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The Effect of Foliar Spray with Manganese and Elements and Bio-Fertilization on Some of the Elements Absorption of Wheat Plant (*Triticum Aestivum* L.)

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Abstract: *A pot experiment was conducted in the autumn season 2019/2020 in a wire-house sunshade / the Technical College / Al-Mussaib, the experiment factors include: three levels of Manganese (0,40,80) mgm Mn.L^{-1} symbolized as (Mn_0,Mn_1,Mn_2) respectively , three levels of copper (0,10,20) mgm Cu.L^{-1} symbolized as (Cu_0,Cu_1,Cu_2) respectively , and two levels of biological fertilization (with and without) symbolized as (B.F_0 , B.F_1), the results showed that manganese spray when concentrating (80 mg .Mn L^{-1}) to a significant increase in all the studied parameters, including plant height as it increased from(76.83 to 83.55 cm), flag leaf area as it increased from(31.05 to 36.41 cm^2) , dry matter weight as it increased from (13.71 to 16.51 gm), manganese concentration in leaves as it increased from(706.47 to 750.86 µg.gm^{-1}) ,and copper concentration in leaves as it increased from(4.25 to5.60 µg.gm^{-1}) , also spraying with copper at a concentration of (10 mg .Cu L^{-1}) to a significant increase in all the studied parameters , including plant height as it increased from(77.42 to 82.88 cm), area of the flag leaves as it increased from(31.95 to 35.70 cm^2) , dry matter weight as it increased from (13.86 to 16.98 gm) , manganese concentration in leaves as it increased from (699.33 to 759.61 µg.gm^{-1}) ,and copper concentration in leaves as it increased from(4.55 to 5.20 µg.gm^{-1}), also the addition of biological fertilizer significantly affected all the studied parameters, including plant height as it increased from (75.39 to78.96 cm), flag leaf area as it increased from(31.71 to 32.62 cm^2) ,dry matter weight as it increased from(14.39 to 15.00 gm) , manganese concentration in leaves as it increased from(699.03 to 712.91 µg.gm^{-1}) , and copper concentration in leaves as it increased from (4.12 to 4.35 µg.gm^{-1}) .*

Keywords: *foliar nutrition; manganese; copper; bio fertilization; wheat.*

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a herbaceous plant and belongs to the Graminaceae family, and it is one of the most important strategic grain crops in Iraq and the world for its role in achieving food security. It is to deem one of the main sources of energy that the human body needs, and one of the most important advantages that made it important in food is the outstanding balance between proteins and carbohydrates in its grains [1], The total production for 2019 in Iraq was (4343). One thousand tons and the cultivated area reached (1.582 thousand hectare), while the average yield was (686.1 kg) [2].

Researchers have recently turned to introducing some modern technologies in agriculture, including foliar nutrition. Research has confirmed that 85% of the plant's need can be given through foliar nutrition by two methods, first by cytoplasmic bridges located under the cuticle layer to the epidermal cells and then the cytoplasm, second It travels through the stomata between the leaf cells and the inter-distances in the leaf down to the phloem [3].

Manganese plays an important role in many vital interactions within the plant, as it contributes to regulating the osmotic effort of plant cells, as it contributes to the Krebs cycle and to building the chlorophyll molecule, besides its participation in the process of protein and fat formation and has a role in activating a group of enzymes and important functions of manganese It works to increase the proportion of vitamin C and in regulating the closing and opening of stomata [4].

Copper is the other element of the micro-elements that play an important role in plant nutrition and plays an effective role in physiological biological processes inside the plant. It enters building nucleic acids RNA and DNA and raises the ability of plants to fix atmospheric nitrogen biologically. It involves polyphenol oxidase in the metabolism of photosynthesis and carbohydrate metabolism [5].

Many studies and research in the last four decades have dealt with the bio-fertilizers, as the scientific leap and recent discoveries have allowed scientists to develop new technologies to be introduced into agriculture which is called as integrated agriculture or sustainable agriculture to achieve an increase of yield eco-friendly and protection of the environment. Biological fertilizers as a group of microorganisms or addition of biological origin added to the soil or seeds or both in order to supply the plants with nutritional needs . Microbial inoculants have spread the use of biological fertilization recently worldwide by adding some beneficial and efficient microbes and using them as bacterial or fungal biological fertilizers, or both together. help to increase production as the amount of increase in the resulting crop can reach over 40% and the biological fertilizers increase the content of the crop of nutrients and compounds Energy compared to mineral fertilizers and the lower costs of producing animal fertilizers This increases the profit and income of the farmer and the investor, and the use of mineral fertilizers often leads to harmful environmental effects, including washing of nitrates into the groundwater. Therefore, there is a need for a nutrient management system to maintain productivity as it is consider vital fertilizers, including bacteria and root fungi from Successful alternatives in increasing nutrient readiness and stimulating plant growth [6]. In the light of above mentioned facts, the current this study came with the aim of:

1- Study the effect of adding biological fertilizer and relationship to the growth and concentrations of elements in the Wheat (Barcelona variety).

2- Study the effect of spraying with manganese and copper and the overlap between them and with the addition of biological fertilizer in the Wheat.

3- Determination of the best concentration for manganese and copper.

2. MATERIALS AND METHODS

The experiment was carried out on November 27, 2019 in mixed-tissue soil, as a sample of soil was taken before planting and brought from the surface horizon 0-30 cm to one of the fields of the technical college / Al-Mussaib to determine some chemical and physical characteristics as in (Table 1), the experiment was designed according to the design Completely Randomized Design (CRD) included three factors with three replicates, one repeater included 18 randomly distributed treatments and the number of experimental units reached 54 units, and the experiment factors were: The first factor (spraying manganese element in the form of manganese sulfate $MnSO_4.H_2O$ (Mn 32%) in three levels:(Mn_0 = 0 mg Mn. L^{-1} , Mn_1 = 40 mg Mn. L^{-1}, Mn_2 = 80 mg Mn .L^{-1}), while the second factor spraying copper element as $CuSO_4.5H_2O$ (Cu 25%) in three levels: (Cu_0 = 0 mg Cu. L^{-1}, Cu_1 = 10 mg Cu. L^{-1}, Cu_2 =20 mg. Cu L^{-1}), and the third factor: biological fertilizers in the form of fungal and bacterial fertilizers (mycorrhiza and a mixture of azotobacter bacteria) And azosperlum) and in two levels: ($B.F_0$ without addition, $B.F_1$, adding biological fertilizer) ,The experiment soil was dried air, milled, passed through a sieve with a hole diameter (4 mm), mixed well for the purpose of homogenization and placed in pots with dimensions (45 * 45) cm. Chemical fertilizers were added (NPK). For all treatments at the level of the full fertilizer recommendation, phosphorus was added at once and mixed with the soil before planting in the form of triple superphosphate (P% 48) and with (150 kg. H^{-1}). As for fertilizer N and K after planting, (100 kg. H^{-1}) potassium was added. In the form of potassium sulfate fertilizer (42% K), as for nitrogen, it was added by (160 kg. H^{-1}) in the form of urea fertilizer (46% N) in two batches, the first after two weeks of planting and the second 45 days after the first batch, according to the quantity The recommended [7], as for adding biological fertilizers, where the fungal inoculum was carried on the peat moss and 50 gm of the pollen was mixed under the surface layer of the potting soil to a depth of (5) cm, then the wheat seeds to be inoculated with the bacterial vaccine were mixed in a sterile plastic container for half an hour With the addition of gum arabic to ensure the adhesion of the pollen to the seeds, and they were sterilized according to the method [8], and then the seeds of wheat were planted, the Barcelona variety. At a rate of 30 seeds per pot, and after a week of germination, diluted to 10 plants in each pot, copper sulfate and manganese sulfate were sprayed in three stages of growth, which are the stage of branching, elongation and lining, using a (2 Liter) hand sprinkler and diluting the concentration with a small amount of Bubblegum to ensure wetness Completing the leaves and increasing the efficiency of the spray solution, the spraying process was carried out early in the morning to avoid high temperatures. As for the non-addition treatment, it was sprayed with distilled water. The wheat plants were harvested on 5/17/2020 after they reached the stage of full maturity.

Table 1. Soil characteristics in experiment

Soil characteristics	**Value**	**Unit**
Electrical conductivity (1:1)	3.2	$ds.m^{-1}$
pH (1:1)	7.8	--
Organic mater	2.1	$gm.kg^{-1}$
$CaSO_4$	45.9	
Available N	1.15	$mgm.kg^{-1}$
Available P	5.20	
Available K	1.109	
Available Mn	1.35	
Available Cu	0.75	
Micorrhiza fungi	36	$spur.gm^{-1}$ soil
Azotobacter	$4.5*10^9$	$CFU.gm^{-1}$ soil
Azosperllium	$3*10^5$	
Sand	540	$gm.kg^{-1}$
Silt	270	
Clay	190	
Texture	Loam	

2-1 Plant properties study

1) Plant height (cm): It was measured from the base of the plant at the level of the soil surface to the top of the spike in the major branch , at the stage of maturity for five plants per experimental unit.
2) The area of the flag leaf (cm^2): I took five flag papers for the main stems and for each experimental unit randomly according to the following equation: The length of the paper * the width of the paper from the middle * 0.95 [9].

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3) The dry matter weight of the plant (gm): I took five plants randomly from each experimental unit and cut the vegetative part with the level of the soil surface and put them in perforated paper bags and then these plant samples were dried in an oven at 70 C° for 48 hours after washing them with regular water and then with water Distilled and recorded her dry weights.
4) The concentration of manganese and copper in the leaves ($\mu g.gm^{-1}$): The plant samples were taken for each of the vegetative part in the stage of expelling the ears, washed with distilled water to remove the suspended substances in it, then it was dried at a temperature of 65 ° C for a period of 48 hours, then the samples were ground and mixed It was well homogenized and taken from it (0.2 g), then digested using concentrated sulfuric acid and perchloric acid. Then, copper and manganese were estimated using the atomic absorption device, according to what was mentioned in [10].

2-2 Statistical Analysis

The experiment were analyzed statistically according to the method of analysis of variance (ANOVA) using a completely randomized design (CRD), and the arithmetic averages were compared to the test of the least significant difference (LSD), at the level of significance (0.05). Genstat program was used in the statistical analysis [11].

3. RESULTS AND DISCUSSION

3-1- Plant height (cm)

The results of Table (2) show significant differences in the effect of the studied factors in the characteristic of plant height, as the biological fertilization treatment of $B.F_1$ exceeded significantly and gave the highest average plant height of (78.96 cm), while the treatment without pollination gave $B.F_0$ the lowest average It reached (75.39 cm), with an increase percentage of (4.73%). The reason for the increase in plant height when adding biological fertilizer is that A. chroococcum bacteria secrete growth-stimulating substances, the most important of which is (IAA) necessary to elongate cells and this is reflected in the increase in plant height, and these results are consistent with [12] , [13] who showed that there was a significant increase in plant height when adding bacterial bio-fertilizers.

As for spraying with manganese, the treatment Mn_2 significantly outperformed at concentration (80 mg. L^{-1}) and gave the highest average plant height of (83.55 cm) compared to the treatment without spraying Mn_0, which gave the lowest average of (76.83 cm) and an increase percentage of (8.74%). The reason may be due to the increase in plant height when spraying with manganese because manganese is an essential element in the process of photosynthesis, respiration and nitrogen metabolism, and therefore it acts as a stimulant for enzymes, and it cannot be replaced by other cations for some metabolic reactions in the plant as some Krebs cycle enzymes such as Malic Dehydogenas and Oxalosuccinic decarboxylase need manganese as a stimulant, and manganese is the predominant ionic mineral in the reactions of the Krebs cycle [14], and these results are in agreement with [15], who got a significant increase in plant height when spraying with manganese.

As for the effect of copper spraying, the treatment Cu_1 significantly outperformed at concentration (10 mg. L^{-1}) and gave the highest average plant height of (82.88 cm) compared to the treatment without spraying Cu_0, which gave the lowest average of (77.42 cm) with an increase percentage of (7.05%). The reason for the increase in plant height when spraying with copper may be attributed to the positive role of copper in the formation and division of plant cells and thus their elongation, through its contribution to the

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formation of important RNA in the protein's construction [16],[17]and these results are in agreement with [18] who got a significant increase in plant height when spraying with copper element.

As for the interaction between biological fertilization and manganese spraying, the treatment $B.F_1Mn_2$ significantly outperformed and gave the highest average plant height of (87.06 cm) compared to the treatment $B.F_0Mn_0$, which gave the lowest average of (71.48 cm) and an increase of (21.79%).

As for the interaction between biological fertilization and copper spraying, the treatment $B.F_1Cu_1$ significantly outperformed and gave the highest average plant height of (80.49 cm) compared to the treatment $B.F_0Cu_0$, which gave the lowest average of (71.77 cm) and an increase of (12.14%).

As for the interference between manganese and copper spraying, the treatment Mn_2Cu_1 significantly outperformed and gave the highest average plant height of (87.32 cm) compared to the treatment Mn_0Cu_0, which gave the lowest average of (74.37 cm) and an increase of (17.41%).

As for the interaction between bio-fertilization, manganese and copper, the treatment $B.F_1Mn_2Cu_1$ significantly outperformed and gave the highest average plant height of (92.40 cm) compared to the treatment $B.F_0Mn_0Cu_0$, which gave the lowest average of (66.97 cm) and an increase of (37.97%).

Table 2. Effect of Mn , Cu , and biofertilizer on plant height (cm)

Bio-fertilizer	**Mn ($mgm.L^{-1}$)**	**Cu ($mgm.L^{-1}$)**			**Average**
		0	**10**	**20**	
$B.F_0$	0	66.97	73.60	73.87	71.48
	40	69.47	77.43	77.00	74.63
	80	78.87	82.23	79.03	80.04
$B.F_1$	0	81.77	81.80	83.00	82.19
	40	84.03	89.83	79.53	84.47
	80	83.40	92.40	85.37	87.06
LSD0.05		3.44			1.98
Bio-fertilizer * Copper					
$B.F_0$		71.77	77.76	76.63	75.39
$B.F_1$		76.70	80.49	79.68	78.96
LSD0.05		1.98			1.14
Manganese * Copper					
0		74.37	77.70	78.43	76.83
40		76.75	83.63	78.27	79.55
80		81.13	87.32	82.20	83.55
LSD0.05		2.43			1.40
Average		77.42	82.88	79.63	
LSD0.05		1.40			

3-2- Flag leaf area (cm^2)

The results of Table (3) show that there are significant differences in the effect of the studied factors on the characteristic of the area of the flag sheet, as the treatment of biological fertilization $B.F_1$ significantly outperformed and gave the highest average for the area of the flag sheet of (32.62 cm^2), while the treatment without vaccination gave $B.F_0$ less Average reached (31.71 cm^2), with an increase of (2.86%). The reason for increasing the area of science leaf when adding biological fertilizers is attributed to the role of micro-organisms in the secretion of growth stimulants such as auxins, gibberellins and cytokines, which is reflected in improving plant growth and production, and these results are in agreement with [19] who got an increase in the leaf area when adding fertilizer. Dynamic analogy with no addition.

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As for spraying with manganese, the treatment Mn_2 significantly outperformed at concentration (80 mg. L^{-1}) and gave the highest average area of the flag leaf of (36.41 cm^2) compared to the treatment without spraying Mn_0, which gave the lowest value of (31.05 cm^2) with an increase of (17.26%) Perhaps the reason for the increase in leaf area when spraying with manganese is that spraying with manganese has caused an increase in the concentration of nitrogen, phosphorous and potassium, and that the increase of all these elements in plant tissues has contributed to increasing the efficiency of photosynthesis for its role in building and activating the work of enzymes involved in many processes. Physiology and building of amino acids and nuclear acids and energy transport compounds ATP and NADPH2 and the formation ofseveral of synthetics [20] and increasing the content of chlorophyll all this worked to increase the metabolism products and then provide a high storage of nutrients, reduce the state of competition between parts One plant and pushed towards better opportunities for the growth and expansion of cells and their elongation, building new cells, and then increasing the area of the flag leaf, and these results were in agreement with [21], which showed an increase in the paper area by increasing Manganese concentration in the nutrient solution.

As for the effect of copper spraying, the treatment Cu_1 significantly outperformed at the concentration (10 mg. L^{-1}) and gave the highest average surface area of the flag was (35.70 cm^2) compared to the treatment without spraying Cu_0, which gave the lowest value, which was respectively (31.95 cm^2) and by increasing its amount (11.73%). The reason for the increase in the area of the science sheet can be attributed to the effect of the micronutrients in performing many functions within the plant through the element's participation in the process of oxidation, reduction, respiration, and the formation of chlorophyll, and it also plays an important role in the electronic transport system of the photosynthesis process, and this leads to increase the manufactured materials that take part in the process of cell division and elongation, and this is consistent with what was observed by [22] who pointed out the effect of spraying with trace elements, including copper, in increasing the area of the flag.

As for the bilateral interaction between biological fertilization and manganese spraying, the treatment $B.F_1Mn_2$ significantly outperformed and gave the highest average for the area of the flag leaf of (39.22 cm^2) compared to the treatment $B.F_0Mn_0$, which gave the lowest average of (29.68 cm^2) and an increase of (32.14%) .As for the bilateral interaction between biological fertilization and copper spraying, treatment $B.F_1Cu_1$ significantly outperformed and gave the highest average leaf area of (34.04 cm^2) compared to the treatment $B.F_0Cu_0$, which gave the lowest average of (30.68 cm^2) with an increase of (10.95%).

As for the bilateral interference between manganese and copper spraying, the treatment Mn_2Cu_1 significantly outperformed and gave the highest average paper area of (40.05 cm^2) compared to the treatment Mn_0Cu_0, which gave the lowest average of (29.72 cm^2) with an increase of (34.75%).

As for the triple overlap between biological fertilization and manganese and copper spraying, the treatment $B.F_1Mn_2Cu_1$ significantly outperformed and gave the highest average for the area of the flag leaf of (44.50 cm^2) compared to the treatment $B.F_0Mn_0Cu_0$, which gave the lowest average of (28.53 cm^2) with an increase of (55.97%).

Table 3. Effect of Mn , Cu , and biofertilizer on flag leaf area (cm^2)

Bio-fertilizer	Mn (mgm.L^{-1})	Cu (mgm.L^{-1})			Average
		0	10	20	

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$B.F_0$	0	28.53	31.70	28.80	29.68
	40	31.40	32.53	31.60	31.84
	80	32.10	35.60	33.10	33.60
$B.F_1$	0	30.90	34.00	32.37	32.42
	40	32.73	35.87	35.57	34.72
	80	36.03	44.50	37.13	39.22
LSD0.05		2.16			1.24
Bio-fertilizer * Copper					
$B.F_0$		30.68	33.28	31.17	31.71
$B.F_1$		31.47	34.04	32.36	32.62
LSD0.05		1.24			0.72
Manganese * Copper					
0		29.72	32.85	30.58	31.05
40		32.07	34.20	33.58	33.28
80		34.07	40.05	35.12	36.41
LSD0.05		1.52			0.88
Average		31.95	35.70	33.09	
LSD0.05		0.88			

3-3-Plant dry matter (gm)

The results of Table (4) show that there are significant differences in the effect of the studied factors in the characteristic of dry matter weight, as the biological fertilization treatment $B.F_1$ significantly outperformed and gave the highest average dry matter weight of (15.00 gm), while the treatment without vaccination gave $B.F_0$ the lowest average. It reached (14.39 gm), with an increase of (4.23%) The reason for this increase in dry matter yield when adding the biological fertilizer is due to the positive effect in improving the nutritional nitrogen status in the plant because of the atmospheric fixation of nitrogen and the production of growth regulators [23],[24] and these results agree with [25] who showed that the addition of biological fertilizer led to a significant increase in dry matter weight.

As for spraying with manganese, the treatment Mn_2 significantly outperformed at concentration (80 mg. L^{-1}) and gave the highest average weight of dry matter, which was (16.51 gm) compared to the treatment without spraying Mn_0, which gave the lowest average of (13.71 gm) with an increase of (20.42%) This result is consistent with the findings of [26] who got a significant increase in the dry matter yield of the wheat when manganese was added to plant.

As for the effect of copper spraying, the treatment Cu_1 significantly outperformed at the concentration (10 mg.L^{-1}) and gave the highest average dry matter weight of (16.98 gm) compared to the treatment without spraying Cu_0, which gave the lowest average of (13.86 gm) with an increase of (22.51%). The reason for the increase in dry matter weight when spraying with copper is because of the positive effect of copper in activating many enzymes that contribute to oxygen reduction in the process of aerobic respiration, which helps in producing the energy needed for plants. Besides the role of copper in increasing plant absorption of many nutrients, especially macronutrients (N, P, K), which play an important role in increasing the weight of dry matter [27].

As for the interaction between biological fertilization and manganese spraying, treatment $B.F_1Mn_2$ significantly outperformed and gave the highest average dry matter weight of (16.89 gm) compared to treatment $B.F_0Mn_0$, which gave the lowest average of (12.79 gm) and an increase of (32.05%).

As for the bilateral interaction between biological fertilization and copper spraying, treatment $B.F_1Cu_1$ significantly outperformed and gave the highest average dry matter weight of (16.39 gm) compared to treatment $B.F_0Cu_0$ which gave the lowest average of (13.42 gm), with an increase of (22.13%).

As for the bilateral interference between manganese and copper spraying, the treatment Mn_2Cu_1 significantly outperformed and gave the highest average dry matter weight of (19.27 gm) compared to the treatment Mn_0Cu_0, which gave the lowest average of (13.03 gm) with an increase of (47.88%).

As for the triple interaction between biological fertilization and manganese and copper spraying, treatment $B.F_1Mn_2Cu_1$ significantly outperformed and gave the highest average the dry matter weight was (19.77 gm) compared to the treatment $B.F_0Mn_0Cu_0$ which gave the lowest average of (12.17 gm), with an increase of (62.44%).

Table 4. Effect of Mn , Cu , and biofertilizer on dry matter of plant (gm)

Bio-fertilizer	Mn (mgm.L^{-1})	Cu (mgm.L^{-1})			Average
		0	10	20	
$B.F_0$	0	12.17	13.23	12.97	12.79
	40	13.47	15.00	14.27	14.24
	80	14.63	18.77	14.97	16.12
$B.F_1$	0	13.90	15.40	14.60	14.63
	40	14.57	19.70	15.40	16.56
	80	14.43	19.77	16.47	16.89
LSD0.05		1.20			0.69
Bio-fertilizer * Copper					
$B.F_0$		13.42	15.67	14.07	14.39
$B.F_1$		14.00	16.39	14.61	15.00
LSD0.05		0.69			0.40
Manganese * Copper					
0		13.03	14.32	13.78	13.71
40		14.02	17.35	14.83	15.40
80		14.53	19.27	15.72	16.51
LSD0.05		0.85			0.49
Average		13.86	16.98	14.78	
LSD0.05		0.49			

3-4- Leaf manganese content (µg.gm^{-1})

The results of Table (5) show significant differences in the effect of the studied factors in the characteristic of manganese concentration in leaves, as the treatment of biological fertilization $B.F_1$ exceeded significantly and gave the highest average of manganese concentration in leaves reached (712.91 µg.gm^{-1}), while the treatment gave $B.F_0$ without inoculation, the lowest value was (699.03 µg.gm^{-1}), with an increase of (1.98%). The reason for this may be attributed to the fact that inoculation with micro-organisms increases the shoots content of manganese [28] , [29].

As for spraying with manganese, the treatment Mn_2 significantly outperformed at concentration (80 mg. L^{-1}) and gave the highest average concentration of manganese in leaves of (750.86 µg.gm^{-1}) compared to the treatment without spraying Mn_0, which gave the lowest average of (706.47 µg.gm^{-1}) With an increase of (6.28%). The reason for the increase in the amount of manganese in the plant is attributed to the increase in the concentration of manganese in the dry matter with the increase in the level of addition,

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and this result was in agreement with the findings of many researchers who showed an increase in the amount of manganese in the plant by increasing the level of addition [30] and the results from both [31] , [32] who got an increase in the concentration of manganese in the dry matter of the plant by increasing the level of addition and this shows the clear response to the addition of manganese by spraying in both the concentration of the absorbed amount of this Element.

As for the effect of copper spraying, the treatment Cu_1 significantly outperformed at the concentration (10 mg. L^{-1}) and gave the highest average concentration of manganese in the leaves of (759.61 µg.gm^{-1}) compared to the treatment without spraying Cu_0, which gave the lowest average of (699.23 µg.gm^{-1}), with an increase of 8.63%. This is attribute to the role of copper in protein formation through its contribution to building nucleic acids and reducing nitrates [33].

As for the bilateral interaction between biological fertilization and manganese spraying, the treatment $B.F_1Mn_2$ significantly outperformed and gave the highest mean concentration of manganese in leaves of (788.21 µg.gm^{-1}) compared to the treatment $B.F_0Mn_0$, which gave the lowest average of (685.64 µg.gm^{-1}) With an increase of (14.95%).

As for the two-way interaction between biological fertilization and copper spraying, treatment $B.F_1Cu_1$ significantly outperformed and gave the highest mean concentration of manganese in leaves of (738.47 µg.gm^{-1}) compared to treatment $B.F_0Cu_0$, which gave the lowest average of (677.67 µg.gm^{-1}) an increase of (8.97%).

As for the bilateral interaction between manganese and copper spraying, the treatment Mn_2Cu_1 significantly outperformed and gave the highest mean concentration of manganese in leaves, which was (801.67 µg.gm^{-1}) compared to the treatment Mn_0Cu_0, which gave the lowest average of (676.27 µg.gm^{-1}) an increase of (18.54%).

As for the triple interaction between biological fertilization and spraying of manganese and copper, the treatment $B.F_1Mn_2Cu_1$ significantly outperformed and gave the highest mean concentration of manganese in leaves of (863.20 µg.gm^{-1}) compared to treatment $B.F_0Mn_0Cu_0$, which gave the lowest average of (666.17 µg.gm^{-1}) an increase of (29.57%).

Table 5. Effect of Mn , Cu , and biofertilizer on leaf manganese content (µg.gm^{-1})

Bio-fertilizer	**Mn (mgm.L^{-1})**	**Cu (mgm.L^{-1})**			**Average**
		0	**10**	**20**	
$B.F_0$	0	666.17	702.60	688.17	685.64
	40	678.13	713.93	701.77	697.94
	80	688.70	740.13	711.67	713.50
$B.F_1$	0	686.37	761.33	734.20	727.30
	40	726.50	776.43	762.07	755.00
	80	749.53	863.20	751.90	788.21
LSD0.05		26.86			15.50
Bio-fertilizer * Copper					
$B.F_0$		677.67	718.89	700.53	699.03
$B.F_1$		684.40	738.47	715.88	712.91
LSD0.05		15.50			8.95
Manganese * Copper					
0		676.27	731.97	711.18	706.47
40		702.32	745.18	731.92	726.47
80		719.12	801.67	731.78	750.86
LSD0.05		18.99			10.96

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Average	699.33	759.61	724.69	
LSD0.05	10.96			

3-5- Leaf copper content (µg.gm^{-1})

The results of Table (6) show significant differences in the effect of the studied factors on the characteristic of copper concentration in leaves, as the treatment of biological fertilization $B.F_1$ exceeded significantly and gave the highest average concentration of copper in the leaves, reaching (4.35 µg.gm^{-1}), while the treatment gave Without $B.F_0$ vaccination, the lowest value was (4.12 µg.gm^{-1}), with an increase of (5.58%). The reason for this may be attributed to the role of these organisms in the secretion of organic acids and chelating materials for many trace elements [34].

As for spraying with manganese, the treatment Mn_2 significantly outperformed at concentration (80 mg. L^{-1}) and gave the highest average concentration of copper in leaves, reaching (5.60 µg.gm^{-1}), compared to the treatment without spraying Mn_0, which gave the lowest average of (4.25 µg.gm^{-1}), with an increase of (31.76%). The reason for this is due to the increase in manganese concentration in dry matter. These results are consistent with the results of [35] and with the results [36] who mention to the significant effect of adding micronutrients to the absorption of macronutrients and micronutrients when adding manganese to the wheat plant.

As for the effect of copper spraying, the treatment Cu_1 significantly outperformed at the concentration (10 mg. L^{-1}) and gave the highest average concentration of copper in the leaves of (5.20 µg.gm^{-1}) compared to the treatment without spraying Cu_0, which gave the lowest average of (4.55 µg.gm^{-1}), with an increase of (14.28%). The increase in the concentration of copper is due to the fact that the fertilizer concentration added by spraying the vegetative total increased the amount of copper in the leaves, which caused an increase in its absorption and thus an increase in its concentration in the dry matter [37]. These results are consistent with the findings of Ars. And the results of [38].

As for the two-way interaction between biological fertilization and manganese, treatment $B.F_1Mn_2$ significantly outperformed and gave the highest average concentration of copper in leaves of (6.68 µg.gm^{-1}) compared to treatment $B.F_0Mn_0$, which gave the lowest average of (3.91 µg.gm^{-1}). And by an increase of (70.84%).

As for the bilateral interaction between biological fertilization and copper spraying, treatment $B.F_1Cu_1$ significantly outperformed and gave the highest average concentration of copper in leaves, reaching (4.58 µg.gm^{-1}) compared to treatment $B.F_0Cu_0$, which gave the lowest average of (3.89 µg.gm^{-1}). An increase of (17.73%).

As for the bilateral interference between manganese and copper, the treatment Mn_2Cu_1 significantly outperformed and gave the highest average concentration of copper in the leaves, reaching (6.11 µg.gm^{-1}) compared to the treatment Mn_0Cu_0, which gave the lowest average of (4.06 µg.gm^{-1}) with an increase of (50.49 %).

As for the triple interaction between bio-fertilization, manganese and copper, the treatment $B.F_1Mn_2Cu_1$ significantly outperformed and gave the highest average copper concentration in leaves of (7.39 µg.gm^{-1}) compared to the treatment $B.F_0Mn_0Cu_0$, which gave the lowest average of (3.69 µg.gm^{-1}) amount is (100.27%).

Table 6. Effect of Mn , Cu , and biofertilizer on Copper leaf content (%)

Bio-fertilizer	**Mn (mgm.L^{-1})**	**Cu (mgm.L^{-1})**			**Average**
		0	**10**	**20**	

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$B.F_0$	0	3.69	4.05	3.99	3.91
	40	3.82	4.08	3.93	3.94
	80	4.15	4.83	4.56	4.51
$B.F_1$	0	4.42	4.82	4.50	4.58
	40	5.59	6.03	5.94	5.85
	80	5.65	7.39	7.01	6.68
LSD0.05		0.32			0.18
Bio-fertilizer * Copper					
$B.F_0$		3.89	4.32	4.16	4.12
$B.F_1$		4.13	4.58	4.33	4.35
LSD0.05		0.18			0.10
Manganese * Copper					
0		4.06	4.43	4.25	4.25
40		4.71	5.06	4.93	4.90
80		4.90	6.11	5.79	5.60
LSD0.05		0.23			0.13
Average		4.55	5.20	4.99	
LSD0.05		0.13			

4. CONCLUSIONS

1) The addition of biological fertilizer to the wheat plant led to a significant increase in all the studied characteristics, including plant height, leaf area, dry matter weight, manganese concentration in leaves, and copper concentration in leaves compared to control.
2) Spray with manganese Mn_2 at a concentration of 80 mg Mn. L^{-1} Achieved significant increase in all the studied traits, including plant height, leaf area, dry matter weight, manganese concentration in leaves, and copper concentration in leaves.
3) The effect of spraying with the copper of the wheat plant was significant in all the studied characteristics, including plant height, leaf area, dry matter weight, manganese concentration in leaves, copper concentration in leaves, and that the concentration was 10 mg Cu. L^{-1} achieved the highest results compared to the control.
4) The results showed that the double and triple interactions were significant for all the studied traits of the wheat, including plant height, leaf area, dry matter weight, manganese concentration in leaves and copper concentration in leaves. The treatment $B.F_1Mn_2$ gave the highest value compared to treatment $B.F_0Mn_0$ Treatment $B.F_1Cu_1$ gave the highest value compared to treatment $B.F_0Cu_0$, while treatment Mn_2Cu_1 gave the highest value compared to treatment Mn_0Cu_0 and treatment $B.F_1Mn_2Cu_1$ gave the highest value compared to the control $B.F_0Mn_0Cu_0$.

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