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EFFECT OF BACTERIAL AND FUNGAL BIO-FERTILIZER AND POTASSIUM FERTILIZER ON IRON, COPPER AND ZINC AVAILABILITY AND YIELD OF *ZEA MAYS* L. CROP

Mohammed Aajmi Salman and Jawad A. Kamal Al-Shibani

University of Al - Qadisiyah, Agriculture College, Iraq.

Abstract

Beneficial microorganisms play a key role in the availability of a wide range of minerals in the soil. The objective of this is the study is to determine the effect of the interaction between *Bacillus* and fungus of *Trichoderma* and three levels of potassium fertilizer (0, 112.5 and 225) kg.h^{-1} on the availability Zn, Cu and Fe for *Zea mays* L. A field experiment on fall season of 2018 was conducted in silty clay loam soil. Results present that *Bacillus* and *Trichoderma* inoculation separately or together made a significant increase in the availability of zinc, copper and iron in the soil. The highest grain yield resulted with the application of bacterial and fungal bio-fertilizer and potassium fertilizers at the level of (225) kg.h^{-1} (5.203, 1.2300 and 16,267), respectively, compared to the control.

Key words: *Bacillus, Trichoderma,* Potaceous Fertilizer, Iron, copper, zinc, mays

Introduction

In terms of economic importance, corn, (*Zea mays* L.) is the third most important cereal crops after wheat and rice. It is one of the most important foods and industrial grain crops in the world, Khaeim Hussein, (2013). The major maize producing areas are North and South America, Eastern Europe, Russia, China, India, and South Africa, Always *et al.,* (2018); Luma A. Alabadi *et al.,* (2018). Yellow maize belongs to the family of Poaceae and the tribe of Tripaceae (Maydeae). It is distinguished from the rest of the tribe by the separation of the male organs from the female. Is a multi-use crop. It is used as food for animals and humans (Directorate of Agricultural Statistics, (2017). Bioreactor treatment, which works to stabilize atmospheric nitrogen, dissolve phosphate and produce growth-stimulating substances such as (IAA) and (GA) additional to decompose plant residues to pre-release and release nutrients and increase the content of humus in the soil. It will be the beginning of a sustainable agricultural system that promotes the growth of plant root populations and high production in whole or in part with time for the use of mineral fertilizers, Verma *et al.,* (2010).

Soil microorganisms play an important role in regulating the movement of decaying organic matter and providing important nutrients for plants such as phosphorus, nitrogen, and carbon. It also increases the availability of different minerals the plants and thus leads to economically increase in the yield. Bio-fertilizers are microorganisms that extend the root system and increase seed germination. Biomass differs from organic and chemical fertilizers in that its production technique is simple and the cost of processing is low and its effect on plant growth is much better, Chen (2006); Hussein Khaeim *et al.,* (2019).

The bacterial strain of (FZB24) *B. subtilis* was used as a vital fertilizer for the cotton plant, which resulted in an increase in plant growth by 30% compared to normal fertilizer of nitrogen, phosphorus, and potassium. This is because of the increase in root plant size and the activation of enzymes in the root zone, as well as the increase in the amount of nutrients, Yao *et al.,* (2006). Samurai (2006) pointed out that the inoculation with Trichoderma spp. played an important role in increasing the efficiency of nutrient absorption in a similar manner to what occurs when vaccinated with mycorrhizal fungi.

Kleifeild and Chet (1992) found that some isolates of Trichoderma spp. have the potential to penetrate the root mass of the plant and form innate structures in the roots similar to those of the microorganisms. The aim of this study was to know the effect of *Bacillus subtilis*

***Author for correspondence :** E-mail : muhammadajmii039@gmail.com, jawad.alshibani@qu.edu.iq

and *Trichoderma* on the growth and yield of maize plant and the concentration of copper, iron and zinc.

Materials and Methods

Field soil was prepared by conducting field the regular field management operations including peritoneal. Three main water channels along the field additional to some sub-drivers channels for each experimental unit were made. The field was divided into three main blocks. Two meters were left between each block and another. Each block was subdivided into (12) experimental unit (3×3)m. A distance of (1m) was left between each experimental unit and another, bringing the total experimental units to 36 experimental units. Corn seeds of the variety of Bihooth (106) were planted on the 24th July 2018. They were planted on lines and the distance between the line and was (75) cm and between planting hole and other was (25) cm. The experimental unit consisted of four lines per each plot and a plant density of 66666 plants.h^{-1}. Three seeds were planted in each planting hole and after 10 days of planting, the seedlings were rug out to one plant. The number of plants in the experimental unit is (48) plants. Urea fertilizer (46)% N was applied as a source of nitrogen (200) kg.N.ha^{-1}. This fertilizer was applied three times, at the stage of six papers, (30) days after the first application and after one month of the second application time, Yusuf and others (2006). Phosphate rock was applied at the level of (80) kg.P.h^{-1} as a source of the phosphorus element once before planting. Potassium sulfate Fertilizer (K_2SO_4) (45)% K was applied as a second-time potassium fertilizer application with urea fertilizer and at three levels (0, 112.5 and 225) kg. ha^{-1}.

Table 1: Physical and chemical proprieties of the soil.

Trait		Value
PH degree		7.8
Electrical conductivity EC		2.87
Interchangeable capacitance of positive ions CEC		18.7
Organic matter O.M		2.9
Available elements	Nitrogen	25.06
	Phosphorus	13.1
	Potassium	190.9
Positive dissolved ions	Calcium	4.5
	Magnesium	2.76
	Sodium	457
Negative dissolved ions	Carbonates	Nil
	Bicarbonates	1.75
	Sulfates	10
	Chlorides	7.0
Soil separators	Sand	185
	Loam	495
	Clay	320
Soil texture	Silt Clay Loam	
Bulk density	1.38	

The Diazinon herbicide was applied to control of the corn stem borer (*Sasamia cvetico*) insect. It was applied on the meristem of the plant on two times, after (25) days after germination, and the second after 10 days of the first one. Cleanings of harmful plants were carried out as needed, as well as the elimination of developing grasses with yellow maize plants. The experimental plots were irrigated on a regular basis and according to the plant's need for water.

Chemical and physical analysis of soil

Soil samples were taken before planting from a depth of (0-30) cm. A compound sample was taken after mixing the mixture to ensurethe uniformity of the sample. It was dried with a wooden hammer and then sieved with a (2) mm diameter sieve for the purpose of completing some chemical and physical tests.

Soil reaction (pH)

Measure in 1 : 1 (soil: water) extract using a pH-meter according to Page (1982) method.

Electrical conductivity (EC)

It was estimated at 1 : 1 (soil: water) extract using an EC-meter according to the method in Page, (1982).

Cation exchange capacity CEC

Estimated by Black method (1965) through soil saturation with Sodium acetate and ammonium acetate.

Soil texture

Was estimated in the international pipette method as reported in Black (1965).

Organic matter

Organic matter was estimated according to the method of Walker-Black, Black (1965b) by oxidation with potassium dichromate solution with concentrated sulfuric acid, and reverse titration with ferrous sulfate using D-phenylamine.

Available nitrogen

The available nitrogen was extracted by potassium chloride (KCl) and nitrogen was determined using the Chaldean device, Page, and others (1982).

Available phosphorus

Phosphorus prepared by sodium bicarbonate $NaHCO_3$ was estimated. The color was developed with ascorbic acid and ammonium sulfides and the Spectrophotometer was used in the estimation of phosphorus ready-made, Page, 1982).

Available Potassium

Soil potassium was extracted using (0.5) molar of calcium chloride and was estimated using Flame Photometer as indicated in, Page (1982).

Available Sodium

Sodium was estimated using sodium chloride solution through the use of the Flame Photometer (Black, 1965).

Calcium (Ca^{+2}) and (Mg^{+2})

They were estimated using a structured solution of ammonium hydroxide and ammonium chloride by adding an EBT detector as reported in Black, (1965).

Chloride (Cl^{-})

It was estimated using the potassium chromate guide and the silver nitrate solution, where a white precipitate is formed according to Black (1965).

Carbonate CO_3 and HCO_3

They were estimated that with the addition of sulfuric acid and the orange-phenol-phenyl-phenylethane reagent when the carbonates are present, the solution color changes to violet and then the sulfuric acid is calibrated according to Black (1965).

Sulfates SO_4^{-2}

The barium chloride solution was used with a standard (1) concentration, hydrochloric acid, and ethanol as reported in Black, (1965).

Table 2: Effect of bio-fertilization (bacterial and fungal) and potassium fertilizer on iron concentration mg.Fe.kg^{-1}soil.

Bacterial biofertilizer (H)	**Fungal biofertilizer (T)**	**Potassium fertilization levels**			**H+T**	**H average**
		K_0	**K_1**	**K_2**		
H_0	T_0	6.567	9.233	7.400	7.411	8.867
	T_1	10.100	8.267	11.633	10.322	13.700
H_1	T_0	10.467	14.633	11.767	11.722	
	T_1	16.133	12.933	16.267	15.678	
L.S.D.			0.2607		0.1505	0.1065

Bacterial bio-fertilizer (H)	**Potassium fertilization levels**			**L.S.D. H+K**
	K_0	**K_1**	**K_2**	
H_0	7.900	8.750	9.950	0.1844
H_1	12.550	13.950	14.600	

Fungalbio-fertilizer (T)	**Potassium fertilization levels**			**Effect ave-rage of T**
	K_0	**K_1**	**K_2**	
T_0	8.517	9.583	10.600	9.567
T_1	11.933	13.117	13.950	13.01
L.S.D.		0.1844		0.1065
Effect ave-rage of K	10.225	11.350	12.275	L.S.D K 0.1304

Cu^{+2}, Zn^{2+} and Fe^{+2}

These elements were estimated using atomic absorption spectroscopy and standard solutions for all elements were introduced by adding extract solution, Black (1965).

Bulk density

The bulk density was estimated using a core sample as reported in Black (1965).

Results and Discussion

The results in Table 2 indicate that the application of the bacterial fertilizer (H_1) increased the concentration of the available iron in the soil. The highest mean was (13.700) mg.Fe.kg^{-1}.soil, compared to control coefficient (8.867) mg.Fe.kg^{-1}.soil. This increase may be attributed to the ability of Bacillus to produce iron-soluble compounds (Siderophores) that are formed with iron soluble complexes of Siderovoros-iron which is the available form of iron in the soil, Zahedi (2015).

T. harzianum bio-fertilizer (T1) resulted in the highest mean of the concentration of the available iron (01.13) mg.Fe.kg^{-1}.soil, compared to the control (9.567) mg.Fe.kg^{-1}soil. The reason is that *T. harzianum* has the ability to increase iron-oxide complex (Fe_2O_3) availability by converting it into the chelate form and reducing the non-available (Fe^{+3}) chelated ion to the available form (Fe^{+2}) to be absorbed by the plant, Altomare *et al.,* (1999). The application of potassium fertilizer resulted in a significant effect on increasing the concentration of the available iron in the rhizosphere soil. The highest values were (12, 275 and 11.350) mg.Fe.kg^{-1}soil (K1 and K2), respectively, compared to the control (K0). This increase in the rhizosphere soil may be due to the role of the sulfate ion produced due to potassium degradation, which reduces soil pH, leading to increased iron availability to the plant.

Di-overlaps between bacterial and fungal fertilizers, potassium fertilizer resulted in significant increases in the availability of iron. Bacterial and fungal overlapping resulted in the highest increase in iron availability in the soil. The reason for the increased concentration of the available iron in the soil dues to the interference of bacterial and fungal fertilizer that has been attributed to the role of Bacillus bacteria, which secrete the secretions of the iron (Fe^{+3}). The chelates iron moves to the surface of the microbial cell and is reduced to (Fe^{+2}) outside or inside the microbial cell, Nielands (1984). Stabilization of the fungus fertilizers on plant roots stimulates the absorption of water and some nutrients (copper, iron, phosphorus, manganese, and sodium) from the soil solution.

This increase in nutrient uptake confirms the improved uptake mechanism of these elements, Yedidia *et al.*, (2001). Treatment of ($H_1T_1K_2$) resulted in the highest mean of the available iron concentration in the soil of (16.267) mg.Fe.kg^{-1}soil compared to ($H_0T_0K_0$) and to other treatments. This increase may be attributed to several reasons, including the reduction of pH as a result of the hydrogen release as a result of nutrient uptake by plants and microorganisms.

The efficient and active isolates of the applied bacteria produce antibiotics, organic acids, and enzymes, all of which operate with different mechanics and mechanisms in the availability of nutrients, including iron in the soil. This result is consistent with what, Glick *et al.,* (2007).

The results in Table 3 indicate that the application of the (H_1) bacterial fertilizer resulted in an increase in the concentration of zinc availability in the soil (3.379) mg.Zn.kg^{-1}soil compared to the control (1.569) mg.Zn.kg^{-1}soil. The reason for this increase may be due to the effectiveness and activity of the applied bacterial fertilizer that contributes to the zinc sorption process of its insoluble compounds, which are ($ZnCo_3$) in calcareous soils, Mishra and Dash (2014). Fungal fertilizer (T1) application has increased the concentration of the available zinc in the soil. The highest obtained mean of the available zinc was (3.212) mg.Zn.kg^{-1}soil compared with the control of (1.737) mg.Zn.kg^{-1}soil. The reason is that the *T. harzianum* has the ability to reduce oxidative stress and release (Zn^{+2}) available form ion to be absorbed by the plant, Harman (2000). These results are consistent with Altomare *et al.,* (1999). There was a significant effect on increasing the concentration of zinc in the soil when potassium fertilizer is being applied (2.586 and 2.940) mg.Zn.kg^{-1}soil (K_1 and K_2), respectively, compared with the control of (1.898) mg.Zn.kg^{-1}soil. The increase in the concentration of the available zinc in the soil after harvesting can be attributed to the role of sulfur (SO_4), which is derived from the degradation of potassium fertilizer. It reduced the number of soil reaction pH and thus increased melting of zinc compounds, which increases its availability for the plants, Al-Shabini (2007).

Di-Overlaps between bio-bacterial and bio-fungal fertilizers and potassium fertilizer have had a significant effect on increasing the availability of zinc in the soil. Bacterial and fungal overlapping resulted in the highest increase in the concentration of the available zinc in the soil. This is due to the fact that fungal and bacterial fertilizers increases the providing process of the soil by micronutrients (Zn, Fe, Cu, N, P, and K) and subsequently compensates for zinc deficiency in the soil, thereby increasing its availability, Altomare *et al.,* (1999). The results shown in the table confirmed that the triple interference of ($H_1T_1K_2$) resulted in a significant increase in the concentration of the available zinc in the soil at (225) mg.Zn.kg^{-1}soil. The highest concentration of the available zinc in soil is (5.23) mg.Zn.kg^{-1}soil compared with control treatment (0.663) mg.Zn.kg^{-1}soil.

Table 3: Effect of bio-fertilization (bacterial and fungal) and potassium fertilizer on Zinc concentration mg.Zn.kg^{-1}soil.

Bacterial biofertil izer (H)	**Fungal bioferti lizer (T)**	**Potassium fertilization levels**			**H+T**	**H average**
		K_0	**K_1**	**K_2**		
H_0	T_0	0.663	1.833	1.000	1.042	1.569
	T_1	2.150	1.463	2.307	2.097	3.379
H_1	T_0	2.043	3.050	2.463	2.431	
	T_1	4.730	2.787	5.203	4.328	
L.S.D.			0.1383		0.0798	0.0565

Bacterial bio-fertilizer (H)	**Potassium fertilization levels**			**L.S.D. H+K**
	K_0	**K_1**	**K_2**	
H_0	1.248	1.575	1.885	0.0978
H_1	2.547	3.597	3.995	

Fungalbio-fertilizer (T)	**Potassium fertilization levels**			**Effect ave-rage of T**
	K_0	**K_1**	**K_2**	
T_0	1.353	1.732	2.125	1.737
T_1	2.442	3.440	3.755	3.212
L.S.D.		0.0978		0.0565
Effect ave-rage of K	1.898	2.586	2.940	L.S.D K 0.0692

The results in Table 4 indicate that the application of the (H_1) resulted in an increase in the concentration of the availability of copper in the soil with the highest mean of (1.0172) mg.Cu.kg^{-1}soil, compared with the control of (0.7644) mg.Cu.kg^{-1}soil. This because of the ability of bacterial fertilizers to increase copper availability in the soil through different mechanisms such as reduced soil reaction degree and the production of compounds that are chelated to iron and copper, Vessey (2003). These results are consistent with, Gupt and Gopal (2008).

The application of (T_1) resulted in an increase in the concentration of available copper in the soil at a mean of (1.0056) mg.Cu.kg^{-1}soil compared with control of

(0.7761) mg.Cu.kg^{-1}soil. The increase in the concentration of the available copper in the soil is due to the ability of the fungus *Tracoderma* to increase the availability of micronutrients such as zinc, iron, and copper to the plants, and producing separate antibiotics that increase the availability of these elements by reducing them, Altomare *et al.*, (1999).

The results in Table 6 indicated that there was a significant effect on the increase in the concentration of copper in the soil when potassium fertilizer is applied (0.8892 and 0.9700) indicated (K_1 and K_2), respectively, compared to control. The increase in the concentration of the available copper in the soil after harvest can be attributed to the provision of an appropriate level of potassium in soil that positively affects the increase of the root secretions of organic and amino acids. These acids reduce the degree of reaction in the soil of the rhizosphere, which increases the copper availability, Rovira (1969). Bilateral interactions between bacterial and fungal fertilizer have had a significant effect on copper availability in soil.

Table 4: Effect of bio-fertilization (bacterial and fungal) and potassium fertilizer on Cupper concentration mg.Cu.kg^{-1}soil.

Bacterial biofertil izer (H)	Fungal bioferti lizer (T)	Potassium fertilization levels			H+T	H average
		K_0	K_1	K_2		
H_0	T_0	0.5867	0.8100	0.6467	0.6533	0.7644
	T_1	0.8700	0.7267	0.9467	0.8756	1.0172
H_1	T_0	0.8267	1.0300	0.8933	0.8989	
	T_1	1.1467	0.9767	1.2300	1.1356	
L.S.D.			0.01093		0.00631	0.00446

Bacterial bio-fertilizer (H)	Potassium fertilization levels			L.S.D. H+K
	K_0	K_1	K_2	
H_0	1.248	0.7583	0.8367	0.00773
H_1	2.547	1.0200	1.1033	
Fungalbio-fertilizer (T)	**Potassium fertilization levels**			**Effect average of T**
	K_0	K_1	K_2	
T_0	0.7067	0.7700	0.8517	0.7761
T_1	0.9200	1.0083	1.0883	1.0056
L.S.D.		0.00773		0.00446
Effect average of K	0.8133	0.8892	0.9700	L.S.D K 0.00547

Table 5: Effect of bio-fertilization (bacterial and fungal) and potassium fertilizer on corn grain yield, tons.h^{-1}.

Bacterial biofertil izer (H)	Fungal bioferti lizer (T)	Potassium fertilization levels			H+T	H average
		K_0	K_1	K_2		
H_0	T_0	5.730	5.850	6.000	5.860	6.058
	T_1	5.800	6.300	6.667	6.256	6.683
H_1	T_0	5.700	6.000	6.133	5.944	
	T_1	6.300	6.667	9.300	7.422	
L.S.D.			0.8823		0.6239	0.3602

Bacterial bio-fertilizer (H)	Potassium fertilization levels			L.S.D. H+K
	K_0	K_1	K_2	
H_0	5.765	6.075	6.333	0.5094
H_1	6.000	6.333	7.717	
Fungalbio-fertilizer (T)	**Potassium fertilization levels**			**Effect average of T**
	K_0	K_1	K_2	
T_0	5.715	5.925	6.067	5.902
T_1	6.050	6.483	7.983	6.839
L.S.D.		0.6239		0.4412
Effect average of K	0.8133	0.8892	0.9700	L.S.D K 0.3602

Bacterial and fungal overlapping resulted in the highest increase in copper availability in the soil. The reason for the increased concentration of the availability of copper in the soil is attributed to the role of bacterial fertilizer, which leads to reduced soil reaction in the rhizosphere area. When the pH decreases, copper becomes more available in the soil. As well as to the role of *T. harzianum* in the availability of nutrients, including copper.

The results confirmed that the triple interference of ($H_1T_1K_2$) resulted in a significant increase in the concentration of the available copper in the soil at (225) kg.h^{-1}. The highest mean of the available copper in the soil was (1.2300) copper compared to the control treatment H0T0K0 which gave the lowest average (0.5867) mg.Cu.kg^{-1}soil.

Table 5 present the results of the significant increase in the total grain yield due to the application of the bacterial fertilizer (H_1). It reaching an average of (7.422) tons.h^{-1} in relation to the (H_0) comparison treatment. This is due to the ability of bacteria to produce organic and mineral acids and the secretion of the phosphatase enzyme, which contributes to the increased availability of m elements, especially potassium. Al-Rashidi (1988) refers to the degrading of silicate metals containing potassium by *B. subtilis* bacteria. This is because of their significant role in the process of transferring carbohydrates from the areas of their synthesis to the storage area in the grains, Abu Dahi *et al.*, (1988).

The results present that the application of fungal fertilizer (T_1) resulted in a significant increase in the total grain yield, reaching the highest mean (6.839) tons.h^{-1} in comparison with the (T_0) treatment. This is due to the ability of fungus *T. tracoderma* to increase nitrogen availability and micronutrients such as copper, zinc, and iron and to produce separate antibiotics that contribute to the increased availability of these elements by reducing them, Altomare *et al.*, (1999). As well as stimulating and encouraging the growth and development of the roots, which is reflected in the outcome, Harman (2000).

The application of different levels of potassium fertilizer has resulted in significant differences in the total grain yield compared with the control treatment. They made (6.204 and 7.025) tons.h^{-1} with the application of levels of (K^1 and K^2), respectively, compared to the comparison treatment (K0) (5.883) tons.h^{-1}. This is due to potassium, which plays an important role through its effect on the traits of plant growth, leading to preserve on certain content of chlorophyll. This leads to an increase in the flow of nutrients to the places where the grain is formed, which contributed to the fullness of the fertilized fertilizers and increased numbers, which contributed to the increase in weight. This has a positive effect on the number of starch grains and the number of seed storage cells, which led to an increase in the weight of grains and in turn affected the increase in yield, Abdul Wahahooki (2008). This result is consistent with the findings of Saidi (2011) and Najadi (2010).

Bilateral interference between fungal fertilizers and potassium fertilizer resulted in the highest mean increase in total grain yield (7.983) tons.h^{-1}. This is due to the role of fungal compost which has the potential to increase the development and growth of maize plant roots. This fungus decomposes soil's organic matter and increases the nutrient content in the rhizosphere area. This increases the ability of the plant to absorb nutrients.

Potassium fertilizer has an important role in increasing the total yield by increasing the potassium absorbed in the leaves, which led to the efficiency of photosynthesis process and the transfer of the products of representation to the areas of need in the plant, which reflected positively on the total yield, and this result is consistent with, Abbas and others (2012). The results confirmed that the triple interference of ($H_1T_1K_2$) (*Bacillus* + *T. harzianum* + potassium fertilizer) resulted in a significant increase in total grain yield (225) kg.h^{-1} (9,300) tons.h^{-1} compared with the comparison treatment (5,730) tons.h^{-1}.

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