyll content, although it declined with growth advantages. The impact of farmyard manure exceeded significantly other nutrient sources in the number of spikes m^{-2}, Spike Partitioning Coefficient %, 1000-grain weight, and total grain yield displaying (253.542 spikes m^{-2}, 40.429%, 36.89 g, and 2122.9 kg ha^{-1}). Significant differences were found in Phyto-biochemicals among the effects of nutrition sources and the biostimulator. The outcomes of soil analysis revealed a few enhancements, such as the decline in soil pH as well as an increase in soil organic matter due to organic fertilization and biostimulant application.
Keywords: *organic farming, organic fertilizer, seaweed extract, wheat, biochemicals*

Introduction

Wheat as one of the most essential grains in the world, is not only a source of essential supplements such as carbohydrates, proteins, and vitamins but also a source of antioxidants, such as flavonoids and phenolic acids. Bread wheat is the most critical market cereal species, but there is a deficiency of suitable varieties for sustainable farming systems almost any modern-bred varieties of bread wheat that are conventionally grown are not ideal for organic farming (Konvalina et al., 2011), while organic farming of wheat preserves the ecosystem and prevents pollution through utilizing natural fertilizers that increase soil water potential and the water availability to plants. Commonly incorporated fertilizers are animal manure, compost, and poultry excrement, which improve the organic matter content of the soil and help avoid soil degradation (Ahmed et al., 2011). The development of the healthy culture of people in different societies, as well as the far-reaching move of ranchers to organic farming as a result of their coordinated awareness of the detrimental effects of conventional farming

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on the soil and its sustainability are the main causes of the global shift away from conventional production systems to modern sustainable production systems (Gatsios et al., 2021). Organic farming is more eco-friendly, though production potential is very low under this system (Konopka et al., 2012).

Plant biostimulators are non-toxic substances that regulate plant metabolic systems and improve plant development and photosynthetic efficiency (Omidbakhshfard et al., 2020). Many biostimulants enhance plant nutrition (nitrogen, phosphorus, potassium, and other essential micronutrients), thus improving the grain quality and yield. These biostimulant products include seaweed extracts, beneficial microbes (bacteria and fungi), organic acids, amino acids or protein hydrolysates, and chitosan (Sible et al., 2021).

The biochemical components in wheat incorporate phenolic acids and flavonoids which are the most sorts of health prevention agents found in cereal grains their utilization has been connected to a decrease in certain constant diseases among people (Del Rio et al., 2013). There were differences among wheat varieties in the entire antioxidant properties and phenolic content, and they found a relationship between antioxidant status and nutrient quality (Li et al., 2015; Žilić, 2016). Results from the studies demonstrated the impact of organic fertilization in increasing the grain protein content, while the influence of mineral fertilization was shown in reducing the phenolic and flavonoid concentrations (Abad et al., 2004; Rempelos et al., 2020; Lacolla et al., 2021).

The application of fertilizers in modern agriculture is based on the requirements of different genotypes (Panayotova et al., 2014); moreover, these fertilizers enhance the photosynthetic efficiency, chlorophyll, and carotenoid contents of plants (Yang et al., 2002; Duan et al., 2007).

Agroecology and soil quality are interconnected and largely depend on the application of organic and inorganic nutrient sources. Additionally, organic sources of fertilizer increased sustainability in the production system, whereas the high use of chemicals in modern agriculture increased soil degradation, which negatively affected the soil and water resources, therefore, one of the most important ways of soil regeneration involves adding organic materials to conserve organic matter and maintain or enhance soil fertility (Melero et al., 2007).

The aim of the investigation is to assess the yield and physio-biochemical properties of bread wheat varieties grown conventionally and organically, as well as the impact of biostimulator application in improving those properties.

Materials and methods

Two different field experiments were conducted in the open field at the Sulaimani Agricultural Research Center in Bakrajo district in the Sulaimani region- KRG\IRAQ (Lat. 35° 34’ 19”; N, Long 45° 22’ 16”; E, 754 m.a.s.l.) during the winter season of 2021–2022. The meteorological data for the growing season is presented in *Table 1*. Different organic and inorganic sources of fertilizer were applied to four distinctive bread wheat varieties, Sulaimani-2, Rizgari, Wafi, and Sham-6, which were received from the directorate of field crops research-Sulaimani represented as (V1, V2, V3, and V4) respectively. The varieties Sulaimani-2 and Rizgari were bred in ICARDA and developed at the Field Crops Research Center in Sulaymaniyah and Erbil, while the two varieties Wafi and Sham-6 were bred and developed in France and ICARDA

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research centers. The wheat varieties were treated with four different sources of organic and inorganic fertilization, including NP as inorganic fertilizer (F1), 120 kg nitrogen used as urea (46%), and 80 kg P_2O_5 as Triple Super Phosphate (TSP) 48% as well as three various organic sources, Farmyard Manure FYM (F2), Poultry Manure PM (F3), and Biofertilizer (Azotobacter chroococcum 1%) (F4) produced by Corax-Bioner Zrt. Hungary, they were applied by (4000 kg ha^{-1}, 800 kg ha^{-1}, and 32 kg ha^{-1}) respectively.

The field experiments were conducted by a split-plot design in which the four fertilization sources (F1, F2, F3, and F4) were allotted to the main plots, while the four wheat varieties (V1, V2, V3, and V4) were distributed in the sub-plots. Line sowing of wheat maintaining the 20 cm row spacing, was ensured on 24th November 2021, in a net plot size of 2 m^2, using 50 kg seed ha^{-1} in five rows to ensure a plant density of 400 plants per 1 m^2 for all varieties. The same treatments used in the first field experiment (Exp.1) were repeated in the second experiment (Exp.2) in the open field with a biostimulator soluble seaweed extract powder (Alga600) produced by LEILI Marine Bioindustry INC., China which was applied at 0.5 g L^{-1} every two weeks beginning from the stem elongation stage. The growth stages were identified according to the Zadoks Growth Scale, which is a 0-99 scale of development that is recognized internationally for research.

Soil analyses were carried out at the soil laboratory of the Directorate of Agricultural Research Center Bakrajo-Sulaimani. The soil samples were collected from the experimental field (0 to 30 cm depth) after harvesting for each treatment by an auger, where each sample was thoroughly mixed and crushed gently. The samples were air-dried and sieved to pass through a 2 mm sieve. The soil texture was determined with the Bouyoucos hydrometer method (Bouyoucos, 1962), while the soil pH was measured by a pH-meter using a glass electrode (McLean, 1983), and the EC was determined using the conductance mete according to methods mentioned by (Richards, 1954). The total nitrogen was estimated according to the Kjeldhal method (Bremner and Mulvaney, 1983). The concentration of phosphorus in soil was determined with the Olsen method (Olsen, 1954). Potassium was determined by using a flame photometer (Richards, 1954). Total organic matter was estimated according to the Walkley and Black method as described in (Jackson, 1958; FAO, 1974). The soil calcium carbonate ($CaCO_3$) was determined according to the titration method (Drouineau, 1942). Whereas Calcium, Magnesium, and Chloride were measured by (Richards, 1954).

The soil texture of the experimental site before cultivation was silty clay loam with a pH of 8.15, an electrical conductivity of 0.25 dS m^{-1}, and an organic matter content was 1.55%. The initially available nitrogen (N), phosphorous (P), and potassium (K) content were 0.25%, 8.84 mg Kg^{-1}, and 0.115 mmol L^{-1}, respectively.

Three different readings were taken on 12th April, 27th April, and 10th May to determine the chlorophyll contents of flag leaf using the SPAD-502Plus (KONICA MINOLTA, Japan) meter at three different growth stages (stem elongation, booting, and flowering). The flag leaf area was measured at the flowering stage according to the following equation:

$$\text{Flag leaf area (cm}^2\text{)} = \text{L max x W max x Index factor (0.905)} \qquad \text{(Eq.1)}$$

where L max is the maximum length of the flag leaf and W max is the maximum width of the flag leaf.

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The studied biochemical include Soluble Sugar Content (µg g^{-1}), Total Phenolic Content (µg g^{-1}), Total Flavonoid Content (µg g^{-1}), Carotenoids Content (µg g^{-1}), and Protein%.

Table 1. *Some meteorological data for the growing season 2021-2022*

Year	Months	Temperature °C			Relative humidity %			Precipitation mm
		Max	Min	Average	Max	Min	Average	
2021	October	27.80	20.20	23.88	65.00	17.00	32.82	13.50
	November	27.10	8.30	15.27	61.00	36.00	44.48	8.40
	December	16.70	1.50	5.81	66.00	51.00	59.94	105.10
2022	January	13.00	-2.40	3.31	81.00	54.00	67.06	119.30
	February	20.80	1.40	6.51	76.00	55.00	65.04	33.10
	March	19.10	3.60	7.19	74.00	49.00	62.97	51.30
	April	22.80	14.60	18.84	49.00	26.00	34.08	35.80
	May	24.80	14.30	19.87	60.00	34.00	44.05	15.70
	June	33.80	26.80	29.43	34.00	22.00	29.44	0.90

The total quantity of rain during the season 2021-2022 was 383.10 mm

Biochemical tests

Soluble sugar content (SSC)

Soluble sugar content was determined by the method described by (Yemm and Willis, 1954; Zheng et al., 2008; Lateef et al., 2021). Grind grains (1 g) were soaked in 5 mL of deionized water and boiled at 100°C for 30 min followed by cooling and centrifugation at 4000 rpm for 15 min. After, 300 µL of the supernatant sample was taken and mixed with 2.7 mL of Anthrone reagent and boiled for 5 min, cooled the mixture. The standard solution was prepared to produce a standard curve and calculated at 620 nm using a UV-visible spectrophotometer (UV-365, SHIMADZU, Japan), and the soluble sugar content was calculated by a formula:

$$\text{SSC } (\mu g/g \text{ FM}) = \frac{V}{W} \text{ x C} \quad \text{(Eq.2)}$$

where V = volume of extract (mL), W = weight of the sample (g), and C = concentration from the standard curve.

Total phenolic content (TPC)

The content of TPC was determined according to (Lateef et al., 2021) using the Folin-Ciocalteu method with some modifications. The grind grains (1 g) were extracted with 80% Methanol incubated for 16 h at 10°C and centrifuged for 19 min at 14000 rpm at 4°C. An aliquot of 25 µL of each sample was mixed with 2 mL of 1:9 Folin-Ciocalteu reagent: water (v/v) and reacted for 6 min, 1.6 mL of 10% saturated Na_2CO_3 solution was added and left for 53 min in the dark at 38°C. Then, read at 750 nm using a UV-visible spectrophotometer (UV-365, SHIMADZU, Japan). The

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standard solution was prepared to the standard curve, and TPC was calculated by the formula:

$$\text{TPC } (\mu\text{g GAE/g FM}) = \frac{\text{V}}{\text{W}} \text{ x C} \qquad \text{(Eq.3)}$$

where V = volume of extract (mL), W = weight of the sample (g), and C = concentration of Gallic acid from the standard curve.

Total flavonoid content (TFC)

The TFC was calculated using the $AlCl_3$ method according to (Lateef et al., 2021) with some modifications. The grind grains (1 g) were extracted with 80% methanol incubated for 16 h at 10°C, and centrifuged for 19 min at 14000 rpm. The extract solution of (40 µL) was mixed with (0.90 mL of methanol (80%), 0.30 mL of 2% Aluminum Chloride, 0.08 mL of 1 M Potassium Acetate, and 1.72 mL of deionized water). After incubation for 32 min at room temperature, the absorbance of the solution was read at 415 nm using a spectrophotometer. The standard solution was prepared to the standard curve, and calculate TFC by this equation:

$$\text{TFC } (\mu\text{g QE/g FM}) = \frac{\text{V}}{\text{W}} \text{ x C} \qquad \text{(Eq.4)}$$

V = volume of extract (mL), W = sample dry weight (g), and C = concentration of quercetin from the standard curve.

Carotenoid content (CAC)

The CAC was calculated utilizing the strategies expressed by (Ferrante et al., 2008). Three replications have been utilized to calculate. The carotenoid concentrations were communicated as (µg g^{-1} of dry weight) and evaluated by this equation:

$$\text{CAC} = \frac{\text{The absorbance reading } x \text{ Total volume (mL) x 10000}}{\text{Carotene extension coefficient in methanol } x \text{ weight (g)}} \qquad \text{(Eq.5)}$$

Protein %

The protein content has been determined based on total nitrogen content, while the Kjeldahl method was applied to determine nitrogen content (AOAC, 2000). After estimating the nitrogen in wheat, we calculate the protein percentage using the following equation:

$$\text{Protein\%} = \text{Nitrogen\%} \times 6.25 \qquad \text{(Eq.6)}$$

Correction factor = 6.25

Statistical analysis

The data from the two experiments were statistically analyzed according to the split-plot design using the (JMP pro16) statistical analysis software, as well as the comparison between the effects of the studied treatment means were compared according to the Least Significant Differences (L.S.D) at the probability of < 0.05 and < 0.01(Steel et al., 1997).

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Results

Soil analysis

Organic fertilizers conserve soil health and improve nutrient status. The results shown in *Table 2* indicate a significant difference between soil properties before cultivation, and after Exp.1 and Exp.2 in addition to inorganic and organic sources, it comprised biostimulator application in three various growth stages, stem elongation, booting, and flowering. Reduction in the pH values was noticed with organic fertilization sources from (8.15) before planting to (8.03 in F2, 7.90 in F3, and 7.93 in F4) or while the biostimulator was applied concerning inorganic fertilization showing (8.07 in F2, 7.80 in F3, and 7.79 in F4) beneath the effect of organic sources.

The results in the same table indicate a significant increase in phosphorous content after cultivation, and next to Exp.1 and Exp.2, in which the lowest P content was demonstrated in the soil analysis before cultivation (8.84 mg Kg^{-1}), the highest level of P was illustrated post the Exp.1 was (21.49 mg Kg^{-1}) beneath the effect of inorganic fertilization and 19.39 mg Kg^{-1} with the influence of biofertilizer source. The highest P value in Exp.2 was associated with the effect of the biofertilizer source activated by the biostimulator application.

Table 2. *Some soil chemical characteristics before cultivation, post Exp.1 and Exp.2 with biostimulator application*

Soil characteristics	Before cultivation	Field Exp.1				Field Exp.2 with biostimulator application			
		F1	F2	F3	F4	F1	F2	F3	F4
Soil pH	8.15	8.05	8.03	7.90	7.93	7.86	8.07	7.80	7.79
EC ds m^{-1}	0.25	0.27	0.24	0.25	0.26	0.27	0.24	0.25	0.26
Nitrogen %	0.25	0.14	0.14	0.15	0.15	0.16	0.15	0.15	0.17
Phosphorus (mg Kg^{-1})	8.84	21.49	11.96	16.86	19.39	15.46	9.38	10.86	19.31
Soluble K^+ (mmol L^{-1})	0.115	0.11	0.091	0.103	0.107	0.085	0.089	0.092	0.077
Soluble Ca^{+2} (mmol L^{-1})	1.25	1.20	1.115	1.265	1.235	1.115	1.10	1.085	1.215
Soluble Mg^{+2} (mmol L^{-1})	0.65	0.565	0.75	0.565	0.90	0.53	0.735	0.68	0.635
Chlorine (mmol L^{-1})	0.60	1.03	0.97	0.90	0.53	0.50	0.77	0.67	0.50
Organic matter %	1.55	1.70	1.75	1.74	1.70	1.57	1.71	1.65	1.66
Calcium carbonate %	26.00	28.00	28.20	26.50	27.00	26.70	26.30	25.50	26.50

K^+: Potassium, Ca^{+2}: Calcium, and Mg^{+2}: Magnesium

Flag leaf area and total chlorophyll content

The predominance of the effect of biofertilizer (F4) was clear in Exp.1 within the competition of organic sources versus the inorganic fertilizer on the flag leaf area (FLA), obtaining 35.509 cm^2 obverse 31.983 cm^2 with the inorganic source and 27.519 cm^2 and 29.496 cm^2 by F2 and F3, respectively. The influence of the biostimulator (*Fig. 1*) increased the wheat flag leaf area under the impact of inorganic fertilizer (NP), FYM, and PM, revealing (35.879, 34.459, and 32.788) cm^2. In comparison, there was a reductant in the flag leaf area under biofertilizer treatment with biostimulator application (29.996 cm^2).

There was a gradual decline in the Total Chlorophyll Content (TCC) beneath all fertilization sources from stem elongation to the booting stage and then to flowering in field Exp.1 (*Figs. 2, 3,* and *4*). The effect of biostimulator application in Exp.2 on the

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TCC showed the same path decreasing from stem elongation to flowering with significant differences between various fertilization sources. *Figures 2, 3,* and *4* manifest significant differences between the effect of fertilization sources on the TCC in Exp.1 and Exp.2. At the stem elongation stage, the inorganic fertilization F1 significantly exceeds (55.867) the chlorophyll content compared to the organic sources F2, F3, and F4 with (51.033, 48.450, and 52.147) respectively. However, the influence of biostimulator at that growth stage increased the TCC with the effect of F1 to (59.125), and there was a decline with the impact of F2, F3, and F4 demonstrating (50.650, 47.933, and 49.150), respectively.

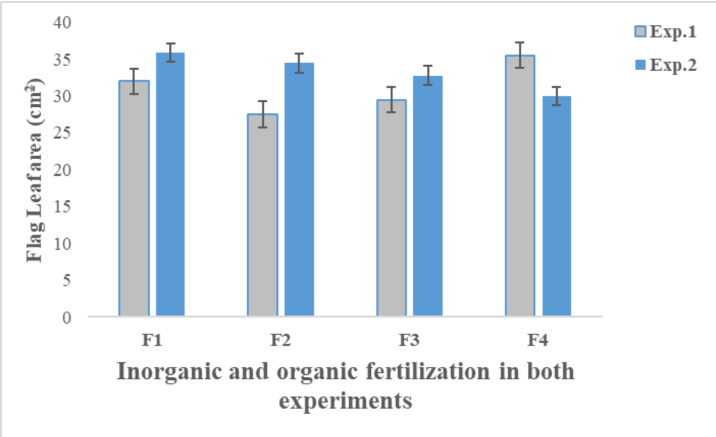

Figure 1. *The impact of inorganic and organic fertilization on the flag leaf area (Exp1: First field experiment), and the effect of the same nutrition sources with biostimulator application (Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}) on the flag leaf area. The comparison of the combination of both experiments was statistically compared with L.S.D value 1.560 at $p < 0.01$, Four fertilizer sources (F1: Inorganic fertilizer Nitrogen 120 kg ha^{-1} and Phosphorus 80 kg ha^{-1}, F2: Farmyard Manure 4000 kg ha^{-1}, F3: Poultry Manure 800 kg ha^{-1}, F4: Biofertilizer 32 kg ha^{-1}). The short length of the error bar indicates high precision and reliability*

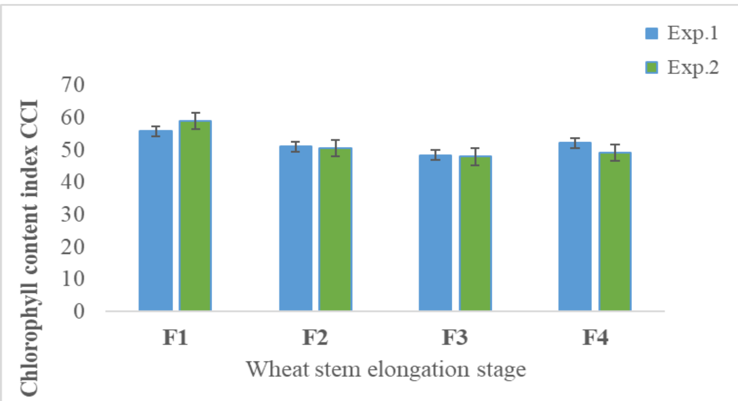

Figure 2. *Total chlorophyll content under the influence of inorganic and organic fertilization (Exp1: First field experiment) and the effect of biostimulator application with the same nutrition sources (Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}) at the stem elongation stage, Four fertilizer sources (F1: Inorganic fertilizer Nitrogen 120 kg ha^{-1} and Phosphorus 80 kg ha^{-1}, F2: Farmyard Manure 4000 kg ha^{-1}, F3: Poultry Manure 800 kg ha^{-1}, F4: Biofertilizer 32 kg ha^{-1}). The short length of the error bar indicates high precision and reliability*

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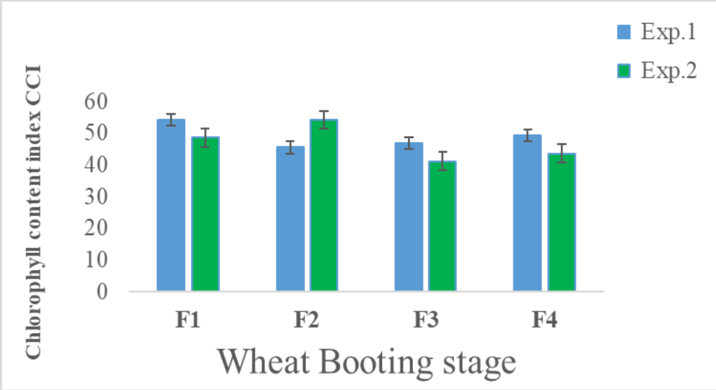

Figure 3. *Total Chlorophyll content under the influence of inorganic and organic fertilization (Exp1: First field experiment) and effect of biostimulator application with the same nutrition sources (Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}) at the booting stage, Four fertilizer sources (F1: Inorganic fertilizer Nitrogen 120 kg ha^{-1} and Phosphorus 80 kg ha^{-1}, F2: Farmyard Manure 4000 kg ha^{-1}, F3: Poultry Manure 800 kg ha^{-1}, F4: Biofertilizer 32 kg ha^{-1}). The short length of the error bar indicates high precision and reliability*

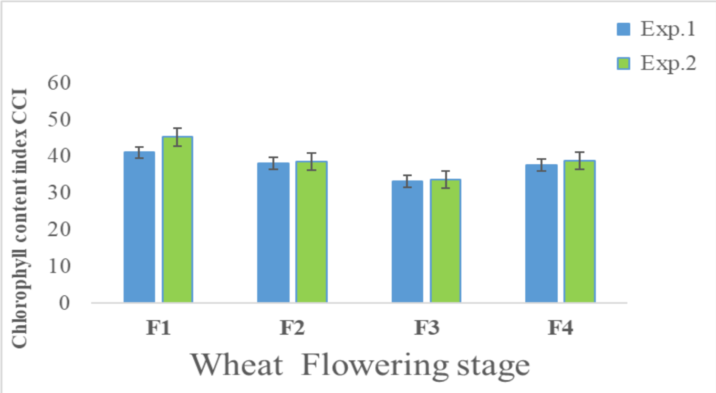

Figure 4. *Total Chlorophyll content under the influence of inorganic and organic fertilization (Exp1: First field experiment) and effect of biostimulator application with the same nutrition sources (Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}) at the flowering stage, Four fertilizer sources (F1: Inorganic fertilizer Nitrogen 120 kg ha^{-1} and Phosphorus 80 kg ha^{-1}, F2: Farmyard Manure 4000 kg ha^{-1}, F3: Poultry Manure 800 kg ha^{-1}, F4: Biofertilizer 32 kg ha^{-1}). The short length of the error bar indicates high precision and reliability*

At the flowering stage (*Fig.* 4), the mineral fertilization (F1) significantly exceeded the organic sources F2, F3, and F4, showing more dropping with and without the application of biostimulator, particularly the organic sources F2 and F3, where TCC was not significantly impacted with biostimulator application by the growth development advantage toward booting and flowering stages.

Wheat grain yield and yield parameters

Table 3 organizes the outcome of Exp.1 and demonstrates some different parameters that contribute directly or indirectly to the total wheat yield. There were significant differences between organic sources in considering inorganic sources on the total number of spikes m^{-2}, spike partitioning coefficient (SPC%), and weight of 1000-grain

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manifesting the impact of organic fertilization (F2) in obtaining the highest number of spikes m^{-2} and the highest percentage of SPC% displayed (253.542 spikes m^{-2}, and 40.429%) respectively, however, the maximum 1000-grain weight was obtained with the impact of the biofertilizer source (F4), on the other hand, there were non-significant variations in the grain yield and biological yield of inorganic sources and all organic sources. The situation under the influence of biostimulator application (*Table 3*) demonstrated general advantages in the studied parameters, significant differences were found between the response of different fertilization sources to the biostimulator application and the efficacy of F3 PM was assured in the highest number of spikes m^{-2} (269.375), SPC% (40.954%) which shared it with F4 (43.142%), total grain yield (2443.52 kg ha^{-1}), and harvest index (0.287) revealing the best response of poultry manure to biostimulator application.

Table 3. *Influence of inorganic and organic sources on the growth and yield parameters and biostimulator application in strengthening some growth and yield parameters*

	Treatments	Total number of spike m^{-2}	Spike partitioning coefficient SPC%	Weight of 1000-grain g	Total grain yield Kg ha^{-1}	Biological yield Kg ha^{-1}	Harvest index
Exp.1	FF1	187.292b	28.093c	34.487b	1548.15	8254.17	0.191c
	F2	253.542a	40.429a	36.890a	2122.9	7050.0	0.294a
	F3	219.792ab	36.516ab	37.052a	1762.04	6968.75	0.260ab
	F4	226.875ab	33.004bc	37.550a	1874.21	7602.08	0.252b
	L.S.D.	39.692*	6.356*	1.602*	N.S.	N.S.	0.039**
Exp.2	F1	243.750ab	32.339b	35.787b	2098.73b	9056.25a	0.230b
	F2	212.708b	35.210b	39.343a	2110.35b	8960.42a	0.242b
	F3	269.375a	40.954a	38.003ab	2443.52a	8520.83a	0.287a
	F4	236.875ab	43.142a	38.320a	2062.96b	7510.42b	0.274a
	L.S.D.	36.428*	3.649**	2.272*	245.623*	630.128*	0.022**

F1: Inorganic fertilizer Nitrogen 120 kg ha^{-1} and Phosphorus 80 kg ha^{-1}, F2: Farmyard Manure 4000 kg ha^{-1}, F3: Poultry Manure 800 kg ha^{-1}, F4: Biofertilizer 32 kg ha^{-1}, Exp1: First field experiment, Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}, NS: Means values not significantly different between themselves, *: Means significant differences between treatments at $p \leq 0.05$. LSD, **: Means significant differences between treatments at $P \leq 0.01$ LSD

The differences among the effects of different fertilizer sources on the biological yield in Exp.1 were non-significant according to the statistical analysis, although the highest biological yield was produced by the effect of chemical fertilizer F1 (9056.25 kg ha^{-1}) in Exp.2 the differences reached the level of significance, in which the biological yield of chemical fertilizer was the highest. The higher biological yield was also produced by all organic sources in Exp.2 compared to their results in Exp.1.

The total grain yield of wheat varieties under the effect of inorganic and organic fertilization in Exp.1 and the response of those varieties to the biostimulator application demonstrate significant differences among wheat varieties in total grain yield. In Exp.1, the V2 variety produced the highest yield 2118.06 kg ha^{-1} followed by V3 with 1972.85 kg ha^{-1}, while V1 showed a minimum grain yield of 1492.69 kg ha^{-1}, variations in the wheat yield varieties may reflect the genetic potential and the nutrient requirements under organic farming, indicating to the proper response of Wafi (V3) and Rizgari (V2) to organic fertilization. *Figure 5* illustrates the influence of biostimulator application on wheat varieties yield performance, indicating higher grain yield for all

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varieties and better response to V3. The impact of a combination between the effect of different fertilization sources and wheat varieties was shown in *Figure 6*, revealing significant differences in the mentioned interactions the maximum grain yield was obtained beneath the combination of F2V2 in Exp.1, while the highest amount of grain yield was produced by the combination between the impact of poultry manure (F3) and execution of Wafi variety (V3) under the influence of biostimulator application in Exp. 2.

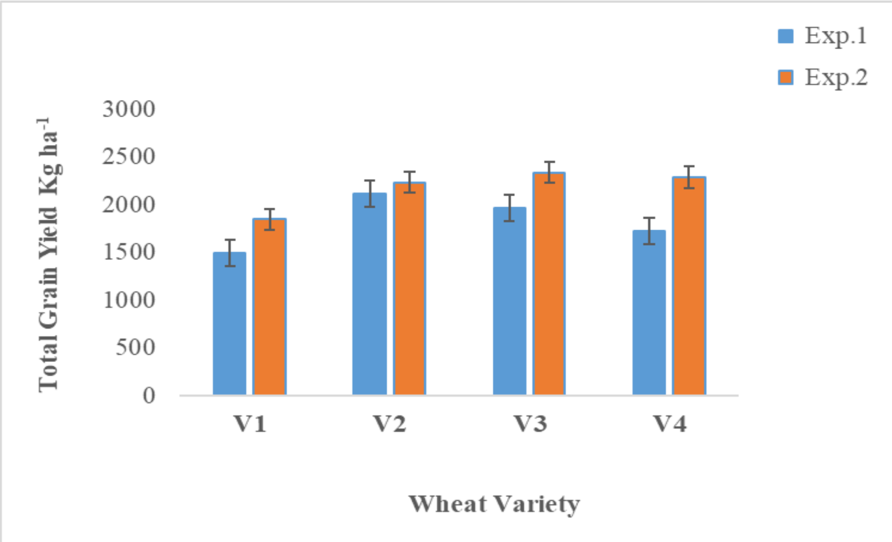

Figure 5. *The total yield of wheat varieties in response to inorganic and organic sources (Exp1: First field experiment) and the influence of biostimulator application (Exp2: Second field experiment with Biostimulator 0.5 g L*$^{-1}$*), Four wheat varieties (V1: Sulaimani-2, V2: Rizgari, V3: Wafi, V4: Sham-6). The short length of the error bar indicates high precision and reliability*

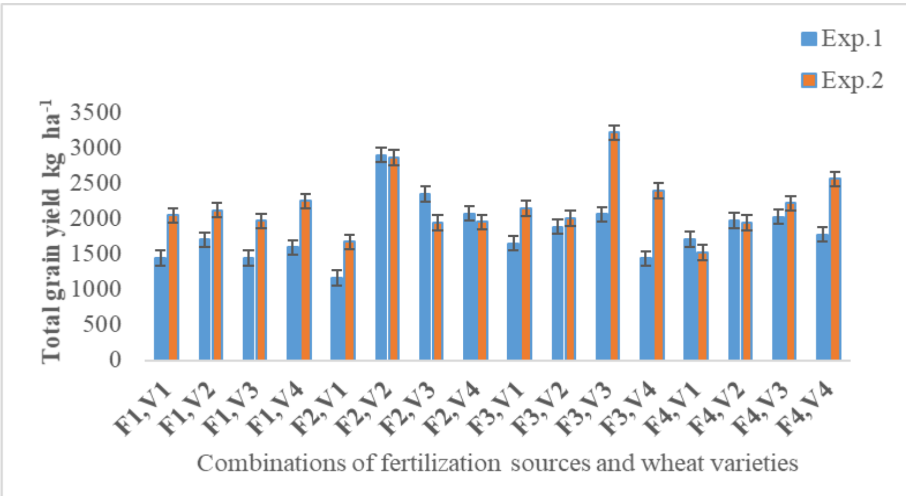

Figure 6. *Influence of the interaction between inorganic and organic fertilization, biostimulator application, and wheat varieties on the total grain yield, Exp1: First field experiment, Exp2: Second field experiment with Biostimulator 0.5 g L*$^{-1}$*, Four fertilizer sources (F1: Inorganic fertilizer Nitrogen 120 kg ha*$^{-1}$ *and Phosphorus 80 kg ha*$^{-1}$*, F2: Farmyard Manure 4000 kg ha*$^{-1}$*, F3: Poultry Manure 800 kg ha*$^{-1}$*, F4: Biofertilizer 32 kg ha*$^{-1}$*) and four wheat varieties (V1: Sulaimani-2, V2: Rizgari, V3: Wafi, V4: Sham-6). The short length of the error bar indicates high precision and reliability*

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Biochemical properties

The impact of biostimulator application and various sources of nutrition on the Phyto-biochemical parameters includes; the soluble sugar content of total phenolic, total flavonoids, carotenoids, and protein of wheat varieties compared to the circumstances underneath the impact of inorganic fertilizer and organic sources FYM, PM, and biofertilizer without biostimulator application was illustrated in (*Fig. 7*). The maximum level of SSC (353.08 µg g^{-1}) was obtained with poultry manure F3 and the effectiveness of biostimulator, while there were significant increases in SSC with organic fertilization sources FYM and biofertilizer with seaweed application revealing (308.75 and 294.009 µg g^{-1}). The total phenol was boosted with an application of a biostimulator to organic nutrition sources, and the highest level of total phenols was obtained with the influence of F3 and the effectiveness of the biostimulator (240.665 µg g^{-1}), and there was a clear increase in the effect of the biostimulant when added to other fertilizer sources to (200.988, 207.097, and 213.184) µg g^{-1} revealed by (mineral fertilization, FYM, and biofertilization) respectively. There was a significant increase in TFC in response to different fertilizations with a biostimulator. The maximum level (27.182 µg g^{-1}) was given with FYM and the effectiveness of the biostimulator. At the same time, there were significant increases in TFC with organic fertilization sources PM and biofertilizer with seaweed application (24.661 and 26.01 µg g^{-1}), respectively. Unlike other biochemicals, there was a decrease in carotenoids with biostimulator application in Exp.2, the fertilization sources effectivity showed (1.683, 1.238, 1.738, and 2.424) µg g^{-1} with mineral fertilization, FYM, PM, and biofertilizer respectively in Exp.1. A significant increase in protein content was detected with biostimulator application due to the impact of different nutrition sources (inorganic fertilization, FYM, PM, and biofertilizers), which boosted to (12.213, 11.976, 10.176, and 11.441) % respectively.

The results obtained from biochemical analyses of grains of four wheat varieties treated with different sources of organic and inorganic fertilizers manifested significant differences in the biochemical properties of soluble sugar content, total phenolic content, total flavonoids, carotenoids, and protein. The wheat varieties Sulaimani-2, Rizgari, Wafi, and Sham-6 manifested significant differences in both field experiments under the influence of different inorganic and organic fertilizations as well biostimulator applications (*Fig. 8*). The significant influence of the biostimulator on the performance of wheat varieties was demonstrated in all biochemical properties except carotenoids. Significant variations in the response of four wheat varieties were found in SSC content in both field experiments, there was a higher achievement in SSC of all varieties with the effect of biostimulator application in Exp.2 except V2 which did not show a response to the seaweed extract (299.012) µg g^{-1} compared to (366.713 µg g^{-1}) in Exp.1, the wheat varieties V1, V3, and V4 responses displayed (356.182, 288.696, and 293.855) µg g^{-1} respectively compared to (297.407, 252.269, and 271.867) µg g^{-1} by the same varieties without the application of the biostimulator in Exp.1, and there was an illustrative response of the wheat variety Sulaimani-2 (V1) to the biostimulator application which significantly exceeded other varieties in the Exp.2 (356.182 µg g^{-1}). There was a significant increase in total phenolic content from (210.796, 176.924, 215.009, and 177.533) µg g^{-1} demonstrated by four wheat varieties under the influence of inorganic and organic sources into (228.305, 195.955, 225.262, and 212.411) µg g^{-1} with the application of a biostimulator. The wheat varieties V1, V2, and V3 showed higher flavonoid means in Exp.2 (26.759, 24.240, and 25.217) µg g^{-1} respectively, compared to (23.46, 19.255, and 21.86) µg g^{-1} in Exp.1.

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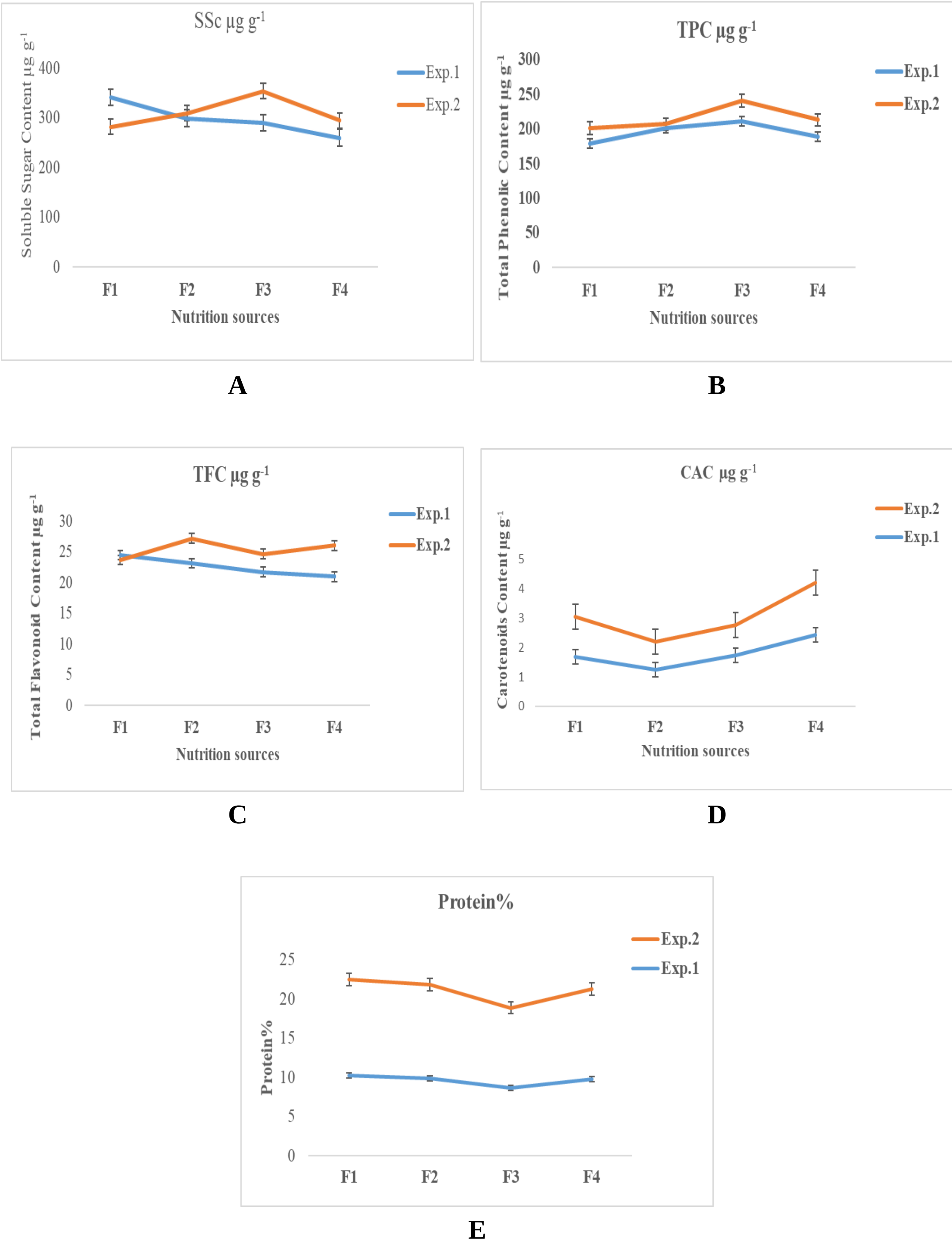

Figure 7. *Effect of different inorganic and organic nutrition sources, and foliar application of biostimulator on A. SSC: Soluble sugar content µg g^{-1}, B. TPC: Total phenol content µg g^{-1}, C. TFC: Total Flavonoids µg g^{-1}, D. CAC: Carotenoids content µg g^{-1}, and E. Protein % a significant difference between the mean values according to L.S.D at ($p \leq 0.01$), Exp1: First field experiment, Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}, Four fertilizer sources (F1: Inorganic fertilizer Nitrogen 120 kg ha^{-1} and Phosphorus 80 kg ha^{-1}, F2: Farmyard Manure 4000 kg ha^{-1}, F3: Poultry Manure 800 kg ha^{-1}, F4: Biofertilizer 32 kg ha^{-1}). The short length of the error bar indicates high precision and reliability*

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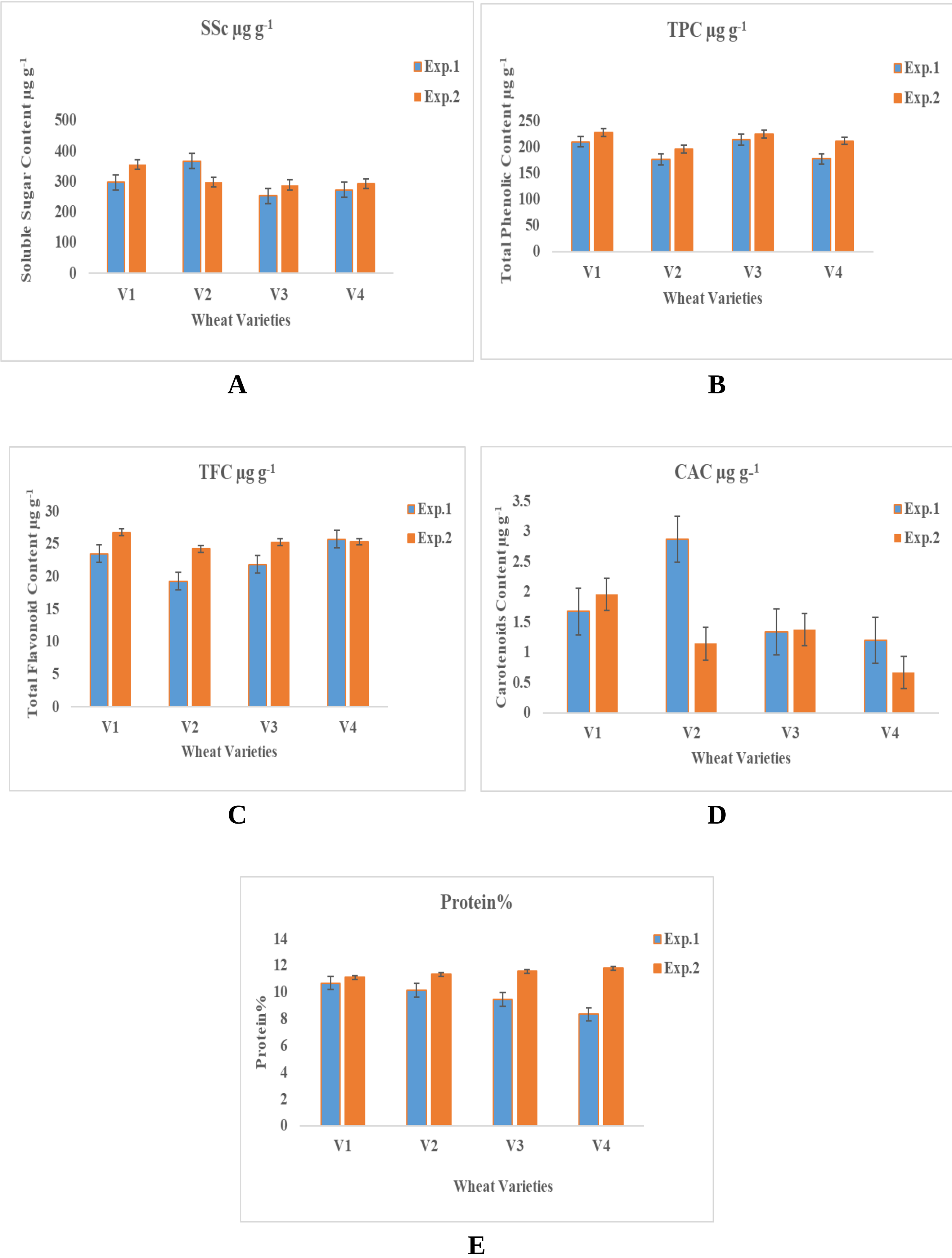

***Figure 8.** Responses of four wheat varieties to the inorganic and organic nutrient sources and foliar application of biostimulator in Phyto-biochemicals include A. SSC: soluble sugar content µg g^{-1}, B. TPC: Total phenol content µg g^{-1}, C. TFC: Total Flavonoids µg g^{-1} D. CAC: Carotenoids content µg g^{-1} and E. Protein % a significant difference between the mean values according to L.S.D at ($p \leq 0.01$), Exp1: First field experiment, Exp2: Second field experiment with Biostimulator 0.5 g L^{-1}, Four wheat varieties (V1: Sulaimani-2, V2: Rizgari, V3: Wafi, V4: Sham-6). The short length of the error bar indicates high precision and reliability*

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The biostimulator application displayed various effects due to the carotenoid content in the wheat varieties, while the Sulaimani-2 (V1) showed raising from 1.673 to 1.959 µg g^{-1} DW, the other varieties (V2 and V4) revealed non-responses to the seaweed application demonstrating drooping in carotenoids content and the V3 variety was with a slight response. Maximum protein% was realized by V4 (11.796%) with seaweed application and exceeded significantly other varieties, the response of wheat varieties to biostimulator was varied, while V4 showed minimum protein content in Exp.1. The variety sham-6 (V4) was stimulated biochemically and obtained the highest protein content in Exp.2.

Discussion

Soil amendment

The soil improvement after utilizing distinctive fertilization sources and biostimulator applications included a reduction in the soil pH value, and a rise in phosphorous availability, and organic matter. There was a slight decrease in the pH value before cultivation to the lower levels under the effect of organic nutrition and biostimulator application, especially with the impact of poultry manure and biofertilizer with adding seaweed extraction. The decrease in soil pH could be due to the impact of the dissolution of organic sources, the pH value decreasing may lead to a boost in the availability of the nutrition status and increase the availability of the soil nutrients for the plants, organic fertilization provides the soil with supplement supply, and stimulates more vital impacts than mineral fertilization, they improve soil porosity and promote soil health (Van Stappen et al., 2015; Alzamel et al., 2022). *Table 2* illustrates more soil amendment with organic fertilization and biostimulator application, the soil organic matter was increased next to both experiments and the highest percentage of organic matter was with the effect of the treatments used in Exp.1. Organic fertilizers are a valuable source of organic material and essential nutrients for plants. The influence of organic nutrition might cause nutrient release at a slow pace, not at all like mineral fertilization, and the phosphorus is accessible all through the edit development period, and the possibility of the survival of these components inside the soil to take advantage of the crops created within the taking after seasons (Mnthambala et al., 2022). The impact of biostimulants as natural preparations improved crop performance and increased the efficiency of plant nutrient utilization (Rouphael et al., 2018). The effective enhancement of physiological processes in the uptake and translocation of various nutrients in the plant cells with the biostimulator impact may result from pH reduction under seaweed application (*Table 2*) that might modify the source-sink relationship and overcome nutrient limitation through improving nutrient availability (De Pascale et al., 2017).

Flag leaf area and TCC

Chlorophyll is an integral part of light harvesting during the process of photosynthesis. The applied biofertilizer with Azotobacter plays a crucial role in fixing atmospheric nitrogen that provides an accessible nutritional component and enhanced plant efficiency, which may lead to an increase in cell division and elongation in the flag leaf area of the wheat varieties (Al-Naqeeb et al., 2018). Our results demonstrate a significant increase in flag leaf area with biostimulator application (*Fig. 1*) except for

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the biofertilizer source (F4), as there was no response to the biostimulant. The high effect of biofertilizer was evident in Exp.1 with no biostimulator application.

Differences in the chlorophyll content upon the effect of different sources of biofertilizer depend on the nutrition availability due to the sensitivity of chlorophyll content to leaf nitrogen signification (Padilla et al., 2018). The chlorophyll content declined gradually as wheat plants proceeded towards new growth stages, and the different fertilization sources revealed lower TCC at the booting growth stage (*Fig. 3*) with non-significant differences between them in Exp.1, while the impact of biostimulator application (Exp.2) was not increase the TCC content but the beneficial impact of applying biostimulants on chlorophyll content was due to their capacity to supply components such as phytohormones and some supplements with a synergistic activity that upgrades endogenous cytokinin, which created a protective impact on chloroplast (Zavaleta-Mancera et al., 2007). There was a gradual decline in the total chlorophyll content beneath all fertilization sources from stem elongation to the booting stage and then to flowering in field Exp.1, (*Figs. 2, 3,* and *4*), revealing the effectivity of growth advantages and environmental conditions. The total chlorophyll content value at the different growing stages was sensitive to relative chlorophyll content, which increased and then decreased with growth stage development and phenotypic modifications (Liu et al., 2019).

Yield and yield components

The impact of competition between distinctive sources stems from the degree to which these fertilizer sources influence soil fertility and the availability of nutrients during different growth stages. The combined effect of organic and inorganic fertilizers on the wheat grain yield and yield-related traits is explained in *Table 3,* showing non-significant differences in the wheat grain yield while applying the farmyard manure (F2) produced a maximum number of spike m^{-2}, SPC%, and harvest index showing up to 253.542, 40.419, and 0.294 respectively.

Wheat yield was highest when farmyard manure was applied. The creation of a favorable root zone with good soil porosity and equilibrium in water retention throughout the growing season positively influenced the partitioning of photosynthates and the rate of assimilation. All of that led to higher spike initiation and spike partitioning coefficient, the results agree with the findings of (Abbas et al., 2022; Liu et al., 2022), and Tehulie and Tola (2020) who indicated the highest wheat grain yield with FYM application. Response of nutrition sources to the biostimulator application manifested significant differences in all studied criteria related to the wheat grain yield, showing the effectivity of PM (F3) with the biostimulator in producing the highest number of spike m^{-2}, total grain yield kg ha^{-1}, and harvest index obtained (269.375, 2443.52, and 0.287) respectively. Compared to other nutrition sources, the improved grain yield under the interaction between F3 and biostimulator was 14% greater than the output of the effect of mineral fertilization F1 and 13% and 15% greater than the biostimulator combination to F2 and F4, respectively.

Application of seaweed increased the wheat grain yield of all wheat varieties compared to those varieties with the different nutrition sources without foliar application of biostimulator, the potential of organic fertilizers may not provide all plant requirements so the biostimulator influence behaved as the fulfillment of the plant demands through boosting the nutrition availability and the modification improvement in synthesizing the protein and essential enzymes for different biochemical and

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physiological functions to proceed more efficiently, so the significant increase in the wheat grain yield might result from the enhancement effect of the seaweed biostimulator that combined with organic nutrition sources on cell development, proper regulation of enzymes, and photosynthetic efficacy (Rasul et al., 2015; Posmyk and Szafrańska, 2016). The biostimulator application Exp.2 caused a significant increase in the grain yield of all wheat varieties compared to Exp.1, which was grown under the effect of different nutrition sources without biostimulator application (*Fig. 5*). The grain and protein yield were more stable in the case of the old wheat cultivars, while the modern varieties of bread wheat were characterized by better and more efficient nutrient management with essential requirements for an adequate supply of micronutrients that biostimulator application accelerates the absorption and relocation of different microelements within the plant, our results of the present study indicate to a significant increase in the interaction effect (*Fig. 6*) between the biostimulator application and PM source (F3) and the Wafi variety (V3) demonstrating the PM potential in providing macro and micronutrients as well as increasing in the wheat plants uptake of essential nutrients that in addition to raising in the dry grain weight, it develops the grain quality through translocating to the main sink and improving the amino acid, starch, carbohydrate, and protein contents (Gao et al., 2020).

Biochemicals

Wheat grain comprises a high concentration of compatible solutes and secondary metabolites as antioxidants that support and protect human health. A significant increase in SSC was recorded with the use of biostimulants in addition to various organic sources (FYM, PM, and biofertilizer) compared to the sole application of organic sources (*Fig. 7*). The maximum level of SSC (353.08 μg g^{-1}) was observed with the combined application of PM with biostimulator could be due to a rise in the nutrient availability and acceleration in the physiochemical processes that lead to an increment of net photosynthesis and growth respiration which provide the accumulation of SSC and starch in wheat grains (Popko et al., 2018).

The phenolic compounds are phenolic acids, flavonoids, coumarins, lignans, stilbenes, and tannins (Žilić, 2016). The highest level of total phenols content under the impact of nutrition sources was manifested with the effect of PM (F3) showing (211.053 μg g^{-1}), while the lowest value of TPC was obtained with the influence of mineral nutrition (F1) (178.727 μg g^{-1}), the previous results confirmed the higher accumulation of total polyphenols in grain with organic farming. The higher values of phenol concentration in wheat grains might be an outcome from the activity of microorganisms in air nitrogen-fixing that stimulate the production of phytohormones and other growth-promoting compounds, our findings were in line with those resulted by (Konopka et al., 2012).

Although the resulting carotenoids in our study matched the carotenoid levels in wheat (1.38 and 1.54 μg g^{-1}), the biostimulator application reduced the carotenoids in the treatments. Reduction in carotenoids content while treated with a biostimulator may result from the nutrition system and depend on the general growth effectivity in metabolic processes and the source-sink relationship (Konopka et al., 2012), during the early stages of grain development, carotenoids from the β, β-branch (zeaxanthin, antheraxanthin, and violaxanthin) were presented at higher levels at 10 days post anthesis and declined gradually in mature grains (Zhai et al., 2016).

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Figure 7 manifests the higher levels of phenols and flavonoids that were exhibited with the application of a biostimulator with organic nutrition impacts, the highest level of TPC was realized with PM application, nevertheless, the FYM displayed a maximum level with the effect of the biostimulator on TFC. The results indicate significant variations in the concentrations of the studied antioxidants TPC and TFC, which might differ according to the effectiveness of these nutrition sources in providing the wheat crop demands, as well as the soil situation and ambient environmental factors. The concentration of phenols and flavonoids in cereals were found in the free and conjugated shapes, the most significant concentration of phenolic acids and flavonoids is within the aleurone layer of cereal grains, but these compounds are moreover found in embryos and seed coats of grains. Our finding agrees with the output of (Rempelos et al., 2020).

There was a significant increase in the TPC concentration of all wheat varieties with the application of a biostimulator, and the maximum concentration was detected with the response V1. The performance of the wheat varieties V1, V2, and V3 with biostimulator application showed a significant boost in flavonoid concentration in comparison to the situation of non-application of biostimulator application. Differences among varieties in showing TPC and TFC originated from differences in genetic potential as well as physiochemical composition and response of wheat varieties to applied fertilization systems, and our outcome was in line with the results of (Zengin et al., 2017). There was a significant increase in protein content when the biostimulator was applied to the effect of different nutrition sources, and the protein percentage was increased to (12.213%, 11.976%, 10.176%, and 11.441%) with seaweed extract application which was boosted from (10.267%, 9.868%, 8.711%, and 9.797%) produced under the influence of nutrition sources (F1, F2, F3, and F4) respectively. The foliar application of the biostimulant increased the protein content due to a higher proportion of amino acids in the biostimulant composition, the results agree with the accomplishment of (Abbas et al., 2022).

Conclusions

The organic fertilization sources completed for the mineral fertilizer successfully in the flag leaf area, chlorophyll content, yield and yield components, and bio-chemicals. The biostimulant application upgraded the physio-biochemical properties and yield of wheat varieties beneath the impact of distinctive organic fertilizers, there was a significant impact of FYM on the yield and criteria related to the total grain yield, while the poultry fertilizer effect overdid other sources within the physio biochemicals. There was obvious boosting in TPC, TF, SSC, and protein due to acceleration in the nutrient availability and enhancement in some metabolites that impact the substrates for secondary metabolism, which led to a rise in phytochemical products. The carotenoid content diminished with the application of seaweed extraction. Significant variations were found in the responses of four wheat varieties to the nutrition sources and biostimulant applications. The wheat varieties Wafi and Rizgari showed a significant response to organic fertilization, while a great response to the biostimulator addition was performed by the Wafi variety.

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