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Effect of fungal inoculation and different levels of chemical fertilization on wheat growth and production (*Triticum aestivum* L.)

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ABSTRACT

The field experiment was carried out in Al-Bidaa area in Al-Shatra district using RCBD design and three replications to study the effect of biofertilizer with *Trichoderma harzianum* and *Mycorhiza* and their interaction and three levels of chemical fertilization are 0, 50% and 100% of wheat fertilizer recommendation (urea 200 kg N ha^{-1}, triple superphosphate 100 kg P_2O_5 ha^{-1} and potassium sulfate 80 kg K_2O ha^{-1}) and its effect on plant height, dry weight of vegetative total, total yield to seed and plant content of NPK at flowering stage. The results showed that fungal inoculation treatments significantly exceeded the rest of the treatments and gave the interaction treatment between *Trichoderma harzianum* and *Mycorhiza* fungi and 100% level of chemical fertilization highest values in dry weight, total wheat yield and plant content of nitrogen and phosphorus by 168.81%, 64%. 69%, 89.54% and 94.76% respectively, compared to the comparative treatment (non-inoculated and non-chemically fertilized), which gave the lowest values for the above studied traits, and the overlap treatment between the mixed fungal inoculation and the level of 50% of the fertilizer recommendation chemical (A3K1) was not significantly different from the treatment of chemical fertilization by 100% of the fertilizer recommendation chemical, whether fungal or non- inoculated. This indicates that the mixed fungal inoculation has reduced the need for chemical fertilization by 50%.

Key words: *Fungal inoculation, Wheat growth, Chemical fertilization*

Introduction

Mixed bio-inoculation between different rhizospheric organisms play a pivotal role in the rhizosphere by dissolving and transporting nutrients and then being absorbed by the plant. Therefore, it is necessary to make a combination of different microorganisms useful in the agriculture system to enhance the effectiveness of some external agricultural interventions (Hayat *et al.*, 2010; AL-Taey *et al*., 2019).

The success of crops relies upon supplement contribution amid development. Sole utilization of chemical fertilizers frequently decays soil fertility and the resultant harvest efficiency because of supplement irregularity in the soil (AL-Taey *et al*., 2017), *Trichoderma harzianum* is a widely used biocontrol fungus, and a number of experiments in many countries have shown that inoculation of plants with *Trichoderma harzianum* has increased the readiness of certain nutrients such as nitrogen, phosphorus and potassium by secreting certain enzymes. It is highly capable of decomposing existing or added organic matter to the soil, as well as increasing the resistance of the plant host against certain pathogens (Harman, 2000; AL-Taey and Majid, 2018).

Al-*Mycorhiza* (AM) is an important part of soil

biology and accounts for more than 50% of its biomass (Smith and Read, 2008), Studies have confirmed the ability of *Mycorhiza* fungi to improve plant growth and increase its dry weight, Increasing dry weight to its ability to produce growth hormones such as indole acidic acid, gibberellins, cytokines, vitamins and amino acids that improve the plant's root total and increase absorption of key nutrients such as nitrogen, phosphorus and potassium, which is reflected in the dry weight increase of the plant (Al-Khaleil *et al*., 2010).

Wheat plant (*Triticum aestivum* L.) belongs to the family of grasslands of strategic crops, which represents the agricultural of the first place among the rest of the crops, because it contains a high nutritional value of proteins and carbohydrates, which are found in balanced proportions in the grain of wheat in addition to containing the grain of wheat on the amounts of fat, vitamins and salts mineral and essential amino acids needed by the human body in its nutrition (Omer *et al*., 2016; AL-Juthery *et al*., 2018). Therefore, the present study aims to show the role of fungal inoculation and chemical fertilization and their interaction in improving the growth and production of wheat crop and its content of major nutrients.

Materials and Methods

The field experiment was carried out in Al-Bidaa area in Al-Shatrah district using RCBD design and three replications to study the effect of fungal inoculation and chemical fertilization levels and their interaction on the growth and production of wheat plants and their content of NPK.

The experiment included two factors, the first four treatments of fungal inoculation are non- inoculation, *Trichoderma harzianum* fungi, *Mycorhiza* fungi and mixed fungal inoculation and symbolized by A0, A1, A2 and A3 respectively, while the second factor three levels of chemical fertilizer are 0, 50% and 100% of the recommendation compost and symbolize by K0, K1 and K2 respectively.

The field was plowed and its soil characteristics are shown in Table 1, then leveled, softened and divided into three replicates. Each repeater included 12 experimental units of 4×3 m and left a distance of 1 meter between the experimental unit and the other.

Inoculates were prepared for *Trichoderma harzianum* fungal after isolation from the soil by dilution and dish countingmethod (Black, 1965) and loaded on organic matter (plant residues). The numerical density of the fungus was 4.1×106 CFU g^{-1} in the prepared fungal Inoculate.

Table 1. Some Physical and Chemical Characteristics of Field Experimental Soil

Traits	Units	Values
Electrical conductivity	dS m^{-1}	4.54
pH		7.3
Organic matter	g Kg $soil^{-1}$	4.35
$CaCO_3$		304.5
Avilable Nitrogen	mg Kg $soil^{-1}$	47.6
Avilable Phosphorus		23.8
Avilable Potassium		215
Analysis of minute volumes		
Sand	g kg^{-1} soil	239
Silt		448
Clay		313
Texture	Loam	

The *Mycorhiza* Inoculate was obtained from the College of Agriculture-University of Diyala, the infested roots were examined under a microscope to confirm the incidence of *Mycorhiza* roots after staining with Trypan Blue according to Phillips and Hayman, (1970), the inoculate using wet sieving and purification method according to Gerdmann and Nicolson, (1963) and the pollen density was 12355 spore per 100 m^{-1} dry soil, while the incidence of roots with *Mycorhiza* was 93.1%.

Added inoculates *Trichoderma harzianum, Mycorhiza* and mixed fungal inoculate to the soil by mixing them with the surface layer of the soil three weeks before planting and three replicates, and some experimental units were left uninoculated as comparative treatments.

Sterilized wheat seeds Class Adana 99 (non-distilled) by washing with distilled water and then sterilized superficially using a minor solution for 5 minutes and then washed with sterile distilled water to remove the residue of sterilization solution and then planted the seeds dated 19/10/2018 on form of a swarm in lines (7 lines by long 4 meters for each experimental unit) and 4.8 g per line based on seed quantity of 120 kg ha^{-1}.

Three levels of chemical fertilization were added by 0, 50% and 100% of the fertilizer recommendation for wheat plant (280 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 80 kg K_2O ha^{-1}) in the form of urea fertilizer (N% 46) in two batches. The first was added in planting, the second was added at the stage of veg-

etative growth after 45 days of planting. Concentrated superphosphate fertilizer (21% P) was added in one batch in planting and potassium sulphate fertilizer added (51% K) in two batches,the first was added in planting and the second in flowering stage.

Two weeks after the growth of plants, the service was carried out to lighten the plants to one plant, and weeding operations were conducted manually to get rid of the bush.

At the maturity of the crop physiologically in 14/4/2019 ten plants were randomly harvested from each experimental unit to study the dry weight of the part vegetation after washing the harvested plants with distilled water andthe samples were air dried and then placed in perforated paper bags in an electric oven at 65 °C for 48 hours until the weight was stabilized and then the dry weight. The total grain yield was calculated from the ten harvested plants after discharging and cleaning of the harvested spikes and was calculated averageone plant product and multiplied by the total number of plants by hectare (El-Sahookie, 1990).

Plant samples were taken (leaf immediately below spike) and milled after drying in oven at 65 °C for 48 hours until weight was established (Page *et al*., 1982), then 0.2 g of leaf samples were taken and then digested with concentrated sulfuric acid according to B-Evenhuis *et al*., (1976). The total nitrogen concentration was estimatedby the method of Kildal according to Chapman and Parker, (1961), the concentration of phosphorus in the plant by the method of ammonium molybdate and measurement by Spectrophotometer according to Haynes, (1980).

Results and Discussion

Dry weight to plant (g^{-1})

The results of Table 2 show that the treatment of mixed fungal inoculation (A3) significantly exceeded all the studied treatments in the average dry weight of the vegetative part of the wheat with the highest dry weight rate of 1251.8 $g.m^{-2}$ and a relative increase of 34.30% compared to the uninoculated treatment (A0) which gave the lowest plant height (932.1 $g.m^{-2}$), this increase in dry weight of vegetative total was due to the role of *T.harzianum* and *Mycorhiza* mushrooms in increasing the activity, growth and development of plant root total and this increases the plant's ability to absorb water and elements,the role of fungi in the decomposition of organic matter in the soil and its secretion of some Vehicles coenzymes that lead to liberation or release of some nutrients such as phosphorus, potassium, iron, and thus increasing the readiness of the plant (Haran, 2011; Newborn, 2012).

Chemical fertilization treatments differed significantly and the fertilization treatment of 100% of the chemical fertilizer recommendation (K2) gave the highest rate of dry weight of wheat and reached 1380.2 $g.m^{-2}$ with a relative increase of 98.25% compared to the non-fertilization treatment (K0) which gave the lowest rate (696.6 $g.m^{-2}$), These results are consistent with (Omer *et al*., 2016) in his study on chemical fertilization of crops.

The interaction between fungal inoculation and chemical fertilization increased the dry weight rate of wheat by more than the effect of individual factors, the interaction between the mixed fungal inoculation and the level of 100% of the fertilizer chemical recommendation (A3K2) was significantly superior to most other interference factors, the highest dry weight was recorded at 1484.9 $g.m^{-2}$ with a relative increase of 168.81% compared to the comparative treatment (non-inoculated and non-fertilized (A0K0)) which gave the lowest dry weight rate of 552.4 $g.m^{-2}$, and the interference treatment between mixed fungal inoculation and chemical fertilization with 50% of the fertilizer recommendation

Table 2. Effect of fungal inoculation and chemical fertilization levels on dry weight to plant ($g\ m^{-1}$).

Fungal inoculation	Nitrogen fertilizer levels			The average
	K0	K1	K2	
A0 Non inoculation	552.4	930.2	1313.6	932.1
A1 Inoculation by *T. harzianum*	621.9	1030.6	1317.0	989.8
A2 Inoculation by *Mycorhiza*	708.5	1114.7	1405.2	1076.1
A3 Mixed Inoculation	903.7	1366.8	1484.9	1251.8
LSD		234.13		
The average	696.6	1110.6	1380.2	LSD
LSD		117.07		74.03

(A3K1) was not significantly different from the treatment of 100% chemical fertilization, whether fungal or non-inoculated, this indicates that the mixed fungal inoculation has reduced the need for chemical fertilization by 50%, this increase in the dry weight of the vegetative total may be attributed to the ability of bio-inoculation to fixed atmospheric nitrogen and thus increase its absorption, this can satisfy the plant's need for this important nutrient, which is involved in the construction of the chlorophyll molecule, nucleic acids (RNA and DNA) and in the synthesis of amino acids and proteins, all of which contribute to the weight of the plant's dry matter, as well as fungal bio-inoculation contribute to the dissolution of compounds containing phosphorus and potassium in the soil and increase the readiness of the plant, as well as the contribution of fungi to improve the growth of the root total and increase the density because production including growth Alaoxinat and Aljprlinat and Alsetukainyin at which increases the plant's ability to absorb water and nutrients from soil solution (Megawer and Mahfouz, 2010).

Total yield plant (kg ha^{-1})

The results of Table 3 show the significant effect of fungal inoculation treatment on the total grain yield compared to non-inoculation, the mixed fungus treatment (A3) recorded the highest rate of total grain yield of 6023 kg ha^{-1} with a relative increase of 15.32% compared to the non- inoculated treatment (A0) which gave the lowest rate of total yield (5223 kg ha^{-1}), this is consistent with the Hadithi, (2012) which received a 20% increase in wheat yield when used *Trichoderma harzianum* stuck compared to non-use, and may also be attributed to the role of *Mycorhiza* fungi in increasing the plant's absorptive capacity through the development of root total and the extension of haifa in the soil and extract nutrients and thus increase the composition of dry matter in the plant, which is reflected in the increase onincrease the total grain yield (Joachim *et al*., 2009).

Chemical fertilization treatments differed significantly and the fertilization treatment at 100% of the chemical fertilizer recommendation (K2) gave the highest total wheat production rate of 6400 kg ha^{-1} and a relative increase of 48.22% compared to the non-fertilization treatment (K0) which gave the lowest rate (4318 kg ha^{-1}), this is due to the role of chemical fertilization in increasing the readiness of the necessary nutrients to build plant cells and the formation of dry matter and thus increase production, which is consistent with Megawer and Mahfouz, (2010).

Bilateral interference between fungal inoculation and chemical fertilization resulted in a greater increase in the total yield of wheat than the effect of individual factors, the interaction between the mixed fungal inoculation and the level of 100% of the fertilizer chemical recommendation (A3K2) was significantly superior to most of the other interference factors, the highest rate of total grain yield was 6790 kg ha^{-1} with a relative increase of 64.69% compared to the comparative treatment (uninoculated and non-fertilized (A0K0)) which gave the lowest rate of total yield of 4123 kg ha^{-1}, and the interference treatment between mixed fungal inoculation and chemical fertilization with 50% of the fertilizer recommendation (A3K1) was not significantly different from the treatment of 100% chemical fertilization, whether fungal or non-inoculated, this indicates that the mixed fungal inoculation has reduced the need for chemical fertilization by 50%, and this increase is due to the total grain yield when inoculated soil *Mycorhiza* and *Trichoderma harzianum* to its role in increasing the readiness of macronutrients and the production of growth hormones that play an important role in increasing the branching of

Table 3. Effect of fungal inoculation and chemical fertilization levels on total yield plant (kg ha^{-1}).

Fungal inoculation	Nitrogen fertilizer levels			The average
	K0	K1	K2	
A0 Non inoculation	4123	5337	6209	5223
A1 Inoculation by *T. harzianum*	4167	5508	6312	5329
A2 Inoculation by *Mycorhiza*	4240	5639	6287	5389
A3 Mixed Inoculation	4742	6538	6790	6023
LSD		396.0		
The average	4318	5756	6400	LSD
LSD		247.4		172.6

roots and its role in cell division and expansion and thus increase plant growth and production, and the presence of these organisms has an important role in increasing the efficiency of photosynthesis by increasing the surface area of the total leaves and increased production of carbon compounds, which in turn move to cereals and increase the amount of grain yield (Sheng *et al*., 2008).

Percentage of nitrogen in the vegetative part of the plant

Table 4 indicates that there was a significant effect of the mixed fungal inoculation (A3) on the percentage of nitrogen compared with the rest of the studied treatments, where the highest percentage of nitrogen was recorded at 3.396 with a relative increase of 24.17% compared to the unfertilized treatment (A0) which gave the lowest rate of percentageof nitrogen (2.735),this increase in plant nitrogen content was confirmed by Haran,(2011) and Hadithi,(2012) who noted that the dense and deep roots formed by plants treated with *T.harzianum* and *Mycorhiza* are able to increase the amount of nitrogen absorbed by the plant.

The chemical fertilization treatments differed significantly and the fertilization treatment at 100% of the chemical fertilizer recommendation (K2) gave the highest percentage of nitrogen percentage to 3.518 and a relative increase of 50.34% compared to the non-fertilization treatment (K0) which gave the lowest rate (2.340), This is due to the role of chemical fertilization in increasing the readiness of the necessary nutrients of the plant and this is consistent with Omer *et al*.,(2016) Which confirmed that the absorption of essential elements such as nitrogen due to increased levels of addition of chemical fertilizer and its reflection on the increase of plant nitrogen content.

Bilateral interference between fungal inoculationand chemical fertilization resulted in an increase in the percentage of nitrogen more than the effect of the individual factors, the interference treatment between the mixed fungal inoculationand the level of 100% of the chemical fertilizer recommendation (A3K2) was significantly superior to most other interference factors and the highest percentage of nitrogen was recorded at 3.823 with a relative increase of 89.54% compared to the comparative treatment (un inoculated and un fertilized (A0K0)) which gave the lowest rate of nitrogen percentage at 2.017, and the interference treatment between mixed fungal inoculation and chemical fertilization with 50% of the fertilizer recommendation (A3K1) was not significantly different from the treatment of 100% chemical fertilization, whether fungal or non-inoculated, this indicates that the mixed fungal in-

Table 4. Effect of fungal inoculation and chemical fertilization levels on %N in the vegetative part.

The average	Nitrogen fertilizer levels			Fungal
	K0	K1	K2	inoculation
A0 Non inoculation	2.017	2.812	3.376	2.735
A1 Inoculation by *T. harzianum*	2.258	3.099	3.409	2.922
A2 Inoculation by *Mycorhiza*	2.323	3.160	3.462	2.982
A3 Mixed Inoculation	2.763	3.603	3.823	3.396
LSD		0.4213		
The average	2.340	3.169	3.518	LSD0.
LSD		0.1973		2907

Table 5. Effect of fungal inoculation and chemical fertilization levels on %P in the vegetative part.

The average	Nitrogen fertilizer levels			Fungal
	K2	K1	K0	inoculation
A0 Non inoculation	0.210	0.274	0.326	0.270
A1 Inoculation by *T. harzianum*	0.243	0.315	0.351	0.303
A2 Inoculation by*Mycorhiza*	0.261	0.328	0.368	0.319
A3 Mixed Inoculation	0.274	0.366	0.409	0.350
LSD		0.4418		
The average	0.247	0.321	0.364	LSD
LSD		0.0412		0.3427

oculation has reduced the need for chemical fertilization by 50%, and the reason for the increase in nitrogen concentration in plants due to the role of fungal inoculation and chemical fertilization in increases the readiness and absorption of nutrients including nitrogen. This is consistent with Smith and Read, (2008) who pointed out that *Mycorhiza* fungus increases the absorption of nitrogen element through several mechanisms, including the external haifs of this fungus, which are spread in the soil that has an important role in Nitrogen uptake by ammonia.

Percentage of phosphorus in the vegetative part of the plant

The results in Table 5 indicate that fungal inoculation significantly affected the percentage of phosphorus in the vegetative part of the plant, the mixed fungal inoculation treatment (A3) recorded the highest percentage of phosphorus at 0.350 with a relative increase of 29.63% compared to the non- inoculated treatment (A0) which gave the lowest percentage of phosphorus (0.270), this increase in the percentage of phosphorus in the vegetative total may be attributed to the role of *T. harzianum* and *Mycorhiza* fungi in increasing the activity and growth of the root total plant and this increases the plant's ability to absorb water and nutrients, including phosphorus which increases its concentration in plant cells, As well as the role of fungi in the decomposition of organic materials in the soil and its secretion of some compounds such as enzymes that lead to the release of certain nutrients such as phosphorus, potassium and iron and thus increase the readiness of the plant (Harman, 2000).

The results of the same Table indicate that the chemical fertilization treatments differed significantly and gave the fertilization treatment 100% of the chemical fertilizer recommendation (K2) the highest percentage of phosphorus in the vegetative part of the plant was 0.364 and a relative increase of 47.37% compared to the non-fertilization treatment (K0) which it gave the lowest rate (0.247), and these results are consistent with Omer *et al*., (2016) Which confirmed that the absorption of essential elements such as phosphorus is due to increased levels of addition of chemical fertilizer.

The bilateral interaction between fungal inoculation and chemical fertilization increased the percentage of phosphorus in the vegetative part of the wheat plant by a higher percentage, as it significantly increased the treatment of the interaction between the mixed fungal inoculation and the level of 100% of the chemical fertilizer recommendation (A3K2) over most other treatments and recorded the highest rate of percentage of phosphorus was 0.409 with a relative increase of 94.76% compared to the comparison (non-inoculated and un-fertilized (A0K0)) which gave the lowest phosphorus percentage rate of 0.210, and the interference treatment between mixed fungal inoculation and chemical fertilization with 50% of the fertilizer recommendation (A3K1) was not significantly different from the treatment of 100% chemical fertilization, whether fungal or non-inoculated, this indicates that the mixed fungal inoculation has reduced the need for chemical fertilization by 50%, and this increase may be attributed to the percentage of phosphorus due to positive interaction between the fungus *T.harzianum* and *Mycorhiza* mushrooms are positively reflected on the plant's phosphorus content. *Mycorhiza* fungi dissolve and absorb phosphorus from the soil, *T. harzianum* secretes some growth hormones, amino acids and some chelating substances of Microelements, such as iron (Evelin *et al*., 2009).

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