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Effect of Biofertilizer with Trichoderma Harzianu Fungus and Zink Foliar Spraying on Cucumber Plant (Cucumis Sativus L.) and Growth Characters

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Abstract : **A Filed experiment was conducted in winter agricultural season 2017/2018 in one of unheated greenhouse in Al-Qadisiyah university / agriculturae college About effect each of Trichoderma harzianum fungus and Zink foliar spraying on Cucumber plant Cucumis sativus L. And interaction between them on cucumber plant type (Sayff F1). The experiment was conducted according to randomize block completely design (R.B.C.D) and by testing the effect of second factors the first factor including biofertilizer treatment Trichoderma harzianum with two Levels (pollination and without pollination), the second factor was Zink foliar spraying with three levels (0,10,20 mg L-1 Zn)K Experiment treatments were six treatments as (T1 distilling water spraying), (T2 T.harzianum),(T3 Zink foliar spraying with concentrate (10)mg L-1 Zn, (T4 T.harzianum pollination + Zink foliar spraying with concentrate 10 mg L-1) ,(T5 Zink foliar spraying with concentrate 20 mg L-1 , (T6 T.harzianum pollination + Zink foliar spraying with concentrate 20 mg L-1) with three Replecats Every experimental unit including 10 plants, and means comparing at least significant differences (L.S.D) in probability level (0.05).The results can be summarized as follows :**

-Biofertilizer T.harzianum treatments exceeded by giving the highest mean in the plant height, flower numbers ,chlorophyll content , Zink content in leaves and numerical density of T.harzianum fungus in soil for the growth two duration amount to 146.88 cm , 34.36 flower plant -1 , 138.79 ml M2 ,% 29.32, 15.22, 14.41 C.F.U* gm soil-1 dried soil respectively.

-While Zink treatment at level (20 mg L-1Zn) was significantly exceeded in vegetation growth characters as the plant height, flower numbers, plants chlorophyll content, Zink content in leaves and numerical density of T.harzianum fungus in soil for the growth two duration was recorded the heights mean amount to 144.09 cm , 34.19 flower plant-1 , 137.45 ml M2 , % 27.83 , 13.66 , 14. 40 C.F.U* gm soil-1 dried.

Keywords: Trichoderma harzianum ,Zink,Cucumber plant.

***The research is part of MSc for 1st author**

I. INTRODUCTION

Cucumber plant belongs to the Cucurbitaceae it is one of the most important summer vegetable crops in the world including Iraq, It is cultured wither in native home India or around all world to get the fruit (1), It is a crop with a high economic return for its importance and increasing the demand for it and for its high consumption as a result for its fame in dishes decorating on tables or pickled in salad and fast food , also it is consumed as a fresh food with a highly nutrition value as known it's rich in minerals and thiamine, Niacin and C vitamin (0.38 gm, 0.3 gm, 0.2 ml, 78 ml to each 100 gm from the fresh fruits which had %95 water, %0.7 protein, 0.1 fats, 3.4 carbohydrates, %0.4 fiber, % 0.4 Ash)(2) .Using biofertilizers in the current time became importance matter, the microorganism play important role in agricultural system especially stimulating microorganism of plants grown from its work on mainly three mechanisms , it's work as biofertilizers, plant stimulator, biological control factors as *T.harzianum* fungus (3,4,5) that has important role to increase of plant capacity to absorbed water and

nutritional elements in soil ,it's increasing plant development and it's growth rate after it's helped plant to germination ,growth rate increases as a result to *T.harzianum* fungus activity that helped to form a deeply roots to absorbed water and nutrients from wide places in soil6) beside fungus roles in the secretion of some hormones and growth stimulating substances(7).Zink plays an important role on formation and production pollin and enzymes activity (8) as well as it role in tryptophan Amino acid formation which is made up from growth hormone Indole actic acid (IAA) that worked in plant elongation (9,10).

II. Materials and methods

A field experiment was conducted in winter agricultural season 2017/2018 in one of unheated greenhouse in Al-Qadisiyah university / agriculturae college. Soil analysis process was conducted after samples taken by randomizing methods from experiments sites at depth (0- 30 cm), physical and chemical characters were analyses as shown in the table (1), Greenhouse land was cultivation well and handily and divided into furrows with three blocks at length 55 m and width 1.25 m and the distance between block and the other 2 m ,50 cm space was left over to each side of greenhouse sides then each blocks was divided into (18) experiments unit at length 2 m with isolation distance (1 m) between unit and the other, and 50 cm distance leftover in the entering and ending of each blocks , so the number of treatments 18 factorial treatments with three replicates.

The experimental units number amount to 54 unit, Drip irrigation system was used to plant watering.the treatments distributed randomly on experimental units on each block and seed beds were prepared, each experimental units contain (10) seedbeds at a distance 40 cm. Phosphate fertilizer was added at amount 100 kg h^{-1} from phosphorus pentaoxide P_2O_2 as a triple superphosphate fertilizer 46% P_2O_5 and by one spurt before culturing. While nitrogen fertilizer was added at rate 200 N h^{-1} to dunum as two spurts, the first after 25 days from germination on the second after flowers appear .The potassium fertilizer was added as potassium sulfate (K_2O) (100 kg h^{-1}) (12).

Cucumber seeds (Sayff F1 type) distributed (at date 9/10/2017) by putting 2 seeds in each hole, and the watering was conducted after seeds culturing completed after germination plants were thinning by leaves one plant in every hole.The experiments involved studying two factors The first Factor seeds treated with *T.harzianum* fungus with two levels (pollination and without pollination) which obtained from AL-Kufa university /agriculture college laboratories. The second Factor was Zink foliar spraying with three levels (0, 10, 20 Zn Mg L^{-1}) as a Zink sulfate ($ZnSo_4$.$7H_2O$) (36% Zn). Experiments included six treatments T1 distilled water spraying) ,(T2 *T.harzianum*),(T3 Zink foliar spraying with concentration 10 mg L^{-1} Zn) ,(T4 *T.harzianum* pollination + Zink foliar spraying with concentration 10 mg L^{-1}) ,(T5 Zink foliar spraying with concentration 20 mg L^{-1} , (T6 *T.harzianum* pollination + Zink foliar spraying with concentration 20 mg L^{-1}).

Spraying process was conducted by using knapsack sprayer with capacity 20 L by four sprayers during the growth season, the first before flowering, the second after 15 from the first, the third 15 days from third, the fourth 15 days from the third. While the control treatments sprayed using distilled water only. Diffuser substance (Zahi) was added to reduced surface extension and guarantee the completely wet to plant leaves. The search was conducted according to factorial experiment design according to randomize completely block design (R.C.B.D) with three replicates, each experiment units involved 10 holes in each hole three seeds were a cultured and thinning process for one plant after 2 weeks from culturing and watering when we needed for it. The differences between treatments were compared according to least significant differences (L.S.D) at probability level (0.05) (12), and the measurements were taken plant Height, floor numbers, plant chlorophyll content, leaves Zink content, *T.harzianum* fungus numbers in the soil after flowering.

III. Plant measurement

1. plant height (Cm)

Plant height was measured for five plants randomly selected from each experimental unit from soil contact place to the developing peak then its mean was recorded.

2. Whole flower numbers in a plant (Flower Plant -1)

The total number of flowers formed in a plant from selected plants was counting randomly to each experiment unit then its mean was recorded.

3. Leaves chlorophyll content (Mg M2)

It was randomly counting after flowering complete to each plant and for all experimental units by using(Spad) device by taking device reading of three flakes to each branch as a rate to three branches per plant and their main was founded and then the following equation was applied :

(ch1)=-80.05+10.4*(spad) according to (13)

4. Zink content in Leaves (%)

Zink in digestive plant samples was determined by using a Spectrophotometer device according to the method mentioned by(14).

5. Number of T.harzianum fungus in the soil after flowering (C.F.U*gm soil-1 dried)

Fungus numbers were determined by dilution method and dishes count by using PDA culture media.

Table (1) Some of the physical, chemical and biological characters to the field soils before culturing to season 2017/2018

Character		Value	Unit
pH		7.6	-
Electrical conductivity EC		3.79	ds/m^{-1}
Cation exchange capacity		265	$cmol_c/kg\ soil^{-1}$
Organic material		7.1	$Gm\ Kg\ Soil^{-1}$
Carbonares minerals		19.0	$Gm\ Kg^{-1}$
Gypsum		1.20	$Gm\ Kg^{-1}$
Prepared ions	**Nitrogen**	37.0	$mg\ Kg\ Soil^{-1}$
	Phosphorus	9.08	
	Potassium	190.59	
	Zink	1.2	
Dissolved cations	**Calicium**	3.12	$cmol/L^{-1}$
	Magnesium	3.57	
	Sodium	4.7	
	Carbonates	Nil	
	Bicarbonate	3.6	

Dissolved negative ions	**Sulfates**	2.60	
Soil separators	**Clay**	100	gm Kg Soil^{-1}
	Silt	220	
	Sand	680	
Soil texture		Sandy Lome	Sand mix
Bulk density		1.41	gm cm^3
Biogenic determines	**Total number of fungi**	2.1*10^4	C.F.U gm soil^{-1} dried
	Numbers of *T.harzianum*	0.3*10^4	C.F.U gm soil^{-1} dried

IV. Results and Discutions

1- plant height (cm)

The results in the table (2) showed biofertilizers T1 (Bio Inoculation) significantly exceeded and given the highest mean in a plant height amount 146.88 cm as comparing with control treatment which giving the least mean to 138.66 cm at increasing rate to 5.92% , the reason may be due to the bio fungus working to secretion hormonal compounds which regulating plant growth (phytohormones) as gibberellins, citoxins, oxins, and gibberellins which increased growing and expansion of roots and therefore increase absorption rates to water and mineral nutrients from roots circumference and therefore increased their accumulation in plant tissue.

The nutrients and plant hormones and their interaction with each other the most limited factor to growing that increased a plant height (15,16) and this results agreed with what brought by (17) and (18) when they treated cucumber plant with fungus *T.harzianum* biofertilizers and other plants and obtained a significant increasing in a plant height . From table (2) we noticed that the mineral fertilizers with Zink elements at concentration (20 mg L^{-1}) was significantly exceeded which recorded the highest mean in a plant height to 144.09 cm as comparing with (0 , 10 Mg L^{-1}) concentration that to 140.83 , 143.38 cm respectively at increasing rate amount to 2.31 , 0.49 % and the reason of increasing due to two active action of Zink element in tryptophan amino acid formation which considerable the starter in oxin formation who responsible for plant height increasing because of their direct effect on the splitting and expansion of plant cells (19) and this results agreed with what brought by(20)when they used Zink foliar spraying in their studies on cucumber plant obtained a significant increasing in plant height.

The duplicate interaction between fungus *T.harzianum* biofertilizers and Zink foliar spraying had significant effect as the mix (T1+ Zn2) (Bio Inoculation + 20 Mg L^{-1} Zn) was significantly exceeded which recorded the highest mean in plant height 150.02 cm as comparing with control treatments T0 + Zn0 which gave the least mean to 136.60 cm at increasing rate 9.82% the reason may be due to the treatment worked on nutrient analyzed and make them ready for absorption from plants according roots hairs as long as their role in plant hormones formation especially Oxins which worked on cells elongate and division and improved the vegetation growth to the plant from plant height increasing (21).

Table (2) Effect of *T.harzianum* fungus and Zink foliar spraying and their interaction in a plant height Total numbers of plant flowers (Flower plant^{-1})

T.harzianum	Znic	T*Zn	T rate	Zn	Zn rate
T0	Zn 0	136.60	138.66	Zn 0	140.83
	Zn 1	141.21			
	Zn 2	138.17		Zn 1	143.38
T1	Zn 0	145.07	146.88		
	Zn 1	145.55		Zn 2	144.09
	ZN 2	150.02			
L.S.D value		**L.S.D T x Zn=0.233**	**L.S.D T =0.134**	**L.S.D Zn =0.164**	

Table(2)results explained T1 Bio pollination significantly exceeded which gave the highest mean in flower numbers 34.36 Flower plant^{-1} as comparing with control treatment which gave least mean in flower numbers 27.11 flower plant^{-1} at rate 26.74 % , the reason may be due to fungus role on activity increasing, growing and development of plant roots and this increasing plants ability to absorbing nutrients as long as plant content from plant hormones , as well as increasing nitrogen efficiency in plant which increased phosphorus dissolving therefore it helped to ions decomposition as Zn , Cu , Fe , Mn and micronutrient which plant needed for its growing and make them more accessible for improved a plant growing also it increased photosynthesis process efficiency which worked on carbohydrates accumulation increasing and all these factors help to increased flower numbers in plant (21). While the second level (20 Mg L^{-1} Zn) significantly exceeded which recorded the highest mean in flower numbers amount to 34.19 flower plant^{-1} as comparing with control treatment which gave the least mean of flower numbers amount to 28.15 Flower plant^{-1} at increasing rate amount to 21.45% the reason may be explained to Zink role in many biochemical and physiological processes which Contributed on metabolism interactions of oxins, proteins and carbohydrates ,Also it's entering on formation of cellular membrane skeletal and Contributed on proactive cell from adverse effect of some oxins interactions, as well as its role in plant reproductive parts growing and developing as believed it had a vital role in flowering and fertilization process, it has been found it concentration in plant parts during growing of reproductive parts highest from it concentration in vegetation growth stage (10) (22) and this result agreed with what found by (23). While the duplicate interaction T1+Zn_2 (Bio pollination +20 ml L^{-1} Zn) it had been significantly exceeded which recorded a highest mean in this character amount to 34.43 Flower plant^{-1}, while (T0+Zn0) gave least mean amount to 23.43 Flower plant^{-1} , at increasing rate amount to 59.82% , this reason may be due to *T.harzianum* fungus role and Zink foliar spraying which increasing availability of nutrients as Fe, Mn, and K that had important role in photosynthesis process and production of nutritional substances inside plant which improving growing and increasing the flowers numbers (21).

The data in the table (4) showed T1 (Bio pollination) significantly exceeded which gave the highest mean in chlorophyll content amount to 138.79 mg M^2 as comparing with control treatment which gave the least mean amount to 128.54 mg M2 at increasing rate 7.97, and may be due to ability of fungus biofertilizer to product substances similar to Oxins, Gibberellin and Cytokines which stimulating chlorophyll degradation, as well as these substances, collect nutritional substances assembled in leaves, therefore, increasing Synthesis of chlorophyll molecule and this result agreed with (18).From the same tables we noticed Zn_2(20 mg L^{-1} Zn) significantly exceeded which recorded the highest mean in chlorophyll content amount to 137.45 ml M^2 while the two concentration (0, 10 mg L^{-1} Zn) recorded the least means amount to 132.62, 130.93 mg M^2 respectively at increasing rate amount to 3.64 , 4.97 %, the reason may be due to direct effect of Zink on process formation of amino acids ,

carbohydrates , energy compounds, therefore, it contributes in chlorophyll molecule built,these result agreed with what founded by each of (20),(24),(25).While the duplicate interaction T1+Zn_2 (Bio pollination +20 ml L^{-1} Zn) significantly exceeded and recorded the highest mean in chlorophyll content amount to 145.73 mg M^2 , while (T0+Zn0) gave the least mean amount to 126.88 mg M^2 at increasing rate amount to 14.85.

Table(3) Effect of *T.harzianum* fungus and Zink foliar spraying and their interaction on a flower numbers (Flower plant $^{-1}$)

T.harzianum	Znic	T*Zn	T rate	Zn	Zn rate
T0	Zn 0	23.42	27.11	Zn 0	28.15
	Zn 1	26.95			
	ZN 2	30.96		Zn 1	29.86
T1	Zn 0	32.89	34.36		
	Zn 1	32.77		Zn 2	34.19
	ZN 2	37.43			
L.S.D value		**L.S.D T x Zn=1.083**	**L.S.D T =0.625**	**L.S.D Zn =0.766**	

- chlorophyll content in Leaves (mg M^2)

The reason may be due to cooperative action between biofertilizers and mineral fertilizer on equipment plant with a nutrients as Iron, Copper, and Magnesium which had important role in chlorophyll synthesis , Iron also contributed in bioprocess of chlorophyll synthesis and increasing chloroplast number and size as well as increase pods number, Copper also considerable necessary to Iron porphyrin synthesis which considered the basic in chlorophyll molecule synthesis thus it's worked on pigment protection from degradation as about 70% from total leaf copper found in chloroplast and magnesium also from the basic elements on chlorophyll synthesis (26).

Table(4) Effect of *T.harzianum* fungus and Zink foliar spraying and their interaction on the plant chlorophyll content (mg M^2)

T.harzianum	Zink	T*Zn	T rate	Zn	Zn rate
T0	Zn 0	126.88	128.54	Zn0	132.62
	Zn 1	129.57			
	ZN 2	129.17		Zn1	130.93
T1	Zn 0	138.37	138.79		
	Zn 1	132.29		Zn2	137.45
	ZN 2	138.37			
L.S.D Value		**L.S.D T x Zn=0.174**	**L.S.D T =0.100**	**L.S.D Zn =0.123**	

2. Zink content in Leaves (%)

Table (5) showed T1 (Bio pollination) significant exceeded which gave the highest mean in Zink content in leaves amount 29.32 very high as comparing with control treatment which gave the least mean amount to 19.91 very high at increasing rate amount to 9.41 very high , the reason may be due to plant supplied with nutrient from *T.harzianum* fungus such as Zink and form a well and dense roots from plant treated with it which helped to increase Zink absorb from plant and increase its concentration in leaves and this agreed with (27). From table (5) results showed Zink foliar spraying Zn_2 (20 mg L^{-1} Zn) recorded the highest mean on Zink content in leaves amount to 27.83 very high as comparing with control treatment which gave least mean amount to 23.43 very high , at increasing rate amount to 18.77, and may be due to Zink direct addition on spraying solution which increase absorb process from leaves tissues and increase Zink assembly in leaves because its slow movement element in plant as well as increase its concentration in leaf area and chlorophyll content in leaves at same treatment and concentration table (5 , 6) that helped to increase surface exposed to spraying ,increasing of photosynthesis production and vegetation growth that lead to Zink absorbed from plant (28) and this agreed with what was brought by (29), While the duplicate interaction T1+Zn_2 (Bio pollination +20 ml L^{-1} Zn) significantly exceeded than others which recorded the highest mean in Zink content inleaves amount to 36.96% , While (T0+Zn0) treatments gave the least mean amount to 17.62% at increasing rate amount to 109.76% , the reason may be due to role of *T.harzianum* fungus and Zink on available nutrients in plant and dry weight increasing to vegetation growing table (3) which resulted in Zink content increasing as well as their role in many biological processes which attached to enzymes and amino acids formation, All factors helped to increase Zink concentration and assembly in leaves (30).

3. Numbers of *T.harzianum* fungus in soil after 35 day and in season ending (C.F.U* gm day $^{-1}$ dried).

The results in table (6) showed T1 (Bio pollination) significantly exceeded than others in growth two durations which gave the highest means to this character amount to 15.22 , 14.41 C.F.U* gm day^{-1} dried soil respectively as comparing with control treatment which gave the least mean for two durations amount to 9.50 , 9.55 C.F.U* gm day^{-1} dried soil respectively at increasing rate amount to 6.21 , 50.89 % . the reason of increase may be due to addition of *T.harzianum* fungus leads to increasing Trichoderma fungus activity that found in the soil through available suitable environmental conditions for growth as moisture , temperature and nutritional source for Fungi settlement (fungus and bacteria) through microorganism secretion that used as a biofertilizer beside roots secretion and organic matter which feeding fungi and then increase their numbers, this indicates to activity and availability of fungus during study duration and two durations (31) and this result agreed with (32) and (33), Table(6) results showed Zink foliar spraying Zn_2 (20 mg L^{-1} Zn) significantly exceeded which recorded the highest means in *T.harzianum* fungus numbers through growth two durations which amount to 14.40, 13.66 C.F.U* gm day^{-1} dried soil respectively as compared with control treatment which gave the least means amount to 10.77, 10.30 C.F.U* gm day^{-1} dried respectively at increasing rate amount to 33.70 , 32.62 % respectively to both durations respectively,

This reason may be due to plant supporting with nutrients and Zink entering in synthesis of enzymes, proteins and amino acids which supporting cellular division that helped provided suitable conditions beside roots formation well that helped to *T.harzianum* fungus numbers increasing through study two durations as a result to holding substance which provided available basic nutritional base to fungus growth and thus increasing its number in the root region (34).

While the duplicate interaction T1+Zn_2 (Bio pollination +20 ml L^{-1} Zn) significantly exceeded in fungus numbers through both two durations which amount to 18.20,16.44 C.F.U* gm day^{-1} dried soil respectively ,while (T0+Zn0) treatment gave the least means amount to 7.24, 6.36 C.F.U* gm day^{-1} dried soil respectively with increasing rate amount to 151.38, 158.49 % respectively , the reason may be due to role of *T.harzianum* fungus and Zink foliar spraying on available of basic nutrients to plant and increasing their accessibility which helped to growth and development roots and prepare suitable conditions that increase their numbers in soil through available suitable conditions as moisture and

temperature for growth through fungus mechanisms and secretion which worked with it and equipped a necessary elements for plant growth as nitrogen, phosphorus and potassium and then increase of their numbers (31).

Table(4) Effect of *T.harzianum* fungus and Zink foliar spraying and their interaction on Zink content in Plant (%)

T.harzianum	Zink	T*Zn	T rate	Zn	Zn rate
T0	Zn 0	17.62	19.91	Zn0	23.43
	Zn 1	23.40			
	ZN 2	18.70			
T1	Zn 0	29.25	29.32	Zn1	22.57
	Zn 1	21.75			
	ZN 2	29.25			
				Zn2	27.83
L.S.D Value		**L.S.D T x Zn=0.668**	**L.S.D T =0.385**	**L.S.D Zn =0.472**	

Table (5) Effect of *T.harzianum* fungus and Zink foliar spraying and interaction between them (C.F.U* gm day^{-1} dried soil) after 35 day.

T.harzianum	Znic	T*Zn	T rate	Zn	Zn rate
T0	Zn 0	7.24	9.50	Zn 0	10.77
	Zn 1	10.67			
	ZN 2	10.60		Zn 1	11.91
T1	Zn 0	14.31	15.22		
	Zn 1	13.15		Zn 2	14.40
	ZN 2	18.20			
L.S.D value		LSD T x Zn=0.233	LSD T =0.134	LSD Zn =0.164	

Table (6) Effect of T.harzianum fungus and Zink foliar spraying and interaction between them (C.F.U* gm day^{-1} dried soil) season ending

T.harzianum	Znic	T*Zn	T rate	Zn	Zn rate
T0	Zn 0	6.36	9.55	Zn 0	10.30
	Zn 1	11.42			
	ZN 2	10.87		Zn 1	11.98
T1	Zn 0	14.24	14.41		
	Zn 1	12.55		Zn 2	13.66
	ZN 2	16.44			
L.S.D value		LSD T x Zn=0.224	LSD T =0.129	LSD Zn =0.159	

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