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RESEARCH ARTICLE

INTERACTION OF *BACILLUS SUBTILIS* AND *TRICHODERMA HARZIANUM* WITH MYCORRHIZA ON GROWTH AND YIELD OF CUCUMBER (*CUCUMIS SATIVUS* L.)

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ABSTRACT

Experiment was conducted in field to study the influence interaction of *B. subtilis* bacteria and *T. harzianum* fungi with AM fungi *G. mosseae* in growth and yield parameters of cucumber *Cucumis sativus*. The present study showed that the relationship between *B. subtilis* and *T. harzianum* with *G. mosseae* was positive due to getting a significant increase in the number of spores and the percentage of colonization and the infection index of AM fungi and interaction among (*G. mosseae* + *B. subtilis* + *T. harzianum*) give highest increase in the number of *G. mosseae* spores (3950) spore, mycorrhizal root (14.57)% and mycorrhizal dependency (79.8)%. The result showed that the interaction between *B. subtilis* and *T. harzianum* give significant increase in the growth and yield parameters, and all treatments with or without mycorrhiza increased the growth and yield of cucumber plant excepting fresh root weight. This increase is due to get a significant increase in chlorophyll a,b and total chlorophyll in all treatments comported with control without any addition, and in mycorrhizal treatment there is a significant increase in weight of mycorrhizal roots and mycorrhizal dependency.

INTRODUCTION

Cucumber (*Cucumis sativus*) it is one of important economic crops in Iraq and Middle East countries, but according to statistical of F.A.O. (2013) the cultivated area reached to 273000 Ha with low productivity. This due to wrong application of pesticide and chemical fertilizer which is destroyed the natural balance of soil and cause upset the activity of the soil microorganisms (George and Marschner, 1996). There for became necessary to search for alternative methods which it is effective, specialized, cheap and not harmful to the environment one of these methods it is used Biofertilizers (Pandey *et al*., 2013). Arbscular Mycorrhiza (AM) fungi one of this biofertilizers. In a study carried out by Ortas (2010) he has tested the response of cucumber plant to inoculated to different type of *Glomus* spp. which are *G. mosseae, G.etunicatum, G. clarum, g. caledonium* and the mixture of these four species in field experiment the results that mycorrhiza inoculation caused significantly increased in cucumber seedling, fruit yield, P and Zn shoot concentration and indigenous mycorrhiza inoculum successful in colonizing cucumber roots.also Tüfenkç *et al*. (2012) found the ability *G.intradices*, *G. etunicatum* and *Gigaspora margarita* in increasing the nutrient uptake and some seedling traits of four cucumber hybrids. Plant growth -promoting bacteria such as *Bacillus subtilis* can act either indirectly or indirectly in improvement plant growth (Bars *et al*., 2004) by production of growth stimulating Phytohormones, solubilization and mobilization of phosphate, production of antibiotics and induction of plant systemic resistance to pathogens (Idris *et al*., 2004; Erturk *et al*., 2010) the result of Waheed *et al*. (2014) study showed the plant which treated with *B. subtilis* recorded maximum shoot length, root length, fresh and dry weight of plant, rate of fruits weight and plant productivity. In addition to above *Trichoderma* spp. is one of fungi that many studies have proven it is efficiency as biofertilizer and biocontrol of many phytopathogen and enhance plants tolerance to wide range of a biotic stresses (Mastori *et al*., 2010; Murali *et al*., 2012) and increased chlorophyll content and concentration of nutrients uptake (Azarmi *et al*., 2011). The aim of this study to evaluate the ability of *G. mosseae* fungus, *B. subtilis* bacteria, *T. harzianum* and interaction between them in some growth and yield parameters of cucumber plant under field conditions.

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MATERIALS AND METHODS

Field experiment were carried out on Diyala - Iraq in clay - loam soil during the growth season 2012-2013. Their purpose is to study the effect of Plant Growth Promoters (PGP) *Glomus mosseae*, *Bacillus subtilis* and *Trichoderma harzianum* and their interaction between them in some parameters of growth and yield of cucumber plant, the experiment included the following steps.

Preparation the inoculation of PGP

T. harzianum funga has been added to soil after growing it on corn greish which added to the soil by 2 gm /hole. as for mycorrhizal fungi *G. mosseae* we used in this experiment two isolation of it which growing on white corn plant, the mycorrhizal inoculate (spores + hypha + mycorrhizal root) are applied at rate 10 gm / hole. Bacterial suspension of *B. subtilis* (11×10^{-9}) were applied at rate 10 ml / hole. All these inoculation were added to soil before 2 days from seeded.

Application field experiment

Cucumber seeds are surface sterilized by using Sodium hypochlorite solution 2% for 2 min then seeds washed twice with distilled water and dried by using filter paper and seeded at rate 3 seeds / hole, and 18 seeds for each treatment, in 8 treatment with 3 replicate for each treatment.

Plant and Mycorrhizal analysis

The following growing and yield parameters were recorded at the end of the experiment .the percentage of germination seeds were recorded after 10 days from seeded, length of shoot, fresh and dry weight of shoot, leaf area, number of flower, number of fruits and fruits weight, fresh and dry weight of root and For the measurements of root parameters (length, average diameter, surface area) we are using EPSON scanner V700. according to methods of Agarwal *et al*. (1986) chlorophyll a ,b and total chlorophyll are identified. For mycorrhizal analysis we are account the number of *G. mosseae* spores in soil by using the wet sieving and decanting method (Gerdemann and Nicolson, 1963), to measurement the percentage of mycorrhizal infection the root staining with acid fuchsine (Phillips and Hayman, 1970) and the Percentage of mycorrhizal colonazation determination by formula in (Giovannetti and Mosse, 1980), weight of mycorrhizal roots (Pairunan *et al*., 1980) and calculation of mycorrhizal dependency (Graham and Svertsen, 1985).

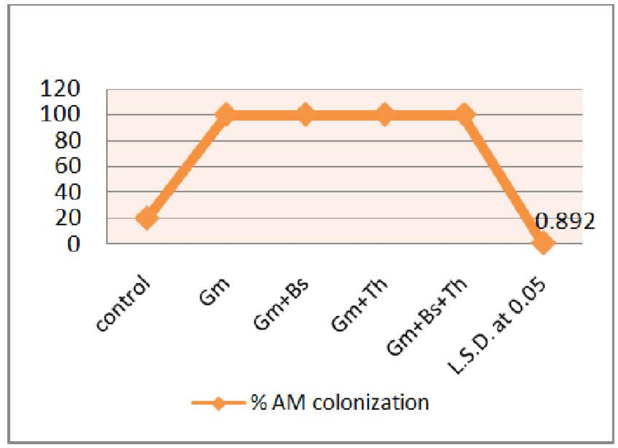
Statistical Analysis

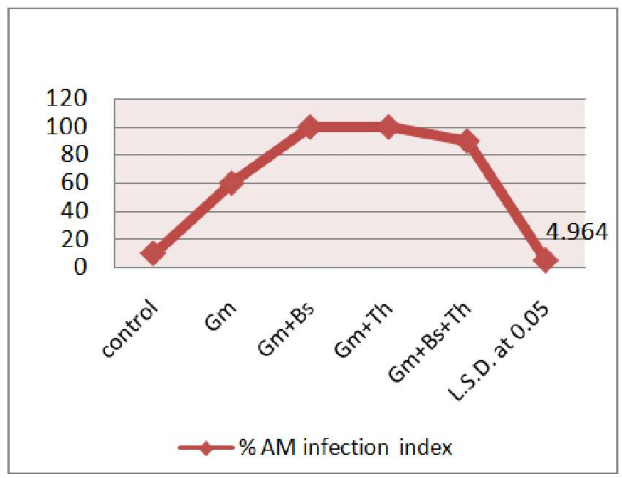
The experiment were conducted and analyzed as factorial with three replications using a Completely Randomized Design (CRD) for laboratory experiments, and a Completely Randomized Block Design (CRBD) for greenhouse and field experiments. The mean values were compared by using LSD test at probability of 5 % ($p \leq 0.05$).

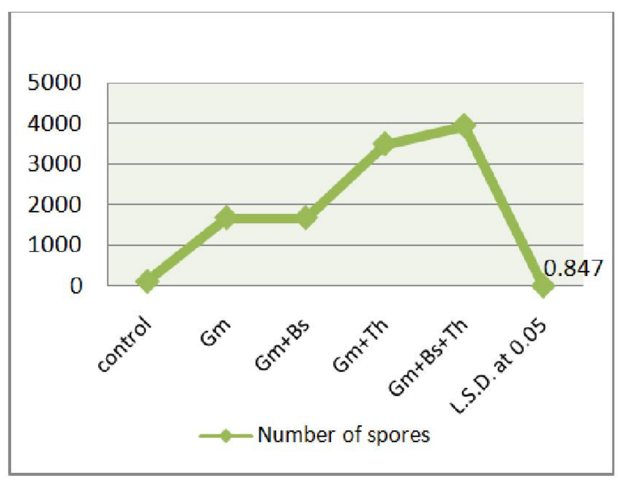
RESULTS

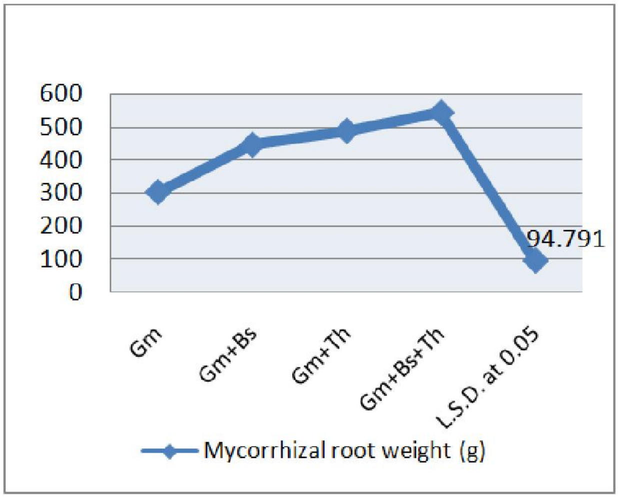
Mycorrhizal parameters

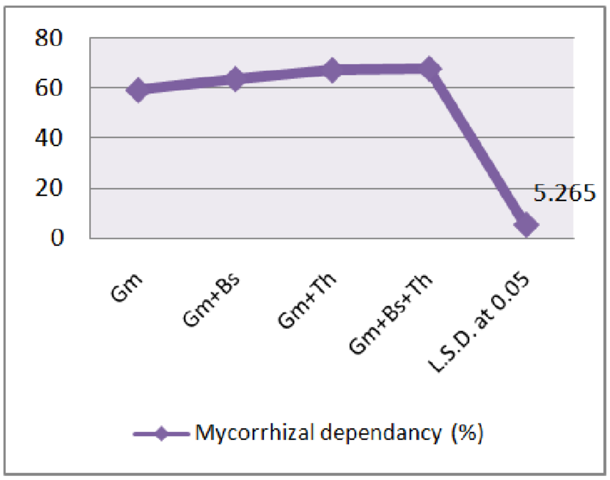
Figure (1) showed the effect of interaction of *T. harzianum* and *B. subtilis* with *G. mosseae* in AM fungi colonization, infected index of AM fungi, number of spores, weight of mycorrhizal root and mycorrhizal dependency under field condition. The result showed. Significant increase in the percentage of AM fungi colonization that was 100% for all treatments and for infection index by AM fungi it is reached to 100% in treatments of interaction (*G. mosseae* + *B. subtilis*) and (*G. mosseae* + *T. harzianum*) comparison with 60% in g. mosseae only and the figure showed that the interaction among (*G. mosseae* + *B. subtilis* + *T. harzianum*) give highest increase in the number of *G. mosseae* spores followed by treatment of (*G. mosseae* + *T. harzianum*) which was (3950, 3500) spores respectively, while we can see the same number of spores in treatments of (*G. mosseae* only) and (*G. mosseae* + *B. subtilis*) which was (1680) spores for each treatments.











Figuer 1. Influence of interaction between *T. harzianum*(Th) and *B. subtilis* (Bs)with mycorrhizal fungi *G. mosseae* (Gm) in % AM colonization, % AM infection index, Number of spores, Mycorrhizal root weight and Mycorrhizal dependancy

From the same figure we can see that the treatment of (*G. mosseae* + *B. subtilis* + *T. harzianum*) give a significant increase in the weight of mycorrhizal root flowed by treatments (*G. mosseae* + *T. harzianum*) and (*G. mosseae* + *B. subtilis*) which the percentage of increase is (79.8, 61.49, 47.35) % respectively compared with the treatment of (*G. mosseae* only). For mycorrhizal dependency the result showed that all treatments give significant increasing in the mycorrhizal dependency and the percentage of increase for (*G. mosseae* + *T. harzianum*) and (*G. mosseae* + *B. subtilis*) and (*G. mosseae* + *B. subtilis* + *T. harzianum*) are (13.51, 14.57, 14.57) % respectively.

Shoot growth and yeild parameters

The results appear in Table (1) showed the ability of PGP to increasing the growth and yield of cucumber plant which grown under field conditions. The result showed that the treatment of (*B. subtilis* + *T. harzianum*) caused a significant increase in shoot height, dry weight, leaf area, flower number, fruit number and fruit weight and the percentage of increasing was (94.56, 221.01, 242.22, 307.75, 218.95, 218.95) % respectively in comparison with control treatment. In Figuer (2) we can see that PGP shows a significant increase in the content of chlorophyll in cucumber plant and all treatments have caused a significant increase in chlorophyll a, b and total chlorophyll in variabl increase among treatments.

Root growth parameters

Table (2) showed that the treatments of (*B. subtilis*+ *T. harzianum*) caused a significant increase in all root parameters which are root length, surface area, average diameter, fresh and dry weight and the percentage increase was (294.87, 277.14, 106.78, 112.69, 207.26) % respectively compared with control treatment.

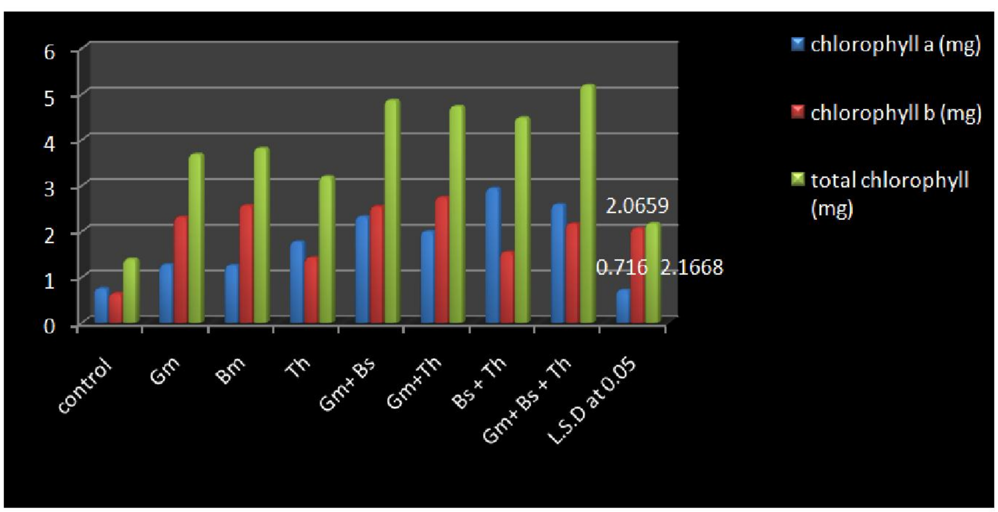


Figure 2. Influence of *B. subtilis* (Bs), *T. harzianum*(Th) and mycorrhizal fungi *G. mosseae* (Gm) and the interaction between them in the content of chlorophyll a , b and total chlorophyll in cucumber plant which grown under field condations

Table 1. Influence of *B. subtilis* (Bs), *T. harzianum*(Th) and mycorrhizal fungi *G. mosseae* (Gm) and the interaction between them in shoot growth and yeild of cucumber plant under field condations

Treatments	Height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	No. of flower	No. of fruits	Fruits weight (g)
control	22.033	13.96	3.380	184.400	4.66	5.33	399.75
Gm	33.867	32.76	8.340	630.466	16.00	13.33	999.75
Bs	31.300	24.50	7.950	370.580	12.66	10.66	750.00
Th	29.367	25.37	8.190	279.356	14.33	12.33	924.75
Gm+Bs	31.933	26.13	9.360	319.020	17.33	15.33	1149.75
Gm+Th	33.400	29.43	10.310	285.620	13.00	13.00	975.00
Bs+Th	42.867	29.57	10.850	631.060	19.00	17.00	1275.00
Gm+Bs+Th	36.330	32.46	10.503	597.220	18.00	16.33	1224.75
L.S.D. at 5%	2.854	1.786	0.569	128.125	2.621	2.601	0.438

Table 2. Influence of *B. subtilis* (Bs), *T. harzianum*(Th) and mycorrhizal fungi *G. mosseae* (Gm) and the interaction between them in root growth parameters of cucumber plant under field condations

Treatments	Length (cm)	Fresh weight (g)	Dry weight (g)	Serves area (cm^2)	Average diameter (mm)
control	38.42	5.36	1.79	11.68	0.369
Gm	95.16	8.60	3.04	24.03	0.699
Bs	66.31	8.73	3.22	16.80	0.628
Th	119.03	8.26	3.31	25.08	0.509
Gm+Bs	137.7	1.07	4.47	28.51	0.559
Gm+Th	124.58	10.60	4.90	24.87	0.755
Bs+Th	151.71	11.40	5.50	44.05	0.763
Gm+Bs+Th	127.13	11.13	5.46	25.59	0.701
L.S.D. at 5%	16.975	NS	0.688	6.744	0.072

DISCUSSION

The present reserch showed that the relationship between *T. harzianum* and *B. subtilis* with mycorrhiza fungi *G. mosseae* were positive which reflected in the increased the number of *G. mosseae* spores, percentage of root colonaziation by mycorrhiza. some studies showed that phosphate solubilising bacteria (PSB) such as *B. subtillis* and *B. circulans* together with AM fungi g. intraradices can increase the establishement of AM fungi in the onion root(Singh and Kapoor, 1998) Through induced the expression of some genes like GmFox2 which encoding to multifunctional protein (Gianinazzi *et al*., 2002). Vivas *et al*. (2003) suggested that should be using co- inoculated between AM fungi *G. mosseae* and *G. intraradices* with *Bacillus* sp. to optimize the formation and function of AM fungi symbiosis in normal and stress environments, saprotrophic fungi such as *Trichodermia* spp. mainly influence in the development of AM fungi symbiosis (Garicia- Romera *et al*., 1998). Al- Kurtany *et al*.(2008) found that the percentage of AM fungi *Glomus* spp. colonization increased when there *T. harzianum* in the same treatment which reflected on increased growth and yeild of eggplant which grown under feild conditions . also the result of this study showed the ability of all PGP and the interaction between them to increasing some growth parameters for shoot and root part and increaseing some yield parameters in cucumber plant. The maize plant shown response to inoculation with AM fungi through increasing of growth and yield parameters which is (biomass, length, circumference of spikes, number of grains per cob, grain yield and grain size), and increased in content of some biochemical such as protein, lipid and starch (Berta *et al*., 2014). Bhuvaneswari *et al*. (2014) reported that the interaction between *G. mosseae* and *T. harzianum* give significant increase in growth parametares of chilli plant such as length of shoot and root, dry weight of shoot and root, number of leaves, number of branching and number of *G. mosseae* spores. These are due to the ability of *T. harizanum* to production phytohormones such as auxins and gibberelin which enhance plant growth (Abood *et al*., 2009), increase nutrant uptake (Al- Kurtany *et al*., 2008) induction of plant resistance (Hibar *et al*., 2007) biocontrol of many bathogens (Yang *et al*., 2012) mycorrhiza fungi can improved plant growth by many strateg such as induced plant resistance for biotic and abiotic stress (Hafez and Abdel-Fattah, 2013) incrase nutrint uptake (Ortas, 2010) and the result of this study showed increase of mycorrhizal depandacy It is thought that the determination of high- mycorrhizal depandacy cultivars could lead to improvements in cucumber seedling production in the future. (Tüfnkçi *et al*., 2012) the result of important the inoculation by *B. subtilis* in this study agree with (Zongzheng *et al*., 2009) and these due to ability of *Basillis* spp. to production phytohormones (Idris *et al*., 2004).

REFERENCES

Abood H.M., Saeed F.H. and Abood M.R. 2008. Derection of gibberellins and auxins like compounds and the phyrohormoethylene in culture filterate of three isolates of *Trichodermma harzianum*. *The Iraq journal of agricultural science* 3.9(2) : 12-18.

Agarwal R. M., Dias R. R. and Chauhan R. A. 1986. Growth of *Vigna unguiculates* L. var GWL .K 3 Bin Sub- optimal moisture condition as influenced by certain anti transpirants. Plant and Soil. 91: 31-42.

Al- kurtany A K. E., AL- Zubaide N.A and Al- Thehaby R.M. 2008. The effect of inoculation by *Penicillium*, *Trichoderma*, *Aspergillus* fungi and their interaction with Mycorrhiza fungus *Glomus* on growth of Eggplant and its yield. *Tikret University Journal for Agriculture Sciences*. 8(1):254-261.

Azarmi R., Hajieghrari B. and Giglou A. 2011. Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology*. 10(31): 5850-5855.

Bars H.P., Fall R., and Vivanco J.M. 2004. Biocontrol of *Bacillus subtilis* against Infection of Arabidopsis Roots by *Pseudomonas syringae* Is Facilitated by Biofilm Formation and Surfactin Production. *Plant Physiology*. 134: 307–319

Berta G., Copetta A. and Ganalero E. 2014. Maize development and grain quality are differentially effected by Mycorrhizal fungi and a growth - promoting pseudomonad in the field. Mycorrhiza. 24: 161-170.

Buensanteai N., Sompong M., Saengchan C. and Thumanu K. 2014. The cellular components of cucumber seedlings after primed with plant growth promoting rhizobacteria, *Bacillus subtilis* Bs008. *African j. of microbiology Research*. 8(10):1006-1011.

Erturk Y., Ercisli S., Haznedar A., and Cakmakci R. 2010. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. Biol. Res. 43: 91-98.

F.A.O. 2013. World Food and Agriculture organization of United Nations. Book Rome, Italy.

Garcia-Romera I., Garcia-Garrido J., Martin J., Fracchia S., Mujica M., Godeas A. and Ocampo J. 1998. Interactions between saprotrophic *Fusarium* strains and arbuscular mycorrhizas of soybean plants. Symbiosis 24:235–246

George, E., Marschner, H., 1996. Nutrient and Water Uptake by Roots of Forest Trees. P.Z.Pflanzenernahr Bodenk 159:11-21.

Gerdemann, J. W. and Nicolson T. H. 1963. Spores of mycorrhizal endogon species extracted from soil by wet-sieving and decanting. *Trans. Br. Mycol. Soc*. 46 : 235 - 239.

Gianninazzi S., Schuepp H., Barea J.M. and Haselwandter K. 2002.Mycorrhizal technology in agriculture: from genes to bioproducts. Bosten - Berlin. pp:20-21.

Giovanneti, M. and Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489 -500.

Graham, J. H. and Syvertsen J. P.1985. Host determinants of mycorrhizal dependency of citrus rootstock seedlings. New Phytologist. 101:667 -676.

Hafez E.E. and Abdel-Fattah G.M. 2013. Molecular defense response of mycorrhizal bean plants infected with *Rhizoctonia solani*. Ann. microbiology .63:1195-1203.

Hibar, K., Daami-Remadi M., Jabnoun-Khiareddine H., and El Mahjoub M. 2005.In vitro and in inhibitory effect of *Trichoderma harzianum* on *Fusarium oxysporum* f. sp. *Radicis-lycopersici*. *Biotechnol. Agron. Soc. Environ*. 9: 163-171.

Idris E.E., Bochow H., Ross H., and Borriss R. 2004. Use of Bacillus subtilis as biocontrol agent. VI. Phytohormone likeaction of culture filtrates prepared from plant growth promoting *Bacillus amyloliquefaciens* FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. *Journal of Plant Diseases and Protection*.111 (6): 583–597.

Mastouri F., Björkman T., Harman G. 2010. Seed Treatment with *Trichoderma harzianum* Alleviates Biotic, Abiotic, and Physiological Stressesin Germinating Seeds and Seedlings. *Phytopathology j.* 100(11):1213-1221 .

Murali M., Amruthesh K., Sudisha J., Niranjana S., and Shetty H. 2012. Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. *Journal of Phytology*. 4(5): 30-36

Ortas I. 2010. Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. *Spanish Journal of Agricultural Research*. 8(S1): S116-S122.

Pandey, B., Bhattacharya, C. and Singh, S. 2013. Effect of pesticides and insecticides on biofertilizer. *International Journal of Advanced Biological Research*, 3(4): 563-566

Pariunan, A. K., Robson A. D. and Abbott L.K. 1980. The effectiveness of Vesicular-arbuscular mycorrhizas in increasing growth and phosphorous uptake of subterranean clover from phosphorous sources of different solubilities . New Phytol., 84 : 327 - 338.

Phillips, J. M. and Hayman D. S. 1970. Improved procedures for clearing roots and staining parasitic and Vesicular - arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc*., 55: 158 - 161.

Singh, S. and Kapoor, K. 1998. Effects of inoculation of phosphate-solubilizing microorganisms and an arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions. Mycorrhiza. 7: 249-253.

Tüfenkçi S., Demir S., Şensoy S., Ünsal H., Demirer E., Erdinç Ç., Biçer Ş. and Ekincialp A. 2012. The effects of arbuscular mycorrhizal fungi on the seedling growth of four hybrid cucumber (*Cucumis sativus* L.) cultivars. *Turk J Agric For*. 36 : 317-327

Vivas A., Marulanda A., Ruiz-Lozano J., José_ Miguel B. and Juan Manuel Ruiz-Lozano· Barea· Azcón R. 2003. Influence of a Bacillus sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza (2003) 13:249–256.

Waheed A.Q., Hassan H.R., Abbas B.A., and Nawar H.H. 2014. Evalution of some growth promoting bacterial strains exist on Eggplant root *Solanumm elongena* L. against *Rhizoctonia solani*. *Journal of Biotechnology Research Center (Special edition)*. 8(1):14-19.

Yang Z., Yu Z., Lei L., Xia Z., Shao L., Zhang K., Li G. 2012. Nematicidal effect of volatiles produced by *Trichoderma* sp. *Journal of asia-pacific entomology*.15.pp:647-650.

Zongzheng Y., Xin L., Zhong L., Jinzhao P., Jin Q. and Wenyan Y. 2009. Effect of *Bacillus Subtilis SY1* on antifungal activity and plant growth Yang. *Int J Agric and Biol Eng*. 2(4):55- 61.

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