J. Agric. Sci. Mansoura Univ., 31 (4): 2533 - 2549, 2006

NUTRITIONAL STATUS OF ONION AS AFFECTED BY BIOLOGOGICAL CONTROL OF WHITE ROT DISEASE

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ABSTRACT

Pot experiments were designed in two seasons of 2004 – 2005 to study nutritional status of onion as affected by biological control of white rot disease. The experiments involved three different microorganisms, namely *Bacillus subtilis, Trichoderma harzianum* and mixture of *Mycorrhiza glomus* of fungi *Eturicatum glomus* in *Traradices glomus* fasciculatum, alone (individual bioagents) or together (mixture bioagents), which were used as soil drench. Two doses of *B.subtilis* and *T.harzianum*, namely (A) 100 and (B) 50 mL / kg soil and two doses of *Mycorrhiza* namely, (A) 10 and (B) 5 g/kg soil were applied.

The obtained results can be summarized as follow:

1. Application of microorganisms, namely (*Bacillus subtilis, Trichoderma harzianum* and *Mycorrhiza glomus* at the rate A or B increased the availability of macronutrients (N, P and K) in soil, macronutrients uptake, percent yield, efficacy of control white rot disease as compared to control treatment (without inoculation).
2. Application of microorganisms at the rate (A) was superior in all parameter under study to rate (B).
3. At a scale of single treatment application of *Bacillus subtilis* as individual to soil was superior in % efficacy of control white rot disease, N, P and K uptake, availability N, P and K in soil and percent yield than other microorganisms.
4. The mixture of *Bacillus subtilis* and *Mycorrhiza glomus* gave the highest increases in all parameters than those achieved by the mixture of *Trichoderma harzianum* and *Mycorrhiza glomus* or any single treatment.
5. Application of a mixture of *Bacillus subtilis* and *Mycorrhiza* increased the % of efficacy of control white rot disease.

Keywords: Biological control *B. subtilis, T. harzianum, Mycorrhiza glomus*, macro nutrient uptake nutrient availability in soil, onion yield.

INTRODUCTION

Nutrients are part of the "environment" for plant and microbial growth and, although frequently unrecognized, always they have been considered as an important factor in disease control. All the essential nutrients are reported to influence the incidence or severity of some diseases; however, no single nutrient controls all diseases or favors disease control for any one group of plants (Huber, 1991). A particular element may decrease severity of some diseases, but increase others and have an opposite effect in a different environment. The effects of N (nitrogen), P (phosphorus), and K (potassium) on disease are reported more frequently than the effects of minor nutrients because of the limited availability of the macronutrients in many soils relative to the large quantity required for optimum plant growth. Nevertheless, it is through an understanding of the disease interactions with each specific nutrient that the roles of the plant, pathogen, and environment can be effectively modified for improving disease control.

Inhibiting nitrification can increase the efficiency and availability of N in high leaching or denitrifying conditions. Microbial enrichment with mycorrhiza or plant growth promoting organisms also increase nutrient uptake especially P, Zn, and Mn; microbes in the rhizosphere influence minor elements availability through their oxidation reduction reactions or siderophore production (Huber and Mccay Buis, 1993). Biological control of rot pathogens that immobilize nutrients in the rhizosphere, and removing weed and pest competition, can increase nutrient availability.

Nutrient uptake can be modified by genetically improving a plants efficiency in root absorption, translocation, and metabolic efficiency or by increasing tolerance to otherwise excess amounts of a specific nutrient (Huber *et al.*, 1995) at the same time, Sing and Kapoor (1999) indicated that inoculation with *Vesicular – Arbuscular Mycorrhizal* (VAM) fungus *Glomus* sp. 88 improved root colonization. At maturity, grain and straw yields as well as N and P uptake improved significantly following inoculation with Phosphate - Solubilizing Microorganisms (PSM), i.e., *Bacillus circulants* and/or *VAM fungs*. Also the inoculum containing both *Entgrobacter agglomerans* (PSM) and *Glomus etumicatum (VAM)* led to a significant increase in total P and N uptake and plant growth. E. *agglomerans* and *micorrhiza* might convert phosphate into available forms and thus enhance plant P uptake.

On the other hand, Kim *et al.* (1998) showed that dual inoculation with *Bacillus circulants* (PSB) and *VAM* fungus resulted in higher grain and straw yields than when these organisms were used alone..

Inoculation of lettuce, onion and clover with *VA Micorrhizal* fungus *(Glomus mosseae)* increased plant yield and phosphate uptake in 3 soils that had been depleted in phosphate (Owusu and Wild, 1980).

Reddy and Rahe (1982) revealed that seed bacterization significantly increased shoot dry weight (12-94%), root dry weight (13-100%) and shoot height (12-40%) of onion seedlings over controls.

Tawarya *et al.* (2001) indicated that *Allium fistulosum* cultivars were well colonized with *Glomus fasciculatum*, the percentage mycorrhizal colonization ranges from 48% to 81%. Shoot phosphorus (P) uptake and dry mass of plants increased with mycorrhizal colonization and mycorrhizal dependency shoot growth ranged from 73 to 95%.

Inoculation delay onion white rot epidemics by 2 weeks and provided significant protection against the disease for 11 weeks after transplanting. Mycorrhiza plants showed an increase of 22% in yield, regardless of the presence of s.cepivorum (Torres *et al.*, 1996)

Reddy and Rahe (1989) showed that seed bacterization with *Bacillus subtilis* (B-2), UI-1 and B caused significant increases in shoot height and shoot dry weight of onion seedlings over controls.

Onion (*Allium cepal*) represents one of the most important and economic crop in the worldwide. Onion white rot disease is among of the most important diseases, which causes severe yield losses in onions (Villalta *et al.*, 2002).

Onion white rot caused by the soil borne fungus *Sclerotium cepivorum* is a serious disease of onions world wide and the absence of resistant cultivars or efficient chemical control makes this pathogen a good target for biological control (Clarkson *et al.*, 2001).

Mclean *et al.* (2005) used *Trichodermaa* atroviride to control white rot both in greenhouse and in the field. When isolate C_{52} was introduced into *Sclerotium capivorum* infestd soil as both pallet and solid – substrate formulation there was no statistic significant differences in the disease control between these treatments (pallet treatment) doubled the percentage of healthy plants compared with the control treatment.

EL- Haddad *et al.* (2004) evaluated the efficacy of *Glomus intaradices, Glomus mosseae, Glomus clarum Gigaspora margarita* and *Gigaspora gigantea* in controlling onion white rot, either *VAM* mycorrhiza was applied to seed or to soil drenching 14 days before transplanting and found that all the tested *mycorrhize* controlled white rot in onion and increased the yield of the crop under greenhouse and field conditions.

Metcalf *et al.* (2004) stated that *Trichodermaa koningii supperessed, Sclerotium cepivorum* and controled white rot diseas in onion.

Metcalf (2002) found that *Trichodermaa* spp isolates significantly reduced onion white rot caused by *S. cepivorum* infection and increased the yield of onion compared with the untreated control.

Reddy *et al.* (1992) revealed that different isolates of *Bacillus subtilis* controlled onion white rot disease through suppressing of germination of *sclarotil* of *Sclerotium cepivorum* fungus.

The objectives of this study are to evaluate the effect of microorganisms namely *Bacillus subtilis, Micorrhaiza glomus* and *Trichoderma harzianum,*on nutritional status of onion and the biological control of white rot disease.

MATERIALS AND METHODS

1- Inoculum preparation and soil greenhouse infestation

a. Pathogenic fungus:

Pathogenic fungus was isolated from diseased onion seedlings and was identified as *Sclerotium cepivorum.* Identification of the three isolated fungi was carried out according to cultural properties, morphological and microscopic characteristics described by Gilman (1957), Barnett (1960) and Singh (1982).

b. Pathogenicity test:

Pathogenicity test was carried out by using *S. cepivorum* isolates A, B and C the most aggressive pathogenic isolate among them was used for further studies whereas the other isolate was neglected.

Sclerotia of three pathogenic isolates A, B and C were inoculated in bottles (500 ml capacity), each bottle contains 100 mL of potato dextrose agar (PDA) medium. The inoculated bottles were then incubated at 20° C. After 15 days, *Sclerotia* of the three different isolates were harvested using smooth brush. *Sclerotia* of *S. cepivorum* were added to light clay soil at the rate of 100 *Sclerotia* / Kg soil. Pots of 25 cm in diameter were then filled with 3 Kg infested soil each. Two onion seedlings variety Giza 20 were transplanted in each pot. Ten pots were used for each fungus isolate. While ten pots only each contains non- infested 3Kg soil were left to serve as a control treatment. All pots were kept in greenhouse and percentages of both diseased and healthy plants were calculated (Abd El Moity, 1985).

c. Antagonistic microorganisms

Three different biocontrol agents (*Bacillus subtilis, Trichoderma harzianum* and a mixture of Mycorrohizal fungi *Glomus etunicatum, Glomus intraradices* and *Glomus fasciculatum*) were used in this study. *Bacillus subtilis* was grown on nutrient glucose broth Medium (NGB) prepared by Dowson (1957). The bacterial suspension was adjusted to contain 30 $\times 10^6$ cfu. *Trichoderma harzianum* was grown in liquid Gliotoxin Fermented Medium (GFM) developed by Brain and Hemming (1945). Under complete darkness just to stimulate toxin production (Abd EL Moity and Shatla 1981) for 9 days. The suspension of *Trichoderma harzianum* contains 30 $\times 10^6$ cfu. Mixtures of mycorrhizal fungi were used as a commercial product known as "Triton".

Greenhouse Experiment:

The aim of this experiment was to study the effect of inoculation with different beneficial microorganisms namely *Bacillus subtilis, Mycorrhiza glomus* and *Trichoderma harzianum* (bioagents) either individually or in a mixture on nutritional status, yield and biological control of onion white rot disease.

The experiment was in randomized complete design with four replications. Clay soil sample was collected from the surface layer (0-40 Cm) homogenized, air dried and passed through a 2-mm sieve. Pots of 25 Cm diameter were filled with 3Kg soil each. All pots were infested with *S. cepivorum* to soil at the rate of 100 sclerotia /kg soil. All pots where received inorganic fertilizers, ammonium sulfate (20%N), super phosphate (15.5% P_2O_5) and potassium sulfate (48%K_2o) at a recommended doses. Three onion seedlings were transplanted to each pot and ten replicates were used. All bioagents were added as soil drench one day before transplanting for each treatment. Soil infested with pathogen only was used as control treatment.

The experiment was conducted at the greenhouse of Agric, Res. Center (ARC), Giza, Egypt. Onion seedlings (*Allium cepa*) were cultivated in winter seasons (2004-2005). Some physical and chemical characteristics of the tested soil sample are shown in (Table 1).

Experimental analyses:

1- Evaluation of bioagents (beneficial microorganisms) efficacy on controlling onion white rot disease and N, P,K uptake.

In this experiment both antagonists *Trichoderma harzianum* and *Bacillus subtilis* were added as suspension at the rate of 100 mL / Kg soil where as mixture of mycrohiza was added at the rate of 10g/ Kg soil All antagonists were added as soil drench to infested soil before 1 day transplanting Soil infested only with pathogen was used as control treatment.

Diseased incidence was determined weekly for two months as a percentage of efficacy of treatment was calculated according the next formula.

$$A = 100 - \frac{F1}{F2} \times 100$$

A: denotes % of efficacy. F_1: denotes % of diseased plant in treatment.
F_2: denotes % of diseased plant in control.

Table (1): Some physical and chemical characteristics of the experimental soil

Particle size distribution %	
Coars sand	16.8
Fine sand	15.8
Silt	28.1
Clay	39.3
Texture	Clay loam
Chemical properties	
$CaCO_3$ %	3.43
pH (1:2.5 soil- water suspension)	8.20
EC dS/m (at 1:5 soil- water extract)	2.65
Organic matter %	2.00
Available nutrients (mg kg $^{-1}$) soil	
N	100
P	13.0
K	320
Fe	6.5
Cations meq L^{-1}	
Ca^{++}	5.70
Mg^{++}	3.40
Na^{+}	16.80
K^{+}	0.98
Anion meq/L	
$CO_3^{=}$	0
HCO_3^{-}	3.56
Cl^{-}	13.40
$SO_4^{=}$	9.92

At maturity onion plants were collected, washed and oven dried (70C°) for dry weight determination. While, macro nutrient (N, P and K) were determined according to Cottenie *et al.* (1982).

Soil samples for each treatment were subjected to determiner available nitrogen, phosphorus and potassium according to Black (1982).

Obtained results were subjected to statically analysis according to Snedecor and Cochran (1980) and the treatments were compared by using least significant difference (L.S.D) at 0.5 level of probability.

2- Efficacy of different doses of biogents (beneficial microorganisms) on controlling white rot disease and nutritional status of onion.

Individually bioagent was added to soil at two doses of suspension for all *Bacillus subtilis* and *Trichoderma harzianum* at the rate of 100 mL / Kg soil (represent as dose A) and 50 mL / Kg soil (represent as dose B).

Where as in mycorrhiza was used at the rate of 10 g / Kg soil (represent as dose A) and 5g / kg soil (represent as dose B) all parameters were measured as previously mentioned.

3- Effect of different bioagents mixture on the controlling of white rot disease and nutritional status of onion.

In this study different mixtures of bioagent were used. The first group was a mixture of *Trichoderma harzianum* and *Mycorrhiza glomus* at the rate of 100 mL of *T. harziunum* and 10 g spores of *Mycorrhiza glomus* / Kg soil. The second group was amixture of *Bacillus subtilis* and *Mycorrhiza glomus* at the same rate. All treatments were applied as soil drench. All parameters were measured as mentioned above.

RESULTS AND DISCUSSION

I – Biological control of white rot disease

1- Pathogenicity test:

Data in Table (2) show that the three isolates were pathogenic to onion seedlings. It was also noticed that the percentage of disease were significantly higher in onion plants infested with *S. cepivorum* isolate A then those infested with *S. cepivorum* isolate B and C. These data are in harmony with those obtained by Abd El Moity (1976). *S. cepivorum* isolates were varied in their destructive effect. This effect is depended on the ability of isolates to produce different quantity of polyglacturonase and oxalic acid **(Batemen and Beer, 1964).**

Table (2): Percentage of white rot disease in onion caused by the three *S. cepivorum* isolates and healthy survivals of onion plants

Infested soil with *S. cepivorum*	Percent % of diseased plants	Percent % of healthy plants
S. cepivorum A	100	0
S. cepivorum B	76	24
S. cepivorum C	92	8
Un- infested soil	0	100
LSD at 5%	2.11	2.11

2 - Effect of different bioagents on controlling of white rot disease on onion:

The effect of different bioagents and their reaction against *Sclerotium cepivorum*, (the cause agent of white rot of onion) was tested under greenhouse conditions for two seasons. Data presented in Table (3) showed that all tested bioagents reduced significantly the disease incidence, and increased the yield of both fresh and dry weights in both seasons.

Bacillus subtilis was the most effective bioagent in decreasing the percentage of disease incidence and gave 4 % disease incidence and 90% efficacy compared with 40% disease incidence and 0%efficacy in control treatment in first season, while the second season gave 10% disease incidence and 83.4% in the efficacy compared with 60% disease incidence and 0% in the efficacy of control treatment. Data revealed that there was a positive correlation between the efficacy of the treatment and the increases of the yield and dry weight *T. harizianum* and *Mycorriza glomus*.

Trichoderma harzianum and *Mycorrhiza* fungi followed *B. subtilis* in this respect and gave the same percentage of diseased incidence, which were 12% and 20% compared with 40% and 60% in control treatment in both season, respectively.

These results indicated that different antagonists had behaved differently towards efficacy under the same conditions. Treatment of *Bacillus. subtilis* led to the best control of white rot disease. This controlling might be due to that *Bacillus subtilis* produces antibiotic and antifungal material (Johnson *et al.*, 1960) these antifungal materials inhibit growth of pathogenic fungus consequently they become more susceptible to the effect of other microorganisms naturally exist in the soil (Hader and Gorodecki., 1991).

The effect of the *Trichoderma* is due to hyphal contact coiling, deformation and lysis, non volatile metabolites of *Trichoderma* were effective in inhibiting the mycalial growth and sclarotial germination (Peyghami, 2001). In addition, *Trichoderma harzianum* produces antifungal antibiotic substances as gliotoxin and Viridian (Abd- El Moity, 1981 and Abd- El moity *et al.*, 2003). Mechanisms of the antagonistic affected of mycorrhizal fungi were included direct interaction such as competition between the symbiont and pathogen for infection sites (Muchovej *et al.*,1991) or indirect interactions such as alteration of root exudation and or of the mycorrhizal share in microbial community (Hassan- Dar *et al.*, 1997 and Filion *et al.*, 2003).

Table (3): Evaluation of efficacy of some bioagentes on controolling white root disease on onion

Different treatments	First season			
	%of disesase	% of Effecacy	Yield g/pot	Dry weight g/plant
Trichoderma harzianum	12	70	106	2.0
Mycorrhiza glomus	12	70	108	2.2
Bacillus subtilis	4	90	116	2.7
Control	40	0	66	1.54
Second season				
Trichoderma harzianum	20	66.7	108	2.2
Mycorrhiza glomus	20	66.7	114	2.5
Bacillus subtilis	10	83.4	118.7	2.7
Control	60	0	64.0	1.60
L.S.D.	0.054	0.054	0.054	0.134

Data in Table (4) show that adding different doses of bioagents led to different degrees of protection against *Sclarotium cepivorum* pathogen. A positive correlation was observed between doses of antagonist and efficacy of the treatment. The best results of different antagonists were obtained at their recommended doses (A) compared with their half ones (Abd El Moity *et al.*, 1990; Aly *et al.*, 1995 and Rodriguez and Cotes, 1999).

These results can be explained by data of (Wolk and Sorker 1994) who stated that the efficacy of antagonists depends on their capacity comparing with other microorganisms occupied rhizosphere area.

Data in Table (5) show that using a mixture of *Bacillus subtilis* and mycorrhiza led to increase the efficacy of the controlling of the white rot disease and gave 100% efficacy compared with using *Bacillus subtilis* or *mycorrhiza* as single treatment. Data also showed that *Trichoderma harzianum* and mycorrhiza mixture led to increase the efficacy compared with a single treatment. This result is due that compatibilty between *Bacillus subtilis* and *Mycorrhiza* or *Tricodermma harzianum* and *Mycorrhiza*. This compatible relation led to synaregestic effect between mixture of bioagent and increasing the efficacy of the control of disease (Abd El Moity, 1985).

Table (4): Efficacy of different doses of bioagents on controlling white root disease in onion

Different treatments	First season			
	%of disesase	% of Effecacy	Yield g/pot	Dry weight g/plant
Trichoderma harzianum (A)	12	70	106	2.0
Trichoderma harzianum (B)	12	70	101	1.8
Mycorrhiza glomus (A)	12	70	108	2.20
Mycorrhiza glomus (B)	25	37.5	78	1.8
Bacillus subtilis (A)	4	90	116	2.7
Bacillus subtilis (B)	5	87.5	102	2.1
Control	40	0	66	1.54
Second season				
Trichoderma harzianum (A)	20	66.7	108	2.2
Trichoderma harzianum(B)	20	66.7	103	1.9
Mycorrhiza glomus (A)	20	68.9	110	2.5
Mycorrhiza glomus (B)	40	33.4	100	2.0
Bacillus subtilis (A)	10	83.4	118.7	2.7
Bacillus subtilis (B)	10	83.4	106	2.2
Control	60	0	64	1.6
L.S.D.	0.054	0.054	0.054	0.134

(A)*: The recommended dose.
(B): The half dose.**

Table (5): Effect of different mixture of bioagents on controlling white rot disease on onion

Different treatments	First season			
	%of disesase	% of Effecacy	Yield g/pot	Dry weight g/plant
Mix. of T.harzianum and Mycorrhiza	10	75	117.5	2.6
Mix. of B. subtilis and Mycorrhiza	0	100	121	2.8
Control	40	0	66	1.54
Second season				
Mix. of T.harzianum and Mycorrhiza	10	83.4	120	2.8
Mix. of B. subtilis and Mycorrhiza	0	100	127.6	3.1
Control	60	0	64.0	1.60
L.S.D.	0.054	0.054	0.054	0.134

2- Plant nutrient status and yield

1.Evaluation of different microorganisms for their efficacy in macro-nutrients uptake of onion.

Data in Table (6) indicated that inoculation with microorganisms (*Trichoderma harzianuma, Mycorrhiza* and *Bacillus subtilis*) as individually to clay soil at the both seasons led to significant increases in N, P and K uptake by onion plants as compared to control treatment (clay soil without inoculation). Increase of mineral uptake by plant could be due to the increase of the root system area and not to any specific enhancement of the normal ion uptake mechanisms (Murty and Ladha 1988). The maximum N, P and uptake was observed when soil inoculated with *Bacillus subitilis* while the lowest N, P and K uptake was noticed when soil inoculated with *Trichoderma harzianum;* this is due to that *Bacillus subtilis* produces antibiotic and antifungal material as *subtilis* (Johnson *et al.*, 1960).

The percent yield increase over control treatment due to different adding microorganism as individually to soil at the both seasons are shown in Fig. (1). The yield increased significantly along with inoculation with different microorganisms (*Bacillis subtilis, Mycorrhiza glomus* and *Trichoderma harzianum*).

The yield at the first season increased by 75, 63 and 60% respectively, while in the second season the increases were 85, 71 and 68%, respectively. This increase in yield may be due to that rhizosphere organisms produced greater amount of organic acids, such as tartaric, citric and lactic acid which may improve plant productivity and increase of the root system (Wafaa, 2001). Rhizosphere soils receive more organic materials and are characterized by higher microbial activity. (Kim *et. al.*, 1998).

From the above mentioned results it could be concluded that microorganisms inoculation can be arranged regarding N, P and K uptake and yield of onion in descending orders as follows:

Bacillus subtilis > *Micorrhiza glomus* > *Trichoderma harzianum.*

Table (6): Nitrogen , phosphorus and potassium uptake of onion as affected by inoculation with microorganisms

Different treatments	Plant uptake mg / plant					
	First season			Second season		
	N	P	K	N	P	K
Trichoderma harzianum	52	4	54.0	56	4.5	67.5
Mycorrhiza glomus	52.8	4.5	60.6	60.2	5.2	71.8
Bacillus subtilis	78.3	6.7	72.9	80.3	7.0	77.5
Control	32.3	2.5	30.8	43.0	3.00	41.5
L.S.D.	0.095	0.155	0.134	0.095	0.109	0.095

1.Effect of different doses of microorganisms on onion macro- nutrients uptake.

Macronutrients uptake increased significantly by two doses of microorganisms as compared to control treatment (soil without inoculation).Data obtained are recorded in Table (7). Results revealed that, at the recommended dose (A), nutrients uptake (N, p and K) increased significantly in both seasons compared with half recommended dose, this is due to efficacy of microorganisms depend on their capacity comparing with other microorganisms occupied rhizosphere area (Wolk and Sorker, 1994).

The highest value of N, P and K uptake was obtained when soil was *inoculated with Bacillus subtilis* followed by *Mycorrhiza glomus* and. *Trichoderma harzianum* at the recommended dose (A) and (B).

Results shown in Fig. (2) indicate that percent yield increased over control treatment due to different doses of isolates *Bacillus subtilis*, *Mycorrhiza glomus* and. *Trichoderma harzianum* in both seasons.

The inoculation soil with isolate *Bacillus subtilis* increased the yield by (75% and 54%), *Mycorrhiza* isolate (63% and 51) and *Trichoderma harzianum* by (60% and 48%) over control at the rate (A) and (B) in the first season, respectively.

Also, percent yield increases due to different doses of isolates were in the same trend in the second season.

On the other hand, soil inoculation with *Bacillus subtilis* appeared to have maximum increase in percent yield increased of onion at the rate (A) and (B) in the first season as well as in the second season **(El Haddad *et al.*, 2004 and Kim *et al.*, 1998).**

2. Effect of different mixture of microorganisms (*Bacillus subtilis, Trichoderma harzianum and Mycorrhiza glomus*) on N, P and K uptake of onion.

Data in Table (8) show that soil inoculated with different mixtures of isolates increased significantly nutrients (N, P and K) uptake of onion as compared to non inoculated soil. The highest values of nutrients (N, P and K) uptake were (92.4, 7.0, 84 and 97. 72 and 90 mg/plant) in the first and second season, respectively. These high values were obtained after soil being inoculated with a mixture of isolates *B.subtilis* and *Micorrhiz glomus*. Increasing of nutrients uptake in these mixture (*B. subtilis* and *Mycorrhiza glomus*) due to an increase in the number of uptake sites per unit area of colonized roots and a greater ability for these roots to exploit the soil for nutrients and bacteria might affect plant growth, so that actively growing roots are able to explore a greater volume of soil (Kim *et al.*, 1998 and Sing and Kapoor, 1999).

Table (7): Nitrogen , phosphorus and potassium effect of two doses of microorganisms on N , P , and K uptake of onion

Different treatments	Plant uptake mg / plant					
	First season			Second season		
	N	P	K	N	P	K
Trichoderma harzianum (A)*	52	4.0	54.0	56.0	4.5	67.5
Trichoderma harzianum (B)**	44.4	2.8	51.7	46.8	3.0	54.5
Mycorrhiza glomus (A)	52.8	4.5	60.6	60.2	5.2	71.8
Mycorrhiza glomus (B)	43.0	2.8	30.0	48.0	3.1	50.0
Bacillus subtilis (A)	78.3	6.7	72.9	80.3	7.0	77.5
Bacillus subtilis (B)	52.5	3.2	46.2	52.0	4.5	50.0
Control	32.3	2.5	30.8	43.0	3.0	41.5
L.S.D.	0.059	0.155	0.134	0.095	0.109	0.095

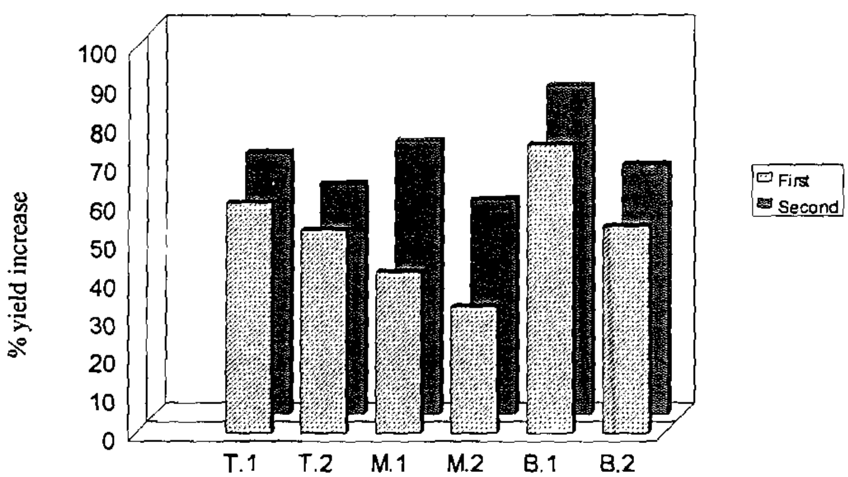
(A)*: The recommended dose. (B): The half dose.**

Table (8): Nitrogen , phosphorus and potassium uptake of onion plants as affected with different mixtures of microorganisms inoculation

Different treatments	N,P and K Plant uptake(mg / plant)					
	First season			Second season		
	N	P	K	N	P	K
Mix. of T.harzianum and Mycorrhiza glomus	78.0	6.2	82.1	81.0	7.0	89.1
Mix. of B. subtilis and Mycorrhiza glomus	92.4	7.0	84.0	97.0	7.2	90
Control	32.3	2.5	30.8	43.0	3.0	41.5
L.S.D	0.095	0.155	0.134	0.095	0.109	0.095

Concerning percent yield increases of onion at both seasons data in Fig (2) indicated that either soil inoculated with mixtures of isolates were percent yield increased significantly as compared to non - inoculated treatment. Inoculation with a mixture of *B. subitilis* and *Micorrhiza glomus* increased the yield by 83 and 90% in the first and second seasons, respectively. These results are in agreement with Wu *et al.* (2005) who reported that application of biofertilizer led to improve some physical and biochemical soil properties. The inoculation with rhizobacteria can significantly increase the root *Mycorrhiza* colonization and P uptake.

Generally, inoculation with different microorganisms as mixtures increased significantly nutrients (N, P and K) uptake and yield of onion. Also, *Bacillus subitilis* inoculation as mixture with *Mycorrhiza glomus* was superior in (N, P and K) uptake and % yield increased as compared to other treatments.



1. **Fig (1): Yield increase percent due to different doses of microorganisms.**
2. **T1: *Trichoderma harzianum (A)*** **B2: *Bacillus subtilis (B)***
3. **T2: *Trichoderma harzianum (B)*** **M1: *Mycorrhiza glomus (A)***
4. **B1: *Bacillus subtilis (A)*** **M2: *Mycorrhiza glomus (B)***

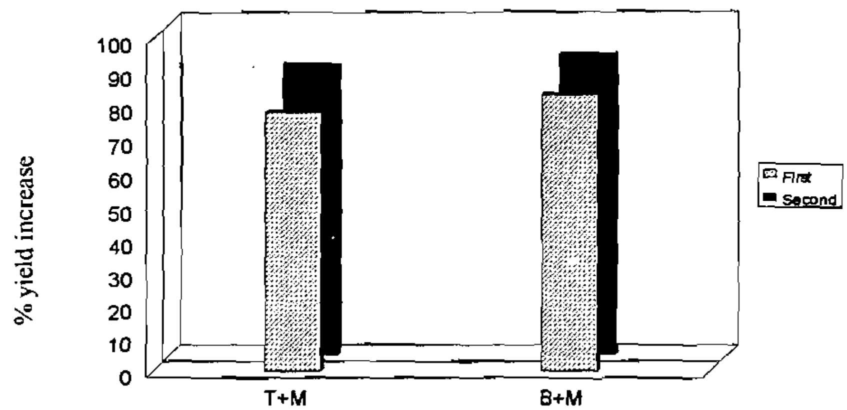


Fig (2): Yield increase percent due to dual inoculation with *Bacillus subtilis*, *Trichoderma harzianum* and *Mycorrhiza glomus*.
T+M: *Mixture of Trichoderma harzianum +Mycorrhiza glomus*

2 - Nutrients availability in soil:

1 – Evaluation of different microorganisms inoculation for their efficacy in availability of nutrients (N, P and K) in soil.

The status and availability of the studied nutritional elements (N, P and K) in soil inoculated with different microorganisms after onion harvesting in both tested seasons are shown in Table (9).

Results revealed that available nitrogen, Phosphorus and potassium in soil inoculated with different microorganisms namely *Bacillus subtilis*, *Mycorrhiza glomus* and *Trichoderma harzianum* increase significantly increase as compared to control treatment (soil without inoculation) in both seasons. Moreover, soil inoculation with *Bacillus subtilis* was superior in available N, P and K as compared to other treatments in both seasons Cannon *et al.* (1995) reported that a significant increase in soil and plant tissue P occurred in a greenhouse study where oxalic acid or salsola leachate was added. Kim *et al.* (1998) reported that available P increased in soils inoculated with phosphate solubilizing microorganisms.

Table (9): Macronutrients availability in clay soil as affected by different microorganisms inoculation

Different treatments	Available macronutrients (ppm)					
	First season			Second season		
	N	P	K	N	P	K
Trichoderma harzianum	195	15	590	198	21	595
Mycorrhiza glomus	200	18	600	204	23	620
Bacillus subtilis	210	21	620	215	28	650
Control	160	12	510	170	18	540
L.S.D.	12.52	3.43	11.23	7.85	2.58	11.28

2 – Evaluation of efficacy for two doses from different microorganisms inoculation in the availability of nutrients (N, P and K) in soil:

There was positive significant effects of different doses of *Bacillus subtilis, Trichoderma harzianum* and *Micorrhiza* glomus on available N, P and K in soil Table (10)

Results indicate that, soil inoculation with different microorganisms at the recommended dose (A) and half dose (B) increased significantly available N, P and K in soil as compared to control treatment. With respect to the effect of doses of microorganism obtained results showed that the dose (A) was more effective on available N , P and K contents in soil as compared to lower rate (B) which explain that low microorganisms inoculation was not favorable as compared to relatively higher rate. Also, results reveal that available N, P and K in soil inoculated with *Bacillus subtilis* were superior in both seasons at both doses (A) and (B) as compared to *Mycorrhiza glomes* and *Trichoderma harzianum.*

3- Effect of different microorganisms mixtures (*Bacillus. subtilis, Trichoderma harzianum* and *Mycorrhiza* glomus) on available nitrogen, Phosphours and potassium in soil.

Data illustrated in Table (11) show that macronutrients availability in soil as affected by mixture inoculation with different microorganisms in two seasons of onion.

Obtained results show that mixture inoculation with *Bacillus subtilis* , *Trichoderma harzianum* and *Mycorrhiza glomus* increased significantly available nitrogen, phosphours and potassium in soil as compared to control treatment (without inoculation)or individually inoculation.

Data also indicate that soil inoculation with *Bacillus subtilist+ Micorrhiza glomus* gave the highest values of available N, P and K (230 , 25 and 660 ppm) in the first season as well as it recorded (245 , 28 and 672 ppm) in the second season . Similar results were obtained by Huber and *et al.* (1993).

Table (10): Macronutrients availability in clay soil as affected by dose of microorganisms application after onion harvesting season

Different treatments	Available macronutrients (ppm)					
	First season			Second season		
	N	P	K	N	P	K
Trichoderma harzianum (A)	195	15	590	198	21	595
Trichoderma harzianum (B)	183	12	570	187	19	580
Mycorrhiza glomus (A)	200	18	600	204	23	620
Mycorrhiza glomus (B)	190	15	587	190	20	600
Bacillus subtilis (A)	210	21	620	215	28	650
Bacillus subtilis (B)	194	19	600	200	22	620
Control	160	12	510	192	18	540
L.S.D.	12.52	3.43	11.23	7.85	2.58	11.28

(A) : The recommended dose. (B) : The half dose.

Generally, microorganisms can be arranged in the following order regarding the availability of macronutrients in soil, yield macronutrients uptake of and biological control of white rot disease of onion.

Mix of *B. subtilis Micorrhiza glomus* + >*T. harzianum* + *Micorrhiza glomus* > *B. subtilis* >*Mycorrhiza glomus*> *T. harzianum*

Table (11) : Macronutrients availability in clay soil as affected by dual inoculation of microorganisms application after onion harvesting in both tested seasons

Different treatments	Available macronutrients (ppm)					
	First season			Second season		
	N	P	K	N	P	K
Mix. of T.harzianum and M. glomus	217	19	635	230	22	650
Mix. of B. subtilis and M. glomus	230	25	660	245	28	672
Control	160	12	510	192	18	540
L.S.D.	12.52	3.43	11.23	7.85	2.58	11.28

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الحالة الغذائية للبصل متأثرا بالمقاومة الحيوية لمرض العفن الأبيض للبصل

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قد أجريت تجارب الأصص خلال موسمى (٢٠٠٤ - ٢٠٠٥) لدراسة المقاومه الحيويـة لمـرض العفن الابيض للبصل وتأثيره على الحالة الغذائية للعناصر بالارض والنبات وقد تضمنت الدراسـه علـى استخدام ٣ انـواع مـن الكائنـات الحيـة (*Bacillus subtilis* *Trichoderma-* *Mycorrhiza glomus*) واضيفت هذه الكائنات الى التربه فى صورتين

١- صوره منفرده . ٢- صوره خليط .

ولقد تم اضافة هذه الكائنات بمعدلين هما :-

١- معدل موصى به ويضاف ١٠٠ ملى / كجم تربه .

٢- نصـف المعـدل ويضـاف ٥٠ ملـى / كجـم تربـةوذلك بالنسـبه لـــ (*Bacillus subtilis* *Trichoderma*) وبمعدل ١٠ جرام / كجم تربة بالنسبه لفطر *Mycorrhiza* وقـد صـممت هـذه التجارب فى قطاعات كامله العشوائيه مع أ خذ ٤ مكررات للمعامله .

وتتلخص النتائج المتحصل عليها فيمايلى :-

١- أضافة الكائنات الحيه الثلاثة السابقة بالمعدل الموصى به او نصف المعدل أدى الى زيـاده الفاعليـه لمقاومه مرض العفن الابيض مما انعكس ذلك على زيادة المحصول.

وزياده امتصـاص العـناصر بالبـصل وزياده محتوى العناصـر الميسـره مثـل N , P, K بالتربه وذلك بالمقارنه بالمعاملات الغير ملقحه .

٢- أضافه الكائنات الحيه بالمعدل الموصى به ادى الى زياده المحصول وزيادة امتصـاص العناصـر بالبصل وزيادة محتوى العناصر الميسرة بالتربة وزيادة الفاعلية لمقاومة مرض العفن الأبيض بالمقارنه بالمعدل المنخفض (نصف المعدل) .

٣- اضافه*Bacillus subtilis* الى التربه كان افضل فى جميع الصـفات المدروسـه يليـه معاملـه *Mycorrhiza* ثم *Trichoderma* .

٤- اضافه خليط *Bacillus subtilis* مع *Mycorrhiza* أعطى افضل النتائج بالنسبه للصفات المدروسه عن خليط *Mycorrhiza* ، *Trichoderma* وكذلك كان افضل من اضافه الكائنات الحيه فى صـوره فرديه مما انعكس ذلك على زياده فاعليه المقاومه لمرض العفن الابيض للبصل وزياده العناصر الغذائيه الكبرى فى التربه وزياده امتصاصها بالنبات .

نستنتج مماسبق :-

ان استخدام الكائنات الحيه فى صوره خليط معا خاصـه البكتريـا *Bacillus subtilis* مـع *Mycorrhiza* واضافتها للتربه يؤدى الى زياده امتصاص العناصر الغذائيه للنبات والارض وزياده فاعلية المقاومه الحيويه لمرض العفن الابيض للبصل وانعكاس ذلك على زياده المحصول .