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EFFECT INOCULATION OF AN ARBUSCULAR MYCORRHIZAL FUNGUS (*CUALOSPORA LEAVIES*) AND NITROGEN FIXER *AZOSPIRILLUM BRASILENS* AND SEAWEED EXTRACT IN PLANT GROWTH ONIONS IRRIGATED WITH SALTY WATER

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ABSTRACT

Pot experiment were carried out to attempt to increase the salinity water tolerance of onion: by using inoculation of AM fungi (*Acualospora leavies*) in combination with a nitrogen fixer *Azospirillum brasilens* and seaweed extract . The effect of these beneficial microbes and extracts, as dual inoculation treatments, was assessed in sterilized sandy loam soil at two types of salt water (0, 5) ds.m^{-1}. this study indicate that triple inoculation with *A. leavies* and *A. brasilens* and seaweed extract can increase the in plant height, root length, wet and dry weight, population of *Azospirillum* and percentage of mycorrhizal infection of onion plants more that dual inoculation with AM fungi or *A. brasilens* or seaweed extract as well as control at with add and no add salt water. Add salt water for dual treatments *A. leavies* + *A. brasilens* (T_3) and *A. leavies* +seaweed extract (T_5) and *A. brasilens* + seaweed extract (T_7)) and the treatment of triple hnteraction *A. leavies* + *A. brasilens* + seaweed liquid extract (T_9) led to no significant reduction in plant height , root length , wet and dry weight, and that the addition of salt water led to a significant reduction in population of *Azospirillum* and percentage of mycorrhizal infection .

KEYWORDS: *Acualospora leavies*, *Azospirillum brasilens*, Seaweed Extract, Salty Water, Onion

INTRODUCTION

The progressive salinization of soil is considered as the major environmental factor limiting plant growth and productivity of the arid and semiarid regions. Increases in the salinity of soil or water supplies used for irrigation result in decreased productivity of most crop plants and lead to marked changes in the growth pattern of plants (Cordovilla *et al*. 1994) , that (5%) of the excess salt content resulting by ground water irrigation (Munns *et al*. 1999) , because of the suffering of Iraq from water scarcity and drought conditions , it has used water drainage and water wells as substitutes for irrigation instead of rivers , and that such water the used extensively the irrigation the crops, especially vegetable crops , including onions in the region of desert and semi-desert of Iraq. So it has influenced the production the onions the many factors, them salinity, as well as the use of toxic chemicals from the chemical fertilizers accumulate in plant products causing health problems in human by bio magnifications (Hansra,1993). Hence, there is a need to study the possible ways of improving the yields of this crop. Biofertilizer have recently gained with momentum for effecting the sustainable increase in crop yield under various agroclimatic conditions, role biofertilizer on the crop growth and yield was confirm by Ramakrishnan and Thamizhiniyan (2009) and Sridev and Ramakrishnan (2010). And the role of organic stimulants (seaweed extract) by Zodape *et al*.(2008) and Thirumaran *et al*.(2009)

Arbuscular mycorrhizal fungi have been shown to over cane the detrimental effect of salinity (Colla *et al*.2008) ,improve drought tolerance (Bryla and Duniway ,1997; Sanchez-Blanco *et al*.2004), and alleviate cultural and environmental stresses (Jeffries *et al*.2003) through greater effective root area and penetration of substrate(s) and activation and excretion of various enzymes by infected arbuscular mycorrhizal fungi roots and / or hyphae (Marschner,1995; Smith

and Read,1997). Many researchers reported that Am fungi could enhance the ability of plants to cope with salt stress (Rabie,2005; Jahromi *et al*.2008) by improving plant nutrient uptake and water (Ruiz-lozano and Azcon,1995; Asghari *et al*.2005) and ion balance (Giri *et al*.2007), protecting enzyme activity (Rabie and Almadini,2005).

Many species of bacteria such as Azospirillum, adapt to saline condition by the intracellular accumulation of low-molecular – weight organic solutes called osmolytes (Fischer *et al*.2003). The accumulation of osmolytes is thought to counteract the dehydration effect of low water activity in the medium, but not to interfere with macromolecular structure or function (Smith *et al*.1994). Bacteria of the genus Azospirillum are free –living nitrogen – fixing rhizobacteria found in close association with plants root, where have been attributed to mechanisms such as nitrogen fixation, phytohormone production, and nitrate reduction (Okon and Vanderlyden, 1997). That dual inoculation with Am fungi and N-fixer Azospirillum can support both needs for N and P, excess of NaCl and will be useful in terms of soil recovery in saline area (Rabie *et al*.2005).

In recent years the use of natural seaweed products as substitutes to the conventional synthetic fertilizer has assumed importance , the seaweed fertilizers are often found to be more successful than the chemical fertilizers (Bokil *et al*.1974), because seaweed fertilizer is quite different from traditional chemical fertilizers because it is a natural product derived from algae materials and it contains algae polysaccharides which complex with heavy metal ions in soil and thus enhance its permeability . these seaweed extract can increase yield of grain crops by 10-20% ,of horticultural crop by 10-40% , of vegetables by 14-46%, and of fruits by 10-30% , as the seaweed fertilizer contains large amounts of non-nitrogen containing organic materials as well as copper, zinc , boron, potassium ,calcium ,magnesium ,iron, iodine and more than 40 mineral elements, particularly highly bioactive materials such as algal polysaccharides, mannitol, highly unsaturated fatty acids and natural growth regulators such as giberellin, cytokinin, auxin, abscisic acid and betaine (Agro- Alliance, 2010) for this the aim of the research in the possibility of increasing salinity tolerance of one of the vegetable crops is irrigated onion salt water, using dual inoculation of mycorrhizal fungi(*Acualospora leavies*) and a nitrogen-fixer bacteria (*Azosspirillum brasilens*)and seaweed extracts .

MATERIALS AND METHODS

A pots experiment was carried out on sandy loam soil during the 2010- 2011 at College Agriculture- University of Diyala in Iraq. The experiment was arranged in a randomized complete design with three replication using onion (*Allium cepa* L). In the 10 treatment, two types of salt water (0, 5) $ds.m^{-1}$ and in combination with two biofertilizers (*Acualospora leavies* and *Azosspirillum brasilens*) and seaweed extract (Table 1).

Sterilized of soil autoclave at $121C^{o}$ and pressure 1.o5 $Kg.cm^{-1}$, then distributed on the pots at capacity (4 Kg) ,add nitrogen fertilizer (urea) , triple super phosphate and potassium sulfate rate (260, 597, 288) $Kg.ha^{-1}$ respectively for all pots.

Added inoculation *A. brasilens* at liquid a culture at rate (20 ml $.pot^{-1}$), the density of the inoculum it was (7.8×10^{5}) CFU. Use of a inoculum fungus mycorrhiza (*A. leavies*) at rate (50 $g.pot^{-1}$) cushion under the surface layer of soil.

Use seaweed extract liquid is the a commercial product from china O cean university organism project Development Co.Ltd .as eased by (1: 1000) and twice, first before agriculture and the second after 20 days. Planted pots follicle local onion (*Allium cepa* L.) by (3) follicles. pot^{-1} , eased after germination to two plants per pot , watered all the treatments ordinary water the first pellet after , germination quench plants to the salt water according to the treatment after (50) day of germination measured plant height , length roots ,fresh weight ,dry weight of shoot , population of *A. brasilens*

and percentage of mycorrhizal infection.

Table 1: Description of the Treatments

Treatment No.	Treatment
T_1	control
T_2	T_1 + salty water 5ds.m^{-1}
T_3	*Acualospora leavies* + *Azosspirillum brasilens*
T_4	T_3 + salty water 5ds.m^{-1}
T_5	*A. leavies* +seaweed liquid extract
T_6	T_5 + salty water 5ds.m^{-1}
T_7	*A. brasilens* + seaweed liquid extract
T_8	T_7 + salty water 5ds.m^{-1}
T_9	*A. leavies* + *A. brasilens* + seaweed liquid extract
T_{10}	T_9+ salty water 5ds.m^{-1}

RESULTS AND DISCUSSIONS

The results show in the table (2) Add salt water(T_2) led to a significant reduction in plant height, root length and wet weight and is morally in dry weight, compared to the treatment of control (T_1), as the rate of decline (17.7% , 19.56% , 10.54%) respectively .it has been widely accepted that plants are stressed in three ways by salinity (1) low water potential of the root medium leads to water deficits in crop plants, (2) toxic effect of ions, mainly Na and Cl and (3) nutrient imbalance caused by depression in uptake and / or shoot transport (Marschner, 1995; Adiku *et al.*,2001)

Add A. leavies + A. brasilens (T3) and A. leavies + seaweed extract (T5) and A. brasilens + seaweed extract (T7) led to a significant increase in plant height, root length and wet weight compared to the treatment control (T1). Add salt water of treatments(T3, T5, T7) led to significant increase in plant height, root length and morally in the wet and dry weight (T4 , T6 , T8) compared to the treatment control (T1) and while decreased in significantly in plant height, root length and dry weight compared to treatment (T3 , T5 , T7) .

Application of *A. leavies* + *A. brasilens* in combination with seaweed extract (T_9) recorded significantly highest plant height, length roots, fresh weight and dry weight (40.0 cm, 32.0 cm 119.02 g.pot^{-1} , 33.32 g.pot^{-1}) respectively, which was not significantly different for plant height, length roots and dry weight (38.0 cm , 31.0 cm , 29.63 g.pot^{-1})respectively , recorded with A. leavies + A. brasilens in combination with seaweed extract and salt water 5.0 ds.m-1 (T10).

As the treatment (T10) is the preferred treatment and the role of triple interaction (A. leavies + A. brasilens + seaweed extract) to increase plant growth presence of salt water . Associative and symbiotic nitrogen fixing bacteria and AM fungi are common beneficial microbes of plants. it is frequently suggested that Am may improve P nutrition, enhance N uptake , or improve disease resistance in their host plants. Other microbes, e.g., N-fixing bacteria, may synergistically interact with Am fungi and thereby benefit plant development and growth. (Rabie *et al*., 2005). Sridevi and Ramakrishnan, (2010) said that over all influences by two beneficial microbes (AM fungi and *Azospirillum*) played a vital role in supplying N and P to the onion and found enhanced the growth and yield over the untreated control, as the effect being registered with the dual inoculation of both the bioinoculants which might be due to the provision of nitrogen and growth promoting substances (IAA , GA ,) by phosphorus by AM fungi created with sustainable growth of the crop. Add extract seaweed with inoculation fungi mycorrhiza led to a significant increase in plant height and fresh and dry weight of shoots compared to the comparison treatment (Kuwada *et al*. 2006). and also studied the effect of extracts of algae on the growth of hyphae mycorrhiza and development and the impact on the processing and absorption of nutrients and its reflection positively on the growth and development of papaya. Kuwada *et al*.(2006) found used all kinds of algae led to a significant

increase in the growth of fungus hyphae Am, which affects positively on the processing and absorption of nutrients and increase the growth and yield of the plant , through greater effective root area and penetration of substrate and activation and excretion of various enzymes by infected arbuscular mycorrhizal fungi root and \ or hyphae (Marschner, 1995 and Smith and Read , 1997) . Noted Barker and Taqu, (2000) that mycorrhiza enhance plant growth through the positive influence of certain substances produced by fungi such as materials of the organization of growth (auxins and gibberellins) , as well as the extracts of seaweed contain auxins, cytokinins, gibberellins (Jensen , 2004).

Ferrini and Nicese, (2002) reported that application of some commercial biostimulates 9 one of the biostimulants they used was a powder mixture of marine brown algae, AM fungi, Vitamins. Amino acid and humic acid) to Oak trees increase N concentration in leaf tissue and thus increase plant growth.

Rabie *et al*.,(2005) said that mycorrhized plants exhibited better improvement in all measurements than that of non- mycorrhized ones at all salinity levels. especially, in the presence of *A. brasilens*. phenol polymers and betaine contained in Alliance seaweed fertilizer can adjust the osmotic pressure of cell fluids and chloroplast and protect the activities of enzymes in plant cells thus enhancing the resistance of plants to pests and diseases and adverse environmental and growing factors(Agro- Alliance, 2010). this study indicates that inoculation with (A. leavies + A. brasilens + seaweed extract) can increase the plant height, length, fresh and dry weight of onion plants more than due inoculation with AM fungi or A. brasilens or seaweed extract as well as control at salty water.

Table 2: Plant Height (cm), Length Roots (cm) Fresh Weight (g.pot^{-1}) and Dry Weight (g.pot^{-1}) as Influenced Different Biofertilizer, Seaweed Extract and Salty Water in Onion

Treatments	Plant Height	Length Roots	Fresh Weight	Dry Weight
T_1 :	31.0 e	23.0 efg	93.4 h	23.38 fgh
T_2 :	25.5 f	18.5 h	83.55 j	20.89 h
T_3 :	36.0 bcd	28.0 abcd	116.07 ab	31.25 ab
T_4 :	33.0 cde	25.0 def	108.7 abc	28.67 bcde
T_5 :	37.0 abc	29.5 abc	112.65 bc	30.08 abc
T_6 :	34.0 bcde	26.5 cde	105.48 ef	27.32 bcde
T_7 :	34.0 bcde	27.0 bcde	111.15 cd	29.48 abcd
T_8 :	32.0 de	26.0 cde	103.67 fg	25.35 defg
T_9 :	40.0 a	32.0 a	119.02 a	33.32 a
T_{10}:	38.0 ab	31.0 ab	113.38 bc	29.63 abcd

From figure (1) shows the results that the highest population of *Azospirillum* (7.27 $\times 10^6$ CFU.g^{-1} soil)was recorded in the treatment with *Azospirillum* in combination with AM fungi and seaweed extract (T_9) compared to the treatments inoculation of *Azospirillum* with AM fungi (T_3) (6.65 * 10^6 CFU.g^{-1} soil) and inoculation of *Azospirillum* with seaweed extract (T_7) (6.12 * 10^6 CFU.g^{-1} soil) and significant increase(9.32% , 18.70%) respectively.

Add salt water(T_3 , T_7 , T_9) (6.65 , 6.12 , 7.27)× 10^6 CFU.g^{-1} soil led to a significant reduction in population of *Azospirillum* (T_4 , T_8 , T_{10}) (5.60 , 5.16 , 6.72)× 10^6 CFU.g^{-1} soil. the highest population of *Azospirillum* (6.72×10^6 CFU.g^{-1} soil) was recorded in the treatment with *Azospirillum* in combination with AM fungi and seaweed extract with salt water (T_{10}) compared to the treatments (T_3, T_4, T_7, T_8)(6.65 , 5.60 , 6.12 , 5.16)×10^6 CFU.g^{-1} soil. seaweed is a highly effective fertilizer because it contains both nutritional materials and growth regulatory matter (giberellin , cytokinin , auxin) and improving other physical characters in soil (Agro- Alliance, 2010) might have significant influence on microbial population .

These results are consistent with what he referred to all of Balota et al.,(1995) and Vazquez et al., (2000) of Hbut having state interference positive in the population of bacteria Azospirillum in the case of biofertilizer mixture with AM

fungi attributed the reason to the positive impact of AM fungi in bacteria in terms of the excreted substances Organization for growth (Barker and Tagu, 2000) as well as their impact is important in supplying bacteria phosphorus sources that are essential for the bacteria as a source of energy to carry out the installation of atmospheric nitrogen (Balota et al.,1995). in the rhizosphere, plant root exudates, which contain various amino acids, sugars and betaines, serve as a source of osmoprotectants for the survival and growth of bacteria at elevated salinity (Miller and Woods, 1996).

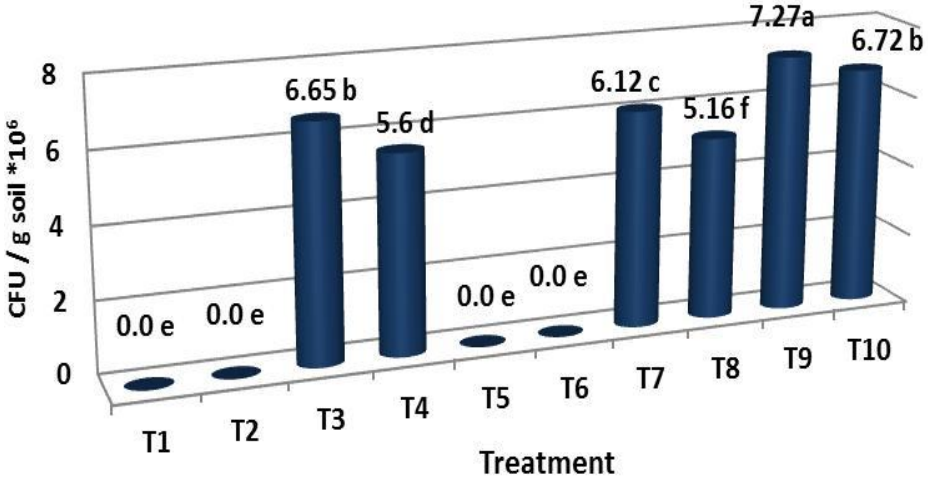

Figure 1: Population of *A.brasilense* (CFU/g Soil *10^6) in Soil as Influenced by Different Biofertilizer, Seaweed Liquid Extract and Salt Water in Onion

The results show in the figure (2) the highest percentage of mycorrhizal infection (94%) was recorded in the treatment with *Azospirillum* in combination with AM fungi and seaweed extract (T_9) compared to the treatment inoculation of Azospirillum with AM fungi (T_3) (90%) and inoculation of AM fungi with seaweed extract (T_5) (86 %) and significant increase (4.4 % , 9.30 %) respectively.

Add salt water (T_3 , T_5 , T_9) (90 % , 86 % , 94 5) led to a significant reduction in percentage of mycorrhizal infection (T_4 , T_8 , T_{10}) (80 % ,78 % , 85 %) . the highest percentage of mycorrhizal infection (85%) was recorded in the treatment with Azospirillum in combination with AM fungi and seaweed extract with salt water (T_{10}) compared to the treatments (T_4 , T_6) (80 % , 78 %). the extracts of seaweed contain auxins , cytokinins, gibberellins (Jensen , 2004). As is know the Auxins role is to control the composition of the roots, as the gibberellins increase the rate of growth of roots and leaves and cytokinins involved in most processes related to formation of mycorrhiza (Paleg and West, 1972). Mycorrhizosphere bacteria may affect AM fungi and their plant hosts through a variety of mechanisms including (1) effects on the receptivity of the root; (2) effects on the root-fungus recognition; (3) effects on the fungal growth; (4) modification of the chemistry of the rhizospheric soil and (5) effects on the germination of the fungal propagules. As the nitrogen fixation further promotes mycorrhizal development (Puppi et al. 1994).Some mycorrhizosphere bacteria may be able to promote mycorrhizal establishment through improved spore germination, the colonization enhancement may also be mutual between associated microorganisms (Rabie et al., 2005), They also noted the mycorrhizal colonization of cowpea plants was significantly increased at relatively low salinity levels.

On the contrary, at higher salinity levels mycorrhizal colonization was significantly reduced although mycorrhizal plants inoculated with Azospirillum still had higher mycorrhizal colonization.Previous research had shown that salinity may reduce mycorrhizal colonization by inhibiting the germination of spores (Hirrel,1981), inhibiting growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen *et al*., 1998), and reducing the number of arbuscules (Pfeiffer and Bloss,1988).

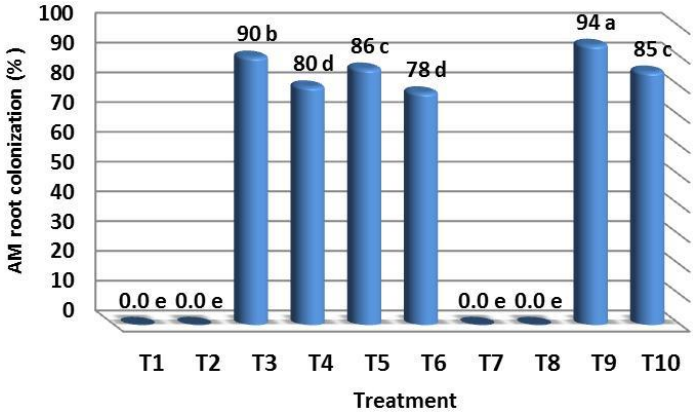

Figure 2: AM Root Colonization (%) as Influenced by Different Biofertilizer, Seaweed Liquid Extract and Salt Water in Onion

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