SYNERGISTIC EFFECTS OF RHIZOBIAL INOCULUM WITH CHEMICAL FERTILIZER ON GROWTH AND YIELD OF WHEAT

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Abstract. The present study was aimed at examining the combined effect of five species of rhizobial inoculation (Control, *Bradyrhizobium sp.* (Vigna), *Rhizobium leguminosarum bv. viciae*, *Bradyrhizobium* Mungbean, *Mesorhizobium ciceri*, and *Rhizobium leguminosarum bv phaseoli*) at three levels (120, 240, 360 kg.ha^{-1}) of NPK on the physiological characteristics, yield components, and leaf chemical content of two wheat species. The results indicated that all physiological characteristics and all nutrient contents of leaf under study, total dry weight of plant at harvest, spike weight, grain number, grain yield, biological yield, and harvest index increased significantly as a result of the interaction between the chemical fertilizer, the biofertilizer, and the wheat species. It was observed that the combination of rhizobial bacteria and wheat species had a positive effect on the physiological characteristics, leaf nutrient contents, and yield components, but not on spike length, spikelet number, and weight of 1000 grains. Our data suggested that a lower level of NPK fertilizer and rhizobial inoculation should be combined in order to achieve the greatest effect on yield components. Also, different types of rhizobial bacteria should be used as biofertilizer in order to improve wheat production.
Keywords: *physiological characteristics, bacteria, production, leaf, Triticum sp.*

Introduction

As one of the first cultivated plants, wheat is regarded as the most significant cereal because it is a major food supply for human. There are numerous species of wheat. For example, macaroni wheat (*Triticum durum*) is a tetraploid wheat species which are cultivated in order to fulfill the demands of the pasta market, whereas *Triticum aestivum* is a hexaploid species that is widely grown throughout the planet (Abou-Taleb and Gomaa, 2012). This species (i.e. *Triticum aestivum*) is one of the most important crops worldwide (Wang et al., 2018). Given the fact that there has been a huge global demand for food as a result of increased world population over the last few decades, a large number of research studies have been aimed at increasing food production so as to meet humanity needs around the world. These diverse researches have included environmental, physiological, agricultural, and genetic studies which all aimed to increased crops productivity (Elghair, 2012).

In order to increase the yields, there has been an increase in the use of mineral fertilizers which are very costly and cause pollution in many ways. Therefore, researchers have always attempted to control such threats by producing alternative substitutes which are more economical, environmentally friendly, and properly improved. In this regard, use of beneficial microbes or plant growth promoting rhizobacteria has been proposed as the best approach to improve crop yield (Adnan et al., 2014). Another widely utilized method to enhance soil fertility and crop productivity is the use of chemical fertilizers which have a negative effect on the complex system of biogeochemical cycles. One of their negative effects, for example,

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is environmental degradation due to runoff and leaching of nutrients, particularly P and N (Yildirim et al., 2011). Another method to increase crop productivity is to use biofertilizers which are substances that contains living microorganisms and applied to plant surface, seeds, or soil. Biofertilizers add nutrients by stimulating plant growth through the synthesis of growth promoting substances and through the natural processes of nitrogen fixation, solubilizing phosphorus. By utilizing biofertilizers, it is expected that there will be a remarkable decrease in the use of chemical fertilizer and pesticides. It is stated that natural nutrient cycle of the soil can be restored and the soil organic matter can be built through the microorganisms in biofertilizers (Al-Shamma and Al-Shahwany, 2014). Moreover, soil contains a wide range of microbes that can act in symbiosis or non-symbiosis association with their host plant (Gray and Smith, 2005).

A range of bacterial genera like *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium,* and *Azorhizobium* exist in rhizobia through which a symbiosis can be established with leguminous plants. Special organs that are known as nodules are produced by these genera on the stems and roots of the hosts, leading to a decrease in atmospheric nitrogen that is delivered to the plant (Sessitsch et al., 2002). As plant growth promoting rhizobacteria (PGPR), rhizobia can also be used in non-legumes (Yanni et al., 1997). Moreover, by producing IAA, gibberellins, and cytokinins, it can enhance growth and yield of cereals (Zahir et al., 2010). Moreover, by producing organic acids, it increase the supply of insoluble nutrients such as phosphorus and iron (Fatima et al., 2006), making these nutrients easily available for plant uptake (Biswas et al., 2000). It is widely reported that the growth parameters, yield, components, and chemical constituents in treated plants can be improved through inoculation of such plants with *Azospirillum*, *Azotobacter*, *Rhizobium*, and *Pseudomonas* in dual or different combinations with organic and mineral fertilizers. According to previous experiments, the best results were obtained by mixing the various inoculations and applying on wheat plants, leading to reduced amount of mineral fertilizers (Mitkees et al., 1996).

The present study was carried out in order to assess the effectiveness of rhizobial bacteria in reducing the use of chemical fertilizer and improving wheat production.

Materials and methods

Pot experiment

The experiment was carried out in the Glasshouse of Biology Department, College of Science, University of Salahaddin-Erbil during winter 2016-2017. The experimental plants used in this experiment included wheat (*Triticum aestivum*) cultivar Hawler2 and (*Triticum durum*) cultivar Seminto that were obtained from the Agricultural Research Center in Erbil. The experiments were carried out using plastic pots with a depth of 25 cm and a diameter of 30 cm. Using a 4 mm pore size sieve, the soil was sieved and then sterilized using formalin 40% as described by Elia et al. (1987). Each pot was filled with 8 kg sandy clay loam soil. *Table 1* presents some of the chemical and physical properties of the soil before the treatments. A sufficient number of wheat seeds were separated and disinfected with 95% ethanol for 30 s and then washed with sterilized distilled water at least 8 times (Etesami et al., 2009). In each pot, 5 seeds were sown at a depth of 3-4 cm and later thinned to four plants.

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Table 1. *Some physical and chemical properties of soil under investigation*

Properties		Value
Particle size distribution (%)	Sand	67.8
	Silt	10.9
	Clay	21.3
Soil texture		Sandy clay loam
pH		8.2
Electrical conductivity (dS.m^{-1})		0.60
Total nitrogen%		0.56
Total phosphorous%		0.144
Total potassium%		0.056
Calcium%		0.253
Iron%		0.078

Isolation of Rhizobial sp

Five strains of rhizobia including *Bradyrhizobium sp*. (Vigna), *Rhizobium leguminosarum* bv. viciae, Bradyrhizobium Mungbean, *Mesorhizobium ciceri* and *Rhizobium leguminosarum* bv. phaseoli were isolated from *Vigna unguiculata, Vicia faba, Vigna radiata L., Cicer arietinum L.,* and *Phaseoulus vulgasrous* root nodules, respectively which were growing for 2-3 months under field conditions at a different area of Erbil city. Afterwards, the host plants which had some non-rhizopheric soil on them were uprooted, and they were placed in polythene bags and taken to the laboratory. By shaking the uprooted plants, their non-rhizospheric soil was removed. Moreover, by dipping and shaking them in water, their rhizospheric soil was removed. The nodules were separated from the roots with a sterilized razor blade, and each of them was placed in a separate Petri plates. The surface of the nodules was disinfected by dipping them in ethanol (95%) for 20 s and then in $HgCl_2$ (0.2%) solution for 3 min followed by washing them with sterilized distilled water 6 times. Using a sterilized glass rod, the surface-disinfected nodules of each host were crushed in a sterilized test tube containing sterilized distilled water. The obtained suspension was used to inoculate Petri plates that contained autoclaved and solidified yeast extract mannitol (YEM) media which was then incubated at 28 ± 1 °C for bacterial growth (Mehboob et al., 2011).

Preparation of inoculums

In order to complete the preparation of inoculum, the selected isolates of rhizobia were grown in 250 mL conical flask containing 100 mL YEM broth by incubating at 28 ± 1 °C in an orbital shaking incubator at 100 rpm for 3 days. In order to obtain uniform cell density (i.e. 10^8 to 10^9 CFU mL^{-1}), dilution method was employed in so as to record an optical density of 0.5 at a wavelength of 535 nm (Mehboob et al., 2011). Inoculation of 10 ml (10^8–10^9 CFU.mL^{-1}) per seedling was done at the base of the plant ten days after germination.

Experimental design and treatment

The current experiment included 6 treatments of rhizobial inoculums (i.e. Control, *Bradyrhizobium sp.* (Vigna), *Rhizobium leguminosarum bv. viciae*, *Bradyrhizobium*

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Mungbean, *Mesorhizobium ciceri*, and *Rhizobium leguminosarum bv. phaseoli*) and 3 levels of NPK (20:20:20) fertilizers (120, 240 and 360 kg.ha^{-1}), making a total of 36 treatments per replication. The experiment was carried out using a factorial complete randomized design (Factorial C.R.D.) with 3 replications. The comparisons between means were made using Tukey test at significant level of 5% for field experiment parameters and 1% for laboratory parameters. SPSS version 16 was used for data analysis.

Experimental parameters

Physiological parameters

The spectrophotometric method as described by Lichtenthaler (1987) was employed to measure chlorophyll a, chlorophyll b, and carotenoid based on the flag leaf samples that were collected at flowering stage during the growing period.

$$Chlorophyll\ a = (13.36 \times A664.2) - (5.19 \times A648.6)$$

$$Chlorophyll\ b = (27.43 \times A648.6) - (8.12 \times A664.2)$$

$$Total\ Chlorophyll = Chlorophy\ a + Chlorophy\ b$$

$$Carotenoid = \{(1000 \times A470) - (2.13 \times Ch\ a) - (97.64 \times Ch\ b)\}/209$$

In order to determine the membrane stability index (MSI), the method proposed by Premachandra et al. (1991) was utilized.

$$MSI = [1 - (C1/C2)] \times 100$$

Biochemical contents

Dried leaves at flowering stage were ground by an electrical grinder. Afterwards, 0.3 g of the ground samples was digested by adding 10 ml H_2O_2 and 10 ml concentrated H_2SO_4, and they were heated. After that, Kjeldahl method was employed to determine the total nitrogen, spectrophotometer method was used to estimate the total phosphorus, atomic absorption method was utilized to estimate the total calcium and iron, and flame-photometer method as described by Ryan et al. (2001) was used to determine the total potassium determined. Moreover, by multiplying the value of total nitrogen by 5.75, the total protein was calculated (Dalaly and Al-Hakim, 1987). Also, the Anthron method (Sadasivam, 1996) was utilized to determine the total soluble carbohydrate.

Yield components

At harvest, main spike length (cm), number of grains.plant^{-1}, weight of spikes.plant^{-1}, grain yield (kg.ha^{-1}), biological yield (kg.ha^{-1}), number of spikelets.spike^{-1}, harvest index (%), weight of 1000 grains (g), and increase grain yield (%) were estimated. The following formula proposed by Ye et al. (2005) was employed to determine the increased grain yield.

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$$Increase\ grain\ yield\% = \big((Grain\ yield\ of\ fertilized\ pot - Grain\ yield\ of\ control)/Grain\ yield\ of\ control\big) \times 100$$

Results

Physiological parameters

According to the data presented in *Table 2,* it was detected that soil application of B3 led to the highest levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid, while the highest value of cell membrane stability was obtained by utilizing B5.

Table 2. *Effects of different species of rhizobial bacteria on some physiological characteristics*

Bacterial species	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)	Carotenoid ($mg.g^{-1}$)	Cell membrane stability %
Control	0.950	0.609	1.559	0.422	88.557
B1	1.326	1.061	2.387	0.666	93.079
B2	1.426	1.149	2.574	0.696	93.401
B3	1.438	1.199	2.638	0.725	93.105
B4	1.394	0.961	2.356	0.675	94.083
B5	1.343	1.130	2.473	0.671	94.248
Tukey 0.01	0.125	0.163	0.253	0.105	0.133

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*

The results also indicated that the highest values of chlorophyll a and cell membrane stability were obtained by adding 360 $kg.ha^{-1}$ of NPK (see *Table 3*). On the other hand, using 240 $kg.ha^{-1}$ of NPK led to obtaining the maximum value of total chlorophyll and carotenoid.

Table 3. *Effects of different levels of chemical fertilizers on some physiological characteristic*

Chemical fertilizer ($kg.ha^{-1}$)	Chlorophyll a ($(mg.g)^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)	Carotenoid ($mg.g^{-1}$)	Cell membrane stability %
NPK1	1.216	0.9706	2.187	0.589	92.433
NPK2	1.353	1.052	2.405	0.671	92.700
NPK3	1.370	1.032	2.402	0.668	93.103
Tukey 0.01	0.075	n.s.	0.153	0.063	0.08

NPK1 = 120 $kg.ha^{-1}$, NPK2 = 240 $kg.ha^{-1}$, NPK3 = 360 $kg.ha^{-1}$

The results presented in *Table 4* revealed that *Triticum durum* significantly surpassed *Triticum aestivum* regarding the total chlorophyll and cell membrane stability, while wheat species had no effect on chlorophyll a, chlorophyll b, and carotenoid.

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Table 4. *Effects of wheat species on some physiological characteristics*

Wheat species	Chlorophyll a $(mg.g)^{-1}$	Chlorophyll b $(mg.g^{-1})$	Total chlorophyll $(mg.g^{-1})$	Carotenoid $(mg.g^{-1})$	Cell membrane stability %
T. aestivum	1.289	0.990	2.279	0.637	91.879
T. durum	1.3370	1.0464	2.383	0.6480	93.612
Tukey 0.01	n.s.	n.s.	0.087	n.s.	0.057

As indicated in *Table 5*, Rhizobium inoculation resulted in significant increase in physiological parameters in both wheat species. The highest values of chlorophyll a and carotenoid were respectively 1.501 and 0.750 $mg.g^{-1}$ that were recorded in B2 of *Triticum durum*, while highest value of chlorophyll b was 1.258 $mg.g^{-1}$ and the total chlorophyll was 2.737 $mg.g^{-1}$, which were recorded in B3 of *Triticum durum*. The results presented in *Table 5* also revealed that the highest value of cell membrane stability was 94.417% that was recorded in B1 of *Triticum durum*.

Table 5. *Interaction effects of wheat species and rhizobial bacteria on some physiological characteristics*

Wheat species	Bacterial species	Chlorophyll a $(mg.g)^{-1}$	Chlorophyll b $(mg.g^{-1})$	Total chlorophyll $(mg.g^{-1})$	Carotenoid $(mg.g^{-1})$	Cell membrane stability %
T. aestivum	Control	0.928	0.596	1.523	0.452	86.529
	B1	1.204	0.980	2.184	0.631	91.740
	B2	1.350	1.091	2.441	0.643	92.735
	B3	1.397	1.142	2.539	0.717	92.075
	B4	1.445	0.965	2.410	0.709	93.916
	B5	1.408	1.169	2.577	0.672	94.281
T. durum	Control	0.972	0.624	1.595	0.391	90.586
	B1	1.448	1.142	2.590	0.702	94.417
	B2	1.501	1.207	2.708	0.750	94.067
	B3	1.480	1.258	2.737	0.734	94.136
	B4	1.344	0.958	2.302	0.642	94.250
	B5	1.278	1.091	2.368	0.670	94.215
Tukey 0.01		0.199	0.26	0.405	0.168	0.212

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*

According to the results presented in *Table 6*, different levels of NPK fertilizer led to significant increase in photosynthetic pigments and cell membrane stability of the two wheat species. The highest value of all photosynthetic pigments was recorded at 240 $kg.ha^{-1}$ of *Triticum durum*, while using 360 $kg.ha^{-1}$ of *Triticum durum* resulted in the highest value of cell membrane stability.

The data presented in *Table 7* show how photosynthetic pigments and cell membrane stability were influenced by different combinations of different levels of NPK fertilizer and different species of rhizobial bacteria. As indicated, using 240 $kg.ha^{-1}$ with B4 led

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to highly significant increases in chlorophyll a, total chlorophyll, and carotenoid. However, the highest value of chlorophyll b was obtained as result of using 240 $kg.ha^{-1}$ with B5. Moreover, using 360 $kg.ha^{-1}$ of NPK with B5 led to the highest cell membrane stability.

Table 6. *Interaction effect of wheat species and chemical fertilizers on some physiological characteristics*

Wheat species	Chemical fertilizer ($kg.ha^{-1}$)	Chlorophyll a $(mg.g)^{-1}$	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)	Carotenoid ($mg.g^{-1}$)	Cell membrane stability %
T. aestivum	NPK1	1.227	0.996	2.224	0.605	91.483
	NPK2	1.242	0.918	2.160	0.624	91.988
	NPK3	1.396	1.056	2.453	0.683	92.167
T. durum	NPK1	1.205	0.945	2.150	0.573	93.384
	NPK2	1.463	1.187	2.650	0.718	93.412
	NPK3	1.343	1.008	2.350	0.652	94.039
Tukey 0.01		0.125	0.163	0.253	0.105	0.133

NPK1 = 120 $kg.ha^{-1}$, NPK2 = 240 $kg.ha^{-1}$, NPK3 = 360 $kg.ha^{-1}$

Table 7. *Interaction effect of chemical fertilizer and rhizobial bacteria on some physiological characteristics*

Chemical fertilizer ($kg.ha^{-1}$)	Bacterial species	Chlorophyll a $(mg.g)^{-1}$	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)	Carotenoid ($mg.g^{-1}$)	Cell membrane stability %
NPK1	Control	0.931	0.627	1.558	0.430	87.755
	B1	1.252	0.978	2.230	0.607	93.996
	B2	1.344	1.204	2.547	0.669	92.720
	B3	1.318	1.106	2.423	0.658	91.919
	B4	1.184	0.863	2.046	0.553	94.155
	B5	1.269	1.047	2.316	0.616	94.056
NPK2	Control	0.898	0.495	1.393	0.395	87.843
	B1	1.290	0.977	2.267	0.677	92.460
	B2	1.427	1.094	2.520	0.696	93.486
	B3	1.475	1.252	2.727	0.773	94.135
	B4	1.580	1.206	2.786	0.778	94.005
	B5	1.445	1.291	2.736	0.709	94.273
NPK3	Control	1.019	0.706	1.726	0.439	90.075
	B1	1.436	1.229	2.665	0.715	92.780
	B2	1.506	1.148	2.655	0.724	93.998
	B3	1.522	1.242	2.764	0.745	93.262
	B4	1.419	0.816	2.235	0.695	94.089
	B5	1.315	1.051	2.366	0.687	94.415

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*. NPK1 = 120 $kg.ha^{-1}$, NPK2 = 240 $kg.ha^{-1}$, NPK3 = 360 $kg.ha^{-1}$

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Leaf photosynthetic pigments and cell membrane stability increased significantly in both wheat species as a result of *Rhizobium* inoculation and chemical fertilizer treatments (see *Table 8*). *Triticum durum* with 240 kg.ha^{-1} of NPK combined with B2 surpassed other interactions and resulted in the highest chlorophyll a and total chlorophyll. Furthermore, *Triticum durum* with 240 kg.ha^{-1} of NPK integrated with B5 and B4 led to the maximum chlorophyll b and carotenoid pigments, respectively, while adding 360 kg.ha^{-1} of NPK with B1 in *Triticum durum* brought about the highest value of cell membrane stability.

Table 8. *Interaction effect of wheat species, chemical fertilizer and rhizobial bacteria on some physiological characteristics*

Wheat species	Chemical fertilizer (kg.ha^{-1})	Bacterial species	Chlorophyll a (mg.g)$^{-1}$	Chlorophyll b (mg.g^{-1})	Total chlorophyll (mg.g^{-1})	Carotenoid (mg.g^{-1})	Cell membrane stability%
T. aestivum	NPK1	Control	0.814	0.615	1.430	0.357	86.978
		B1	1.206	1.009	2.214	0.637	93.604
		B2	1.442	1.286	2.729	0.712	90.955
		B3	1.255	0.991	2.246	0.633	89.425
		B4	1.258	0.935	2.193	0.630	93.875
		B5	1.388	1.141	2.530	0.659	94.058
	NPK2	Control	0.922	0.423	1.345	0.461	85.687
		B1	1.047	0.732	1.779	0.599	90.663
		B2	1.195	0.828	2.023	0.575	93.056
		B3	1.444	1.198	2.642	0.767	94.275
		B4	1.511	1.194	2.705	0.733	93.875
		B5	1.335	1.134	2.469	0.610	94.375
	NPK3	Control	1.047	0.748	1.795	0.538	86.923
		B1	1.359	1.201	2.560	0.658	90.955
		B2	1.413	1.157	2.570	0.642	94.195
		B3	1.492	1.236	2.729	0.749	92.524
		B4	1.566	0.766	2.331	0.764	93.998
		B5	1.501	1.231	2.732	0.749	94.410
T. durum	NPK1	Control	1.049	0.639	1.687	0.504	88.531
		B1	1.299	0.947	2.246	0.577	94.389
		B2	1.245	1.121	2.366	0.625	94.486
		B3	1.381	1.220	2.601	0.683	94.413
		B4	1.109	0.790	1.899	0.475	94.435
		B5	1.149	0.953	2.102	0.574	94.053
	NPK2	Control	0.874	0.568	1.442	0.329	89.999
		B1	1.534	1.221	2.755	0.755	94.258
		B2	1.658	1.359	3.017	0.817	93.915
		B3	1.507	1.305	2.812	0.778	93.996
		B4	1.649	1.219	2.868	0.823	94.135
		B5	1.555	1.448	3.003	0.809	94.170
	NPK3	Control	0.992	0.665	1.656	0.341	93.228
		B1	1.513	1.257	2.769	0.773	94.605
		B2	1.600	1.140	2.739	0.807	93.800
		B3	1.552	1.248	2.799	0.741	94.000
		B4	1.272	0.866	2.138	0.626	94.180
		B5	1.128	0.871	1.999	0.626	94.421
Tukey0.01			0.403	0.528	0.821	0.341	0.431

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri,* B5: *Rhizobium leguminosarum bv phaseoli.* NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360 kg.ha^{-1}

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Biochemical contents

The results presented in *Table 9* demonstrate that the highest values of nitrogen, iron, and protein were recorded by adding B3. Also, phosphorus and carbohydrate reached their highest values as a result of adding B5. Moreover, the maximum values of potassium and calcium were recorded by adding B1 and B4, respectively.

In was also observed that the highest values of nitrogen, phosphorus, iron, and protein were obtained as a result of using 360 $kg.ha^{-1}$ of NPK (see *Table 10*). Furthermore, adding 120 $kg.ha^{-1}$ of NPK resulted in the greatest potassium content of leaves. Also, 240 $kg.ha^{-1}$ of NPK brought about the highest values of calcium and carbohydrate.

According to the data presented in *Table 11*, *Triticum durum* surpassed *Triticum aestivum* significantly in all leaf nutrient contents under study except potassium and calcium which were better in *Triticum aestivum* than *Triticum durum*.

Table 9. *Effects of different species of rhizobial bacteria on some nutrient content of leaves*

Bacterial species	Protein $mg.g^{-1}$	Carbohydrate $mg.g^{-1}$	Nitrogen $mg.kg^{-1}$	Phosphorus $mg.kg^{-1}$	Potassium $mg.kg^{-1}$	Calcium $mg.kg^{-1}$	Iron $mg.kg^{-1}$
Control	182.44	250.47	34064.04	2254.53	8404.92	7443.14	121.62
B1	240.96	308.69	43479.46	2838.69	10481.95	8158.36	178.22
B2	250.70	300.24	44044.08	2569.27	10164.06	8175.40	191.88
B3	258.79	317.42	47026.08	3104.00	9836.24	8142.41	217.45
B4	232.19	324.36	40763.83	2724.08	10350.04	8287.79	183.37
B5	221.68	327.73	41212.83	3818.69	9890.59	8050.33	169.54
Tukey 0.01	2.24	0.58	389.437	9.981	125.046	103.398	3.338

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*

Table 10. *Effects of different levels of chemical fertilizer on some nutrient content of leaves*

Chemical fertilizer ($kg.ha^{-1}$)	Protein $mg.g^{-1}$	Carbohydrate $mg.g^{-1}$	Nitrogen $mg.kg^{-1}$	Phosphorus $mg.kg^{-1}$	Potassium $mg.kg^{-1}$	Calcium $mg.kg^{-1}$	Iron $mg.kg^{-1}$
NPK1	219.93	310.46	38248.73	2748.11	9968.80	7832.05	178.94
NPK2	236.26	312.80	41087.88	2791.14	9792.77	8208.84	165.82
NPK3	264.26	291.19	45958.54	3115.39	9802.33	8087.83	186.27
Tukey 0.01	1.35	0.35	234.783	6.017	75.388	62.336	2.012

NPK1 = 120 $kg.ha^{-1}$, NPK2 = 240 $kg.ha^{-1}$, NPK3 = 360 $kg.ha^{-1}$

Table 11. *Effect of wheat species on some nutrient content of leaves*

Wheat species	Protein $mg.g^{-1}$	Carbohydrate $mg.g^{-1}$	Nitrogen $mg.kg^{-1}$	Phosphorus $mg.kg^{-1}$	Potassium $mg.kg^{-1}$	Calcium $mg.kg^{-1}$	Iron $mg.kg^{-1}$
T. aestivum	230.17	293.05	40029.07	2750.18	10045.62	8248.84	168.64
T. durum	250.13	316.58	43501.04	3019.57	9663.65	7836.98	185.39
Tukey 0.01	0.96	0.25	167.715	4.299	53.852	44.529	1.437

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It was also observed that the nutrient contents of the leaves in both wheat species increased significantly as a result of different species of Rhizobial inoculation compared with the control species (see *Table 12*). The highest values of N (50079.67 mg.kg^{-1}), Fe (263.34 mg.kg^{-1}), protein (287.96 mg.g^{-1}), and carbohydrate (356.79 mg.g^{-1}) were observed in B3 of *Triticum durum*, while the highest values of P (4350.08 mg.kg^{-1}), K (10555.82 mg.kg^{-1}), and Ca (8841.02 mg.kg^{-1}) were respectively recorded in B5 of *Triticum durum*, B1 of *Triticum aestivum*, B4 of *Triticum aestivum*.

Table 12. *Interaction effect of wheat species and rhizobial species on some nutrient content of leaves*

Wheat species	Bacterial species	Protein mg.g^{-1}	Carbohydrate mg.g^{-1}	Nitrogen mg.kg^{-1}	Phosphorus mg.kg^{-1}	Potassium mg.kg^{-1}	Calcium mg.kg^{-1}	Iron mg.kg^{-1}
T. aestivum	Control	184.24	230.56	32041.55	2336.67	9047.43	7618.78	129.89
	B1	241.62	314.89	42021.59	2750.52	10555.82	8085.04	177.47
	B2	254.52	291.72	44264.17	2598.20	10210.15	8737.96	210.69
	B3	252.84	278.05	43972.50	2866.62	10153.99	7968.21	171.57
	B4	235.59	299.09	40972.13	2661.77	10469.07	8841.02	171.90
	B5	212.19	344.01	36902.48	3287.30	9837.22	8242.00	150.31
T. durum	Control	207.50	270.37	36086.53	2172.39	7762.40	7267.50	113.36
	B1	258.39	302.49	44937.34	2926.86	10408.07	8231.68	178.97
	B2	251.99	308.76	43824.00	2540.35	10117.96	7612.83	173.07
	B3	287.96	356.79	50079.67	3341.37	9518.49	8316.61	263.34
	B3	233.19	349.63	40555.52	2786.40	10231.02	7734.57	194.83
	B5	261.76	311.45	45523.18	4350.08	9943.96	7858.67	188.77
Tukey 0.01		3.57	0.926	621.566	15.931	199.582	165.029	5.327

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*

The results also revealed that NPK fertilizer had a significant effect on all nutrient contents of the leaves in both wheat species (see *Table 13*). In addition, using 360 kg.ha^{-1} of NPK for *Triticum durum* led to the highest values of nitrogen, phosphorus, and protein. Moreover, 120 kg.ha^{-1} NPK of *Triticum aestivum* and 240 kg.ha^{-1} NPK of *Triticum aestivum* led to the maximum values of potassium and calcium, respectively. Furthermore, adding 120 kg.ha^{-1} NPK of *Triticum durum* brought about the highest iron and carbohydrate content of the leaves.

Table 14 presents the data related to the effects of the chemical fertilizer combined with biofertilizer on the nutrient contents of the leaves. As observed, the highest values of nitrogen and protein contents of the leaves were obtained at 360 kg.ha^{-1} of NPK with B3. On the other hand the maximum value of phosphorus contents of the leaves was obtained at 360 kg.ha^{-1} of NPK with B5, while the greatest values of potassium and calcium were obtained in 240 kg.ha^{-1} of NPK with B4. Moreover, adding 120 kg.ha^{-1} with B3 led to the maximum values of iron and carbohydrate.

The interaction effects of different species of rhizobial bacteria and different levels of NPK on the two wheat species are presented in *Table 15*. Adding different types of rhizobial bacteria to the soil at different levels of NPK led to significant increase in Fe, P, N, Ca, K, protein, and the leaves' carbohydrate contents in both wheat species. Application of 360 kg.ha^{-1} of NPK with B3 led to the highest nitrogen and protein

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contents of the leaves in *Triticum durum*. The highest values of carbohydrate, phosphorus, and iron were recorded by utilizing 360 kg.ha^{-1} of NPK with B4 of *Triticum durum*, 120 kg.ha^{-1} of NPK with B5 of *Triticum durum*, and (120 kg.ha^{-1} of NPK with B3 of *Triticum durum*, respectively, while the highest values of potassium and calcium were obtained through 240 kg.ha^{-1} of NPK with B4 of *Triticum aestivum*.

Table 13. *Interaction effect of wheat species and chemical fertilizer on some nutrient content of leaves*

Wheat species	Chemical fertilizer (kg.ha^{-1})	Protein mg.g^{-1}	Carbohydrate mg.g^{-1}	Nitrogen mg.kg^{-1}	Phosphorus mg.kg^{-1}	Potassium mg.kg^{-1}	Calcium mg.kg^{-1}	Iron mg.kg^{-1}
T. aestivum	NPK1	217.05	295.59	37747.63	2355.21	10104.45	7965.86	148.11
	NPK2	222.61	317.32	38714.14	2858.63	10035.05	8495.70	161.28
	NPK3	250.85	266.25	43625.44	3036.70	9997.34	8284.95	196.53
T. durum	NPK1	222.81	325.33	38749.83	3141.00	9833.16	7698.24	209.77
	NPK2	249.90	308.28	43461.63	2723.64	9550.48	7921.99	170.37
	NPK3	277.68	316.14	48291.65	3194.08	9607.31	7890.70	176.02
Tukey 0.01		2.24	0.58	389.437	9.981	125.046	103.398	3.338

NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360 kg.ha^{-1}

Table 14. *Interaction effect of chemical fertilizer and rhizobial bacteria on some nutrient content of leaves*

Chemical fertilizer (kg.ha^{-1})	Bacteria species	Protein mg.g^{-1}	Carbohydrate mg.g^{-1}	Nitrogen mg.kg^{-1}	Phosphorus mg.kg^{-1}	Potassiu m mg.kg^{-1}	Calcium mg.kg^{-1}	Iron mg.kg^{-1}
NPK1	Control	172.58	250.71	30014.22	1894.74	9064.47	7483.87	135.78
	B1	239.55	311.26	41661.58	2627.63	10505.41	7885.47	142.63
	B2	225.49	303.63	39215.14	2550.27	9899.13	7882.97	151.98
	B3	246.90	356.81	42938.43	2631.99	10036.23	7728.07	312.42
	B4	220.66	309.11	38375.89	2700.99	10668.13	8163.23	157.33
	B5	214.40	331.24	37287.13	4083.02	9639.46	7848.67	173.52
NPK2	Control	204.37	266.29	35542.27	2371.15	7993.57	7479.29	110.24
	B1	236.24	344.77	41084.41	2955.75	10268.68	8135.42	166.92
	B2	234.00	308.11	40694.83	2512.56	10079.10	7900.04	176.02
	B3	259.33	308.44	45101.62	2725.65	10024.42	8606.24	181.87
	B4	241.08	315.80	41927.17	2926.44	10685.93	8838.98	191.96
	B5	242.52	333.39	42177.01	3255.26	9704.90	8293.08	167.92
NPK3	Control	210.65	234.40	36635.62	2497.70	8156.72	7366.26	118.84
	B1	274.23	270.04	47692.40	2932.69	10671.75	8454.18	225.10
	B2	300.28	288.97	52222.28	2644.99	10513.95	8743.18	247.64
	B3	304.97	287.01	53038.20	3954.35	9448.07	8092.92	158.08
	B4	241.43	348.17	41988.42	2544.82	9696.07	7861.17	200.81
	B5	254.00	318.56	44174.36	4117.79	10327.41	8009.25	167.17
Tukey 0.01		4.65	1.205	808.749	20.728	259.685	214.728	6.932

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli.* NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360 kg.ha^{-1}

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Table 15. *Interaction effect of wheat species, chemical fertilizer and rhizobial species on some nutrient content of leaves*

Wheat species	Chemical fertilizer (kg.ha^{-1})	Bacteria species	Protein mg.g^{-1}	Carbohydrate mg.g^{-1}	Nitrogen mg.kg^{-1}	Phosphorus mg.kg^{-1}	Potassium mg.kg^{-1}	Calcium mg.kg^{-1}	Iron mg.kg^{-1}
T. aestivum	NPK1	Control	177.96	233.90	30950.32	2070.93	9501.22	7836.17	137.28
		B1	241.53	268.36	42005.92	*2231.45*	10734.78	7984.77	149.98
		B2	236.95	314.09	41208.25	2552.93	10088.08	8063.87	137.28
		B3	229.06	313.15	39837.23	2447.81	10054.35	7752.07	166.17
		B4	230.87	289.54	40150.90	*2073.90*	10588.20	8103.87	147.98
		B5	185.92	354.50	32333.16	2754.26	9660.07	8054.37	149.98
	NPK2	Control	167.25	242.17	29087.13	2438.10	8924.14	7399.21	117.59
		B1	229.05	406.81	39834.73	2669.14	9720.69	8215.98	166.17
		B2	226.49	296.13	39389.73	2475.00	9931.38	8150.51	200.56
		B3	241.49	293.03	41998.92	2645.24	10674.82	8093.69	182.37
		B4	234.41	320.56	40766.58	3438.10	11240.79	10583.01	166.67
		B5	236.94	345.23	41207.75	3486.21	9718.50	8531.78	134.28
	NPK3	Control	207.50	215.62	36087.20	2501.00	8716.94	7620.97	134.78
		B1	254.29	269.51	44224.11	3350.98	11212.00	8054.37	216.26
		B2	300.12	264.93	52194.53	2766.67	10611.00	9999.50	294.23
		B3	287.97	227.96	50081.34	3506.81	9732.80	8058.87	166.17
		B4	241.49	287.17	41998.92	2473.31	9578.22	7836.17	201.06
		B5	213.71	332.30	37166.54	3621.43	10133.08	8139.83	166.67
T. durum	NPK1	Control	167.20	267.51	29078.13	1718.55	8627.71	7131.56	134.28
		B1	237.57	354.16	41317.25	3023.81	10276.04	7786.17	135.28
		B2	214.03	293.17	37222.04	2547.62	9710.19	7702.07	166.67
		B3	264.73	400.46	46039.63	2816.17	10018.12	7704.07	458.67
		B4	210.45	328.69	36600.87	3328.07	10748.05	8222.58	166.67
		B5	242.89	307.99	42241.09	5411.79	9618.84	7642.97	197.06
	NPK2	Control	241.49	290.42	41997.42	2304.21	7063.00	7559.37	102.90
		B1	243.42	282.73	42334.09	3242.36	10816.67	8054.87	167.67
		B2	241.50	320.08	41999.92	2550.12	10226.81	7649.57	151.48
		B3	277.17	323.85	48204.32	2806.07	9374.03	9118.79	181.37
		B4	247.75	311.04	43087.77	2414.79	10131.08	7094.96	217.26
		B5	248.09	321.55	43146.27	3024.31	9691.30	8054.37	201.56
	NPK3	Control	213.81	253.19	37184.04	2494.40	7596.50	7111.56	102.90
		B1	294.17	270.58	51160.68	2514.40	10131.50	8853.98	233.95
		B2	300.44	313.02	52250.03	2523.31	10416.89	7486.87	201.06
		B3	321.97	346.06	55995.06	4401.88	9163.33	8126.98	149.98
		B4	241.37	409.16	41977.92	2616.33	9813.92	7886.17	200.56
		B5	294.30	304.81	51182.18	4614.14	10521.74	7878.67	167.67
Tukey 0.01			7.25	1.879	1260.856	32.316	404.854	334.765	10.807

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli.* NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360kg.ha^{-1}

Yield components

The results presented in *Table 16* reveal that, compared with the control treatment, soil application of different rhizobial bacteria had a significant effect on some yield

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components. In addition, using B4 led to the maximum values of total dry weight of 54.31 g.plant^{-1}, spike weight of 8.09 g, grain number of 106.22, and biological yield of 7895.18 kg.ha^{-1}, and utilizing B3 resulted in the highest grain yield of 2823.15 kg.ha^{-1} and harvest index of 38.43%.

Table 16. *Effects of different species of rhizobial bacteria on some yield components*

Bacterial species	**Total dry weight g.pot^{-1}**	**Spike length plant^{-1}**	**Spike weight. plant^{-1}**	**Spikelet number plant^{-1}**	**Grain number plant^{-1}**	**Grain yield kg.ha^{-1}**	**Biological yield kg.ha^{-1}**	**Weight of 1000 grain (g)**	**Harvest index %**
Control	38.80	8.07	5.54	17.33	68.75	1819.77	5492.33	47.99	33.68
B1	52.73	8.51	7.31	18.50	95.28	2550.29	7464.03	48.49	34.28
B2	54.05	8.33	7.59	18.83	97.08	2708.82	7650.86	50.40	36.22
B3	53.95	8.33	7.53	18.33	95.72	2823.15	7636.71	53.47	38.43
B4	54.31	8.60	8.09	18.83	106.22	2780.06	7686.72	47.64	37.04
B5	53.11	8.80	7.76	19.17	105.83	2661.63	7517.50	46.68	35.54
Tukey 0.05	3.31	n.s.	0.70	n.s.	6.578	271.42	468.25	n.s.	4.45

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri,* B5: *Rhizobium leguminosarum bv phaseoli*

According to the results presented in *Table 17*, it can clearly be observed that adding 360 kg.ha^{-1} resulted in the maximum increment of total dry weight, spike weight, grain number, grain yield, and biological yield. Moreover, using 120 kg.ha^{-1} of NPK led to the highest value of harvest index.

According to *Table 18*, some yield components were affected by wheat species, such that *Triticum aestivum* surpassed *Triticum durum* regarding spike length, grain number, and harvest index, while *Triticum durum* surpassed *Triticum aestivum* significantly in terms of total dry weight, spike weight, grain yield, biological yield, and weight of 1000 grains.

Table 17. *Effects of different levels of chemical fertilizer on some yield components*

Chemical fertilizer (kg.ha^{-1})	**Total dry weight g.pot^{-1}**	**Spike length plant^{-1}**	**Spike weight plant^{-1}**	**Spikelet number plant^{-1}**	**Grain number plant^{-1}**	**Grain yield kg.ha^{-1}**	**Biological yield kg.ha^{-1}**	**Weight of 1000 grain (g)**	**Harvest index %**
NPK1	48.26	8.20	6.54	18.42	84.15	2452.47	6830.70	42.64	38.31
NPK2	49.44	8.33	7.16	18.42	93.76	2551.62	6998.19	42.41	37.12
NPK3	55.78	8.80	8.21	18.67	106.53	2632.70	7895.18	41.64	34.22
Tukey 0.05	1.91	n.s.	0.41	n.s.	3.80	156.86	270.6	n.s.	2.57

NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360 kg.ha^{-1}

Table 18. *Effects of wheat species on some yield components*

Wheat species	**Total dry weight g.pot^{-1}**	**Spike length plant^{-1}**	**Spike weight plant^{-1}**	**Spikelet number plant^{-1}**	**Grain number plant^{-1}**	**Grain yield kg/ha**	**Biological yield kg/ha**	**Weight of 1000 grain (g)**	**Harvest index %**
T. aestivum	45.19	9.72	6.47	18.50	105.94	2465.78	6396.43	42.03	38.91
T. durum	57.13	7.16	8.13	18.50	83.69	2648.79	8086.29	56.19	32.82
Tukey 0.05	1.299	0.67	0.28	n.s.	2.58	106.60	183.91	2.4	1.75

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The results presented in *Table 19* revealed that different species of rhizobial bacteria had a significant effect on some yield components of both wheat species. Applying B2 with *Triticum durum* resulted in the highest values of grain yield (2941.26 kg.ha^{-1}), total dry weight of plant (63.04 g.plant^{-1}), and biological yield (8922.86 kg.ha^{-1}). Also, B4 with *Triticum durum* led to the highest value of spike weight (9.21 g). Moreover, the highest value of grain number (127.50) was obtained by utilizing B5 with *Triticum aestivum*, and the maximum value of harvest index (45.76%) was obtained as a result of using B3 with *Triticum aestivum*.

Table 19. *Interaction effect of wheat species and rhizobial bacteria on some yield components*

Wheat species	Bacterial species	Total dry weight g.pot^{-1}	Spike length plant^{-1}	Spike weight plant^{-1}	Spikelet number plant^{-1}	Grain number plant^{-1}	Grain yield kg.ha^{-1}	Biological yield kg.ha^{-1}	Weight of 1000 grain (g)	Harvest index %
T. aestivum	Control	33.02	8.93	4.77	17.33	75.00	1631.83	4673.43	39.12	35.11
	B1	47.39	9.67	6.36	18.33	105.50	2342.69	6707.24	39.56	35.01
	B2	45.07	9.27	6.54	18.67	104.83	2476.37	6378.86	42.63	39.35
	B3	46.61	9.50	6.77	18.00	109.17	2939.37	6597.78	48.63	45.76
	B4	48.05	10.57	6.97	19.33	113.67	2663.52	6800.66	43.36	40.21
	B5	51.01	10.40	7.42	19.33	127.50	2740.90	7220.57	38.88	38.02
T. durum	Control	44.59	7.21	6.31	17.33	62.50	2007.71	6311.24	56.87	32.26
	B1	58.08	7.34	8.27	18.67	85.06	2757.88	8220.81	57.42	33.55
	B2	63.04	7.40	8.64	19.00	89.33	2941.26	8922.86	58.17	33.09
	B3	61.29	7.17	8.29	18.67	82.28	2706.93	8675.63	58.30	31.09
	B4	60.57	6.63	9.21	18.33	98.78	2896.60	8572.78	51.92	33.86
	B5	55.21	7.20	8.09	19.00	84.17	2582.37	7814.42	54.47	33.07
Tukey 0.05		5.409	n.s.	1.15	n.s.	10.76	443.82	765.67	n.s.	7.28

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*

In addition, the two wheat species were significantly different in terms NPK levels (see *Table 20*). In general, the highest values of most yield components were observed at NPK3 for both species, while the highest values of weight of 1000 grains and harvest index were obtained by adding 240 kg.ha^{-1} of NPK of *Triticum durum* and 120 kg.ha^{-1} of NPK *Triticum aestivum*, respectively.

Table 20. *Interaction effect of wheat species and chemical fertilizer on some yield components*

Wheat species	Chemical fertilizer kg.ha^{-1}	Total dry weight g.pot^{-1}	Spike length plant^{-1}	Spike weight plant^{-1}	Spikelet number plant^{-1}	Grain number plant^{-1}	Grain yield kg.ha^{-1}	Biological yield kg.ha^{-1}	Weight of 1000 grain (g)	Harvest index %
T. aestivum	NPK1	39.38	9.35	5.57	17.50	90.25	2346.15	5573.48	45.50	41.83
	NPK2	44.39	9.63	6.18	18.67	104.25	2503.26	6283.56	42.97	40.08
	NPK3	51.80	10.18	7.67	19.33	123.33	2547.93	7332.23	37.62	34.82
T. durum	NPK1	57.14	7.04	7.50	19.33	78.06	2474.48	8087.91	56.28	30.89
	NPK2	54.49	7.02	8.14	18.17	83.28	2648.27	7712.83	56.47	34.21
	NPK3	59.76	7.42	8.76	18.00	89.72	2823.62	8458.13	45.65	33.62
Tukey 0.05		3.31	n.s	0.70	n.s	6.58	271.42	468.25	6.19	4.45

NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360 kg.ha^{-1}

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The results presented in *Table 21* indicate the interaction effects of chemical fertilizer and rhizobial bacteria on some yield components. As observed, the highest values of total dry weight, spike weight, grain number, and biological yield were gained by adding 360 kg.ha^{-1} of NPK with B4, and the highest values of grain yield and harvest index were obtained by adding 240 kg.ha^{-1} of NPK with B4.

Table 21. *Interaction effect of chemical fertilizer and rhizobial bacteria on some yield components*

Chemical fertilizer kg.ha^{-1}	Bacterial species	Total dry weight g.pot^{-1}	Spike length plant^{-1}	Spike weight plant^{-1}	Spikelet number plant^{-1}	Grain number plant^{-1}	Grain yield kg.ha^{-1}	Biological yield kg.ha^{-1}	Weight of 1000 grain (g)	Harvest index %
NPK1	Control	31.36	7.97	4.68	17.50	64.00	1622.55	4439.26	47.55	36.54
	B1	50.36	8.37	6.80	18.00	88.42	2379.34	7127.62	47.34	33.78
	B2	55.73	8.25	7.02	19.00	90.42	2836.99	7888.65	55.11	38.06
	B3	50.95	8.10	6.59	18.50	86.92	2751.12	7211.61	56.83	39.79
	B4	49.64	7.80	6.88	18.50	84.92	2384.05	7026.19	49.81	35.51
	B5	51.51	8.70	7.24	19.00	90.25	2487.85	7290.87	48.70	34.47
NPK2	Control	43.07	8.25	6.31	18.00	78.00	1943.38	6096.25	45.01	31.90
	B1	54.18	8.25	7.21	18.00	99.50	2533.62	7669.26	47.47	33.05
	B2	50.68	8.00	7.25	18.00	93.58	2564.76	7173.39	49.80	36.06
	B3	48.18	8.10	6.96	18.00	87.50	2751.59	6819.53	55.53	41.99
	B4	50.44	8.75	7.780	19.00	103.00	3000.24	7139.42	51.39	42.37
	B5	50.10	8.60	7.44	19.50	101.00	2661.01	7091.30	49.14	37.48
NPK3	Control	41.98	8.00	5.63	16.50	64.25	1893.37	5941.50	51.42	32.61
	B1	53.66	8.90	7.93	19.50	97.92	2737.91	7595.19	50.66	36.02
	B2	55.75	8.75	8.50	19.50	107.25	2724.70	7890.54	46.28	34.54
	B3	62.73	8.80	9.05	18.50	112.75	2966.74	8878.98	48.04	33.49
	B4	62.84	9.25	9.58	19.00	130.75	2955.89	8894.55	41.73	33.23
	B5	57.72	9.10	8.59	19.00	126.25	2836.05	8170.32	42.19	34.68
Tukey 0.05		7.11	n.s.	1.51	n.s.	14.13	583.11	1005.98	n.s.	9.56

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli.* NPK1 = 120 kg.ha^{-1}, NPK2 = 240 kg.ha^{-1}, NPK3 = 360 kg.ha^{-1}

The results presented in *Table 22* indicate that combining different species of rhizobium with different levels of NPK had significant effects on some yield components including total dry weight of plant, weight of spike, number of grains, grain yield, biological yield, and harvest index in both species. As observed, the highest grain number was obtained in *Triticum aestivum* with 360 kg.ha^{-1} of NPK with B5, while the maximum total dry weight and biological yield were observed in *Triticum durum* with 120 kg.ha^{-1} of NPK with B2. Moreover, the greatest grain yield was achieved in *Triticum aestivum* by adding 120 kg.ha^{-1} of NPK with B3, and the highest value of spike weight was recorded in *Triticum durum* by adding 240 kg.ha^{-1} of NPK with B4. Furthermore, the highest value of harvest index was obtained in *Triticum aestivum* by adding 120 kg.ha^{-1} of NPK with B3. *Figure 1* indicates that soil application of 120 kg.ha^{-1} of NPK with B2 increased the grain yield by 111.19% compared to the control. The results also showed that combining the lower level of NPK fertilizer with rhizobial inoculation had the greatest effect on the yield components.

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Table 22. *Interaction effect of wheat species, chemical fertilizer and rhizobial bacteria on some yield components*

Wheat species	Chemical fertilizer ($kg.ha^{-1}$)	Bacterial species	Total dry weight $g.pot^{-1}$	Spike length. $plant^{-1}$	Spike weight. $plant^{-1}$	Spikelet number $plant^{-1}$	Grain number $plant^{-1}$	Grain yield $kg.ha^{-1}$	Biological yield $kg.ha^{-1}$	Weight of 1000 grain (g)	Harvest index %
T. aestivum	NPK1	Control	31.60	8.80	5.12	16.00	81.00	1702.29	4472.75	37.12	38.06
		B1	40.22	9.00	5.13	*17.00*	85.00	2036.33	5692.85	42.31	35.77
		B2	38.16	9.50	5.27	19.00	84.00	2415.66	5401.27	50.79	44.72
		B3	44.68	9.00	6.43	17.00	111.00	3362.11	6324.13	53.50	53.16
		B4	38.80	9.60	5.72	*18.00*	90.00	2346.78	5491.86	46.06	42.73
		B5	42.80	10.20	5.75	18.00	90.50	2213.73	6058.03	43.20	36.54
	NPK2	Control	37.76	9.00	5.27	18.00	86.00	1715.50	5344.66	35.23	32.10
		B1	52.88	10.00	6.96	18.00	122.00	2516.63	7484.78	36.43	33.62
		B2	44.88	9.00	6.37	18.00	107.50	2460.01	6352.44	40.42	38.73
		B3	37.96	9.20	5.48	18.00	86.50	2673.27	5372.97	54.59	49.75
		B4	42.22	10.60	5.55	20.00	92.50	2659.12	5975.94	50.78	44.50
		B5	50.66	10.00	7.48	20.00	131.00	2995.05	7170.56	40.38	41.77
	NPK3	Control	29.69	9.00	3.93	18.00	58.00	1477.71	4202.88	45.00	35.16
		B1	49.06	10.00	6.98	20.00	109.50	2475.11	6944.09	39.92	35.64
		B2	52.16	9.30	7.99	19.00	123.00	2553.43	7382.87	36.67	34.59
		B3	57.20	10.30	8.41	19.00	130.00	2782.73	8096.25	37.81	34.37
		B4	63.12	11.50	9.65	20.00	158.50	2984.67	8934.18	33.26	33.41
		B5	59.58	11.00	9.05	20.00	161.00	3013.92	8433.12	33.06	35.74
T. durum	NPK1	Control	31.13	7.13	4.25	19.00	47.00	1542.82	4405.76	57.98	35.02
		B1	60.49	7.73	8.48	19.00	91.83	2722.34	8562.40	52.36	31.79
		B2	73.31	7.00	8.76	19.00	96.83	3258.32	10376.03	59.432	31.40
		B3	57.22	7.20	6.75	20.00	62.83	2140.13	8099.08	60.16	26.42
		B4	60.48	6.00	8.05	19.00	79.83	2421.33	8560.51	53.57	28.28
		B5	60.22	7.20	8.73	20.00	90.00	2761.97	8523.71	54.20	32.40
	NPK2	Control	48.38	7.50	7.36	18.00	70.00	2171.27	6847.84	54.79	31.71
		B1	55.49	6.50	7.45	18.00	77.00	2550.60	7853.74	58.51	32.48
		B2	56.48	7.00	8.14	18.00	79.67	2669.50	7994.34	59.18	33.40
		B3	58.40	7.00	8.45	18.00	88.50	2829.91	8266.10	56.48	34.24
		B4	58.66	6.90	10.05	18.00	113.50	3341.35	8302.90	52.00	40.24
		B5	49.54	7.20	7.40	19.00	71.00	2326.96	7012.03	57.89	33.19
	NPK3	Control	54.26	7.00	7.32	15.00	70.50	2309.04	7680.11	57.85	30.065
		B1	58.26	7.80	8.88	19.00	86.33	3000.71	8246.28	61.39	36.39
		B2	59.33	8.20	9.02	20.00	91.50	2895.97	8398.21	55.90	34.48
		B3	68.26	7.30	9.69	18.00	95.50	3150.74	9661.71	58.27	32.61
		B4	62.56	7.00	9.52	18.00	103.00	2927.11	8854.92	50.19	33.06
		B5	55.87	7.20	8.14	18.00	91.50	2658.17	7907.53	51.31	33.62
Tukey 0.05			11.12	n.s	2.37	n.s.	22.11	912.23	1573.76	n.s.	14.95

B1: *Bradyrhizobium sp.* (Vigna), B2: *Rhizobium leguminosarum bv. viciae*, B3: *Bradyrhizobium* Mungbean, B4: *Mesorhizobium ciceri*, B5: *Rhizobium leguminosarum bv phaseoli*. NPK1 = 120 $kg.ha^{-1}$, NPK2 = 240 $kg.ha^{-1}$, NPK3 = 360 $kg.ha^{-1}$

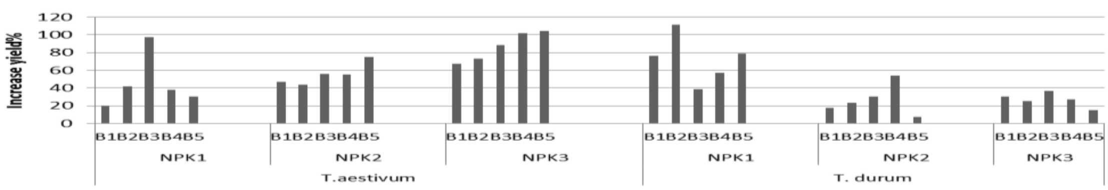

Figure 1. *Interaction effect of wheat species, chemical fertilizer, and rhizobial species on percentage increase of yield*

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Discussion

A common anthropogenic alteration to terrestrial systems is application of fertilizers. An increase in nutrient input can have a direct impact on soil microbial diversity or function by altering soil environments, or an indirect effect through plant-microbe feedbacks with potentially important effects on ecologically-important plant-associated mutualists (Simonsen et al., 2015). Chemical fertilizers can be used in order to increase soil nutrients and crop yields (Jia et al., 2018). Nitrogen (N), phosphorus (P), and potassium (K) are the major nutrients required by the crop, and the reproductive capability, growth, and yield of the plant can negatively be affected by inadequate supply of any of these nutrients during crop growth (Okechukwu, 2011). However, excessive use of mineral fertilizers can generate several environmental problems such as potential pollution to soil, water, and air (Stajković-Srbinović et al., 2014). The present study was aimed at minimizing the use of chemical fertilizer and improving wheat production and environmental risks. The results showed that combining chemical fertilizer and biofertilizer can improve wheat growth and development. The positive effects of the biofertilizer on physiological parameters, biochemical contents of leaves, and yield components under study can be attributed to the fact that rhizobial bacteria are involved in the production of phytohormone, leading to changes in root morphology and physiology that resulted in an increase in nutrient and water uptake of the wheat species from the soil (Mia and Shamsuddin, 2010). Adesemoye and Kloepper (2009) pointed out that plant–PGPR interactions can lead to various benefits such as improvement in seed shoot weights, germination rate, yield, chlorophyll content, leaf area, hydraulic activity, and protein content. Given the production of siderophores and nutrient uptake due to better root system, rhizobial bacteria have mostly been used to promote photosynthetic pigments. Research has indicated that bacterial production of siderophores enhances chlorophyll content and growth of plants due to the selective iron uptake from the solutions of trace elements, and production of siderophores inhibits free radical formation, substantiates the oxidative stress, and prevents the uptake of heavy metals (Ullah et al., 2017). Moreover, the role of *Rhizobium sp.* in enhancement of stress tolerance leads to an increase in cell membrane stability (Mia and Shamsuddin, 2010) because cell membranes are one of the first targets of many plant stresses (Bajji et al., 2002). Due to urgent need for increased global wheat production, wheat tolerance to biotic and abiotic stresses should be should be improved more (ElBasyoni et al., 2017). As pointed out by Mehboob et al. (2011), growth and yield of wheat can be promoted by isolates of *Rhizobium spp*. Rhizobium inoculation can lead to yield enhancement in wheat parameters, which can be attributed to the fact that applying biofertilizers have been proved to lead to high quality yield and avoid environmental pollution (Das et al., 2008). The findings of the present study are in agreement with those of the studies carried out by Agamy et al. (2012) and Adnan et al. (2016). It should be noted that plant species and their genotypes are genetically different in terms of their ability to uptake and metabolize elements.

Conclusion

Based on the results of the present study, it was observed that the combination between lower levels of NPK fertilizer and rhizobial inoculation had the greatest effect on yield components; therefore, such combinations are recommended to be utilized. Recently, biofertilizers have become an important means in agriculture because they use

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beneficial rhizobial bacteria to improve wheat production in a more economical and environmentally friendly way. Therefore, we suggested that the influence of combined effect of NPK fertilizer and rhizobial inoculation on growth and yield of wheat in soil needs further investigation to arrive at a conclusion where the effects are synergistic.

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