Global Journal of Scientific Researches

Available online at gjsr.blue-ap.org
 Vol. 2(5), pp. 144-153, 31 May, 2014

E-ISSN: 2311-732X

Response of barley grown in saline soil to bio-fertilizer as a partial substitutive of mineral fertilizer.

El-Shahat R.M*, Sherif A.E.A and Faten M. Mohamed

Corresponding Author: El-Shahat R.M

Received: 25 April, 2014 *Accepted: 10 May, 2014* *Published: 31 May, 2014*

ABSTRACT

Two field experiments wear designed concluded at the farm of Sahl Husseiniya Research Station. The main target of these experiments was to evaluate the response of barley to 75% NPK, bio-inoculation with Azolla (fresh, dry) and / mixture of halo-tolerant bacteria under saline soil conditions. The mixed bacteria mainly included, nitrogen fixers microorganisms as well as their enzymatic phytohormone, exo-polysaccharid and same enzymatic enzyme activity. High significant were estimated with treatment using (T10) 75% recommended dose of N, P and K + Azolla foliar + mixed bacteria two dose (30 and 60 days after sowing). Inoculation with mixed bacteria (two equal doses at 30 and 60 days of sowing) and Azolla enhanced the biological activity of the soil, and this trend was more pronounced in the treatments received T10. At the end of experiment after barley harvesting, salinity of the upper soil layer 30 cm decreased to be about 21.80 % from the initial soil. On the other hand, pH values decreased with the application of treatment from (T3) 75% recommended dose of N, P and K + Azolla dry to (T10) as foliar. Bio-fertilizer led to the increments in soil N, P and K available contents. The results indicated the import. The obtained results showed that, variation in available micronutrients content soil namely Fe, Mn, Zn and Cu, resulted from mineral –N and bio-fertilizer (Azolla and mixed bacteria) during both planting seasons. All the bio-fertilizers treatments recorded significant increases for grains and straw yield as compared with un-inoculated treatments control. The tested bio-fertilizers attained significantly high values for 1000 grains weight as compared with the control treatments. Treatment T10 reported high significant content and uptake of Fe, Mn, Zn and Cu of grains and straw.

Keywords: *Azolla, Halo-tolerant, Mixed bacteria, 75% N, P and K used, yield and yield component of barley, Saline soil.*

INTRODUCTION

Egypt is present in semiarid region of the world. In addition, human overpopulation becomes a serious constraint for crop production, that crushed by pressure to produce more food per unit area of land.

Egypt faces a noticed reduction in fertile cultivated soil in old Nile valley and delta, which represent about 3-4 % of the total area of Egypt. So an attention was directed towards the desert soil. The fertility status of this soil is poor, in turn, application of bio or organic-fertilization by using micro- organisms or organic supplements to improve it through their activities and providing most of the essential nutrients required to plant growth and crop productivity.

Salinity stress is usually cases decrease in crop production. It inhibits the photosynthesis of plants, causes changes of chlorophyll contents and components and damage of photosynthetic apparatus. It also inhibits the photochemical activities and decreases the activities of enzymes in the calvin cycle (Sairam and Tyagi, 2004).

Apart from alleviating osmotic stress in plants, inoculation with diazotrophs can also enhance oxidative stress tolerance. By oxidativestress it is meant the oxidative dangle caused by reactive oxygen species (Ros) such as superoxide anion radical, hydrogen peroxide hydroxyl radical and singlet oxygen (Sies, 1991 and Stafener, 1995).

Kotpal and Bali 2003. Found that, under field conditions, selected species can fix about 1.2 kg N day^{-1} and in excess of 40 kg N in 35 day, increases soil organic matter, improved soil and supply fixed nitrogen. In order to investigate the effect of Azolla on the physical and chemical properties of the soil, an incubation experiment was carried out in which soil were treated with Azolla at 0, 20, 40, 60 and 80 g kg^{-1}. The soils treated were incubated in the dark 25˚C for eight weeks at field capacity. Soil pH, organic matter, N, P, K, Ca and Mg increased with rate of Azolla. There was reduction in soil bulk density but increased soil porosity (Awodun 2008).

The free nitrogen fixers bacteria are not only able to fix the atmospheric nitrogen, but also are multiple benefits able to synthesize and secrete phytohormone, like substances as thiamin, riboflavin pyridoxine, indol acetic acid, gibberellins, cytokines and abscic acid (Faten and Sherif 2011).

Mehdy, (1994) and Desikan, (1998), they are potentially harmful to plant viability (Bowler, 1992). With the production of antioxidant enzymes like superoxide dismutase (SOD), per-oxidase and catalase the cell can neutralize and thus control free radical formation.

Therefore, the objectives of the current work are to evaluate the potency of application 75% N, P and K with mixed bacteria and Azolla used as solely or in combination with 100 (T1) and 75 % (T2) NPK on barley plants grown in saline soil, and decreases the mineral nutrient uses.

Materials and Methods

Two field experiments were carried out at the farm of Sahl El-Hossinia, Res. Station El-Sharkia Governorate, Egypt, during two winter successive seasons (2010-2011) and (2011-2012). The experiments were designed to study the reduced application N, P and K% about 25% by inoculation of barley with combined mixture of asymbiotic N_2- fixers, as wall as to evaluate the role of Azolla (dry and / or fresh) on pant growth, macro (NPK), and micro-nutrients (Fe, Mn, Zn and Cu) uptake and yield productivity.

Bacterial culture

Nitrogen fixing bacteria of *(Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus polymyxa*, and *Klebsiella pneumonia*) at log growth phase were used in combined mixture as bacterial inoculum according to ***Faten (2011).***

Azolla dry and extract

Wet Azolla were collected from the growth trays in the green house at the log growth, phase and allowed to be air dried in the shade. Azolla fresh was hardly crushed and blended in a mixer till obtaining a suspension. The obtained suspension was filtrated through a sheet of cotton cloth. The obtained filtrate represent Azolla extract to be used in Azolla foliar spray treatment, (Reda, 2011).

Barley seeds

Barley seeds cultivar Giza 123(Hardeum v ulgare) were kindly obtained from Field Crops Res. Inst., Agric. Res. Center, Giza, Egypt.

Soil analysis

The experimental field soil was sampled initially before and after conducting the experiments to determine physical and chemical properties according to Jackson (1967). The results of initial soil analyses are shown in Table (1).

Field experiments

Two field experiments were carried out at Sahl HusseiniyaRes. Station during two winter seasons of 2010- 2011 and 2011-2012, to investigate impact of 75% of recommended dose of N. P and K, Azolla (dry or extract) and mixed bacteria as suspension for seed soaking and extract for foliar treatments (as well as urea as nitrogen fertilizer as control).

Table 1. Physical and chemical properties of the experimental soil

$CaCO_3$ %	O.M %	Clay %	Silt %	Fine Sand %	Crosse sand %		Texture		
2.30	0.52	21.52	56.30	15.70	6.48		Clay loam		
pH	EC	Anion meq L^{-1}				Cation meq L^{-1}			
1:2.5	dS m^{-1}	CO_3	HCO_3	Cl^-	$SO_4^=$	Ca^{++}	Mg^{++}	Na^+	K^+
8.23	16.9	0.00	9.93	190.00	35.58	12.94	20.16	203.00	1.41
Available macro and micro nutrients mg kg^{-1} soil									
N	P	K	Fe			Mn	Zn		Cu
45.90	5.90	198.30	2.96			3.06	0.63		0.11
Biological assay (T0)									
Total count bacteria	T.C. Cyanobacteria			Dehydrogenase		CO_2 evolution		Nitrogenase	
6.50	3.40			12.60		20.75		21.32	

Dry Azolla 0.5 kg/m^2 was incorporated into the upper 5 cm layer of the soil, while Azolla extract and bacterial suspension were surface applied to the soil. Azolla dry treatments were basically added to soil 15 days before barley seeds sowing. Barely seeds were soaked in both Azolla extract and mixed bacteria suspension for 2 hours, and this was carried out for the treatments devoted for soaking process.

After 30 and 60 days from sowing, barley plants were foliar sprayed using both Azolla extract and/or mixed bacteria suspension at the rate of 50 and 20 L fed^{-1}, respectively. Urea (46.5 N %) was applied at the recommended dose (75 kg fed^{-1}), phosphorus was added in the form of super phosphate (15.5% P_2O_5) at the rate 100 kg fed^{-1}, while potassium was added in the form of potassium sulphate (48% K_2O) at the rate of 50 kg K_2O fed^{-1} in control treatment T1 and 75% N, P and K from recommended dose in control T2. Both phosphate and potassium were applied during soil preparation. The amount of added Azolla was calculated due to the Azolla contains 4% N on the dry weight basis.

The experiments were arranged in complete randomized design with three replicates and contained the following treatments:
T1: 100% recommended dose of N, P and K as control (1).
T2: 75% recommended dose of N, P and K as control (2).
T3: 75% recommended dose of N, P and K + Azolla dry.
T4: 75% recommended dose of N, P and K + Azolla foliar.
T5: 75% recommended dose of N, P and K + Mixed bacteria suspension (30 days after sowing).
T6: 75% recommended dose of N, P and K + Mixed bacteria suspension (30 and 60 days after sowing).
T7: 75% recommended dose of N, P and K + Azolla dry + mixed bacteria suspension (30 days after sowing).
T8: 75% recommended dose of N, P and K + Azolla foliar + mixed bacteria suspension (30 days after sowing).
T9: 75% recommended dose of N, P and K + Azolla dry + mixed bacteria suspension (30 and 60 days after sowing).
T10: 75% recommended dose of N, P and K + Azolla foliar + mixed bacteria suspension (30 and 60 days after sowing).

At harvest, random plant samples from each plot (4 X 5 m^2) were collected by using 1 m^2 wooden frame to determine barley yield and its components. Plant samples were then oven dried at 70°C for 72 hours up to a constant dry weight. Macro and micro nutrients contents of both grains and straw were determined after digestion with a mixture of concentrated sulphoric acid and percholoric acid at the ratio of 1:1 (Black, 1965). Total-N in plant samples was determined by Kjeldahel technique (Jackson 1973). Total-K in plant samples was estimated by Flame photometer as described by Jackson (1967). Total content of P, Fe, Mn, Zn, and Cu in plant samples were determined by Inductively Coupled Plasma Spectrometry (ICP) (Ultima 2 JY Plasma)

After barley harvesting, the collected random soil sample from each plot and, air dried and pulverized, then, passed through 2 mash sieve and subjected to determine particle size distribution Table (1) (Piper 1950), soil reaction pH (1: 2.5 suspension) and electrical conductivity (EC) in soil paste (Jackson, 1967). Soil available NPK and Fe, Mn, Zn and Cu were also determined. On the other hand, the humid collected soil sample before drying was used for the determination of soil biological activity in terms of soil total bacterial count (Allen 1959), total cyanobacteria count (Allen and stainer 1968), carbon dioxide evolution (Gaur, 1971), dehydrogenase activity (DHA) (Casida, 1964) and nitrogenase activity (Dart, 1972). All obtained data were statistically analyzed for least significant difference between obtained means as described by (Gomez and Gomez 1984).

Determination of culture growth parameters extracted growth regulators of Azolla and mixed bacteria, culture growth parameters and extracellular growth regulators in culture filtrates were determined in Table (2). Growth regulation substances (IAA, GA and ABA) were fractioned and growth regulator substances excreted by Azolla in the culture filtrate quantified by HPLC according to Kowalczyk and Sandberg (2001).as shown in Table (2).

Table 2. Some characteristics of biological and chemical bio-fertilization used

Strains	IAA $\mu g\ l^{-1}$	Gibbrillic acid	Abscisic acid	Total amino acid mg $100g^{-1}$	Total poly saccharides g l^{-1}	N_2ase μ mol C_2H_4 l^{-1} dry wt./hr	Catalase
Azolla	5564.0	1250.0	211.9	9.95	3.85	96.53	-
Mixed bacteria bacteria	135.0	96.40	16.80	6.67	6.54	186.18	++

RESULTS AND DISCUSSION

Results

Soil biological activity

Densities of total soil microorganisms: Data in Fig.(1), showed that, all the tested soil biological activity parameters under different treatments of mixed bacteria and Azolla were higher than those estimated with control treatment (T1 and T2). Therefore, the treatments (T10) and (T9) succeeded to accelerate soil biological activities; (total bacterial count 275 x 10^6 and 220.26 x 10^6 CFU g^{-1} dry soil, respectively). The same trend was noticed with, total count cyanobacterial; the recorded figures were 292.18 and 271.60 X 10^3 CFU g^{-1} dry soil for two seasons. Treatments (T1) and (T2) were less in total bacterial and cyanobacterial counts.

Data in Fig.(1) showed that, T10 caused an increases of CO_2 evolution (mg CO_2 100 g^{-1} soil), dehydrogenase (DHA) (µg TPF g^{-1} dry soil day^{-1}) and nitrogennase (N_2-ase) activity (µ mol C_2H_4 g^{-1} dry soil hr^{-1}) compared with control. Thereupon, the highest mean values being 190.00 mg CO_2 100 g^{-1} soil, 90.18 µg TPF g^{-1} dry soil day^{-1} (DHA), and 293.11 µ mol C_2H_4 g^{-1} dry soil hr^{-1} (N_2 – ase) were determined.

Generally (T10) succeeded to enhance the biological activity of the soil, whereas, the trend was more pronounced. Also, it was of worth to note that, (T1) and (T2) decreased against the increase noticed with decreasing nitrogen application. Inoculation with mixed bacteria and Azolla sprang increased significantly the soil biological activity over control (T1) and (T2).

Regarding impact of applied mixed bacteria (T6) and (T5) on densities of soil microbes and enzymes activity, the obtained results revealed that, (T6) succeeded to enhance soil microbial densities and active enzymes than (T5).

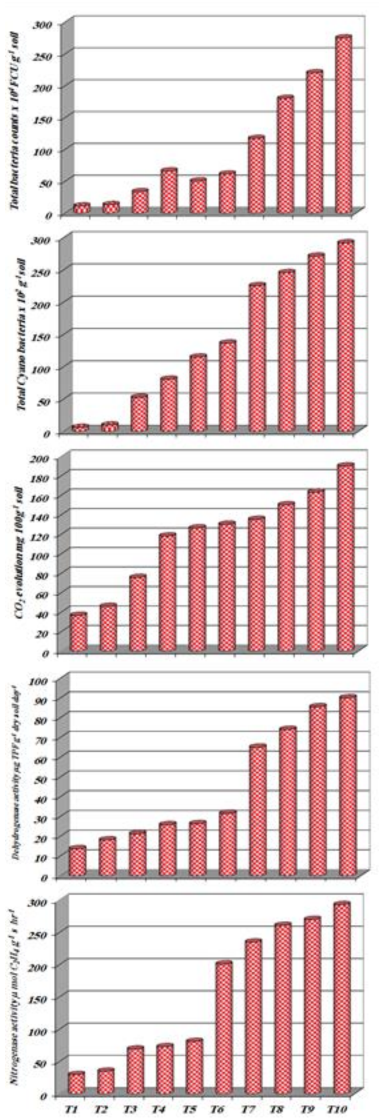


Fig (1) Effect of mixied bacteria and Azolla on soil biological character after barley harvesting

Changes in soil properties
Changes in soil salinity

The trend of salinity as (Fig. 2) shows tented to decrease through two winter seasons of application with bio-fertilizer and cultivated barley. At the end of barley cultivation seasons were foliar with Azolla and mixed bacteria extract was given salinity reduction from 16.90 to 13.30 dS m^{-1}.

All tested bio-fertilizers (Azolla and /or mixed bacteria) treatments decreased both EC. and pH compared to (T1) and (T2) control treatments. However, (T10) decreased EC. From 16.90 to 13.30 dS m^{-1} and while the least pH degree (7.40) was due to the treatment received.

Generally, at the end of experiment after barley harvesting, the upper soil 30 cm layer was the most salinity decreased by about 21.80 % from the initial soil.

Changes in soil pH

Data in Fig.(2) showed that, soil pH values at the beginning was 8.23. The statistical analysis resulted in significant difference among these values. It may be worthy to mention that, slight differences among pH values appeared with used bacterial suspension and Azolla extract.

Foliar, has great effect on soil reaction. Thus trend of soil pH decreased as follows, at the beginning of work, pH value increased with (T1) and (T2) to record pH value of 8.23 to 8.29. On the other hand, pH values decreased with the application of treatment from T3 to T10 as foliar, given from 7.96 to 7.40, those trends were found in sample taken after Azolla and mixed bacteria application.

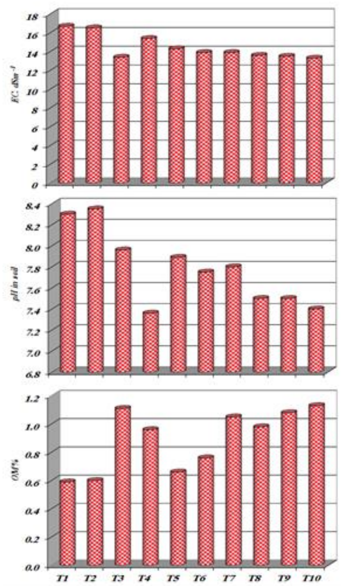


Fig.(2A) EC; pH and O.M% of experimental soil after barley harvesting as affected by mixed bacteria and Azolla as bio-fertilizers.

Soil organic matter

The degree to which microbial organic matter accumulates in soil depends on a balance between production and decomposition of microbial products. That is, the microbial growth efficiency, the degree of protection of microbial biomass in the soil structure and the rate at which bacteria is decomposed by other microorganisms.

Regarding the effect of different treatments on soil organic matter, data in Fig.(2) showed an increase in soil organic matter with treatments T10, T3, T9 and T7 whereas, values being 1.13, 1.11, 1.08 and 1.05 O.M%, were determined, respectively. Generally, the values of soil organic matter, followed the order T10 > T3 > T9 >T7 > T8 > T4 > T6 > T5 > T2 and T1. This may be attributed to the microbial contribution to soil organic matter storage.

Changes in N, P and K availabilities

Data presented in Fig. (2B) show available soil contents macro nutrients of at harvest stage. The available forms of N, P and K increased significantly by using bio-fertilizers (Azolla and / or mixed bacteria). The recorded data showed that, using bio-fertilizers, in the two successive seasons, affected the amounts of some available macronutrients N, P and K (mg kg $soil^{-1}$). Moreover, it was also showed that soils treated with (T10) treatment. In the addition (T6), were more effective than (T1) and (T2) control.

N, P and K available in soil exhibited higher values over the control treatment with all treatments containing bio-fertilizer .The highest N, P and K values available in soil were recorded with (T10) .The corresponding values were 90.17 mg N kg^{-1} soil, 8.19 mg P kg^{-1} and 28190 mg K kg^{-1} soil.
The bio-fertilizer led to the increments in soil N, P and K available contents. The results indicate the import.

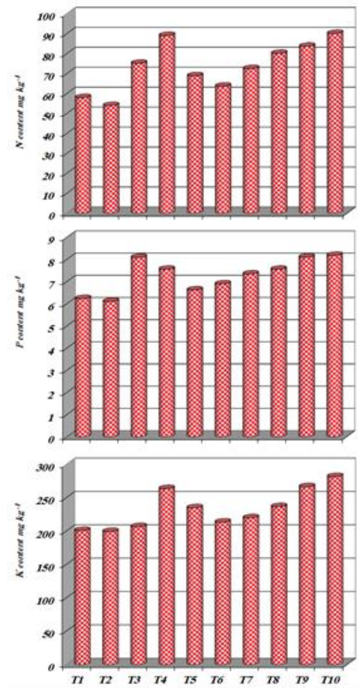


Fig.(2B) N,P And K of experimental soil after barley harvesting as affected by mixed bacteria and Azolla as bio-fertilizers.

Effect of different fertilization sources on available micronutrients in soil

The results of the present investigation showed that, variation in available micronutrients content soil namely Fe, Mn, Zn and Cu, resulted from mineral–N and bio-fertilizer (Azolla and Bacteria) during both planting seasons, Fig.(2). This could be related to the residual effect of bio-fertilizer.

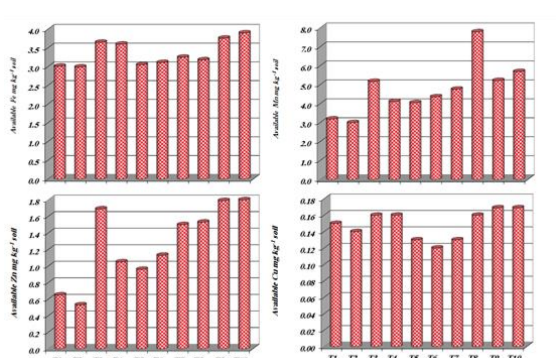


Fig.(2C) Soil content of Fe, Mn, Zn and Cu after barley harvesting as affected by mixed bacteria and Azolla.

Data depicted in Fig (2C) reveled, the relative increases of Fe, Mn, Zn and Cu in the soil as affected by mixed bacterial and Azolla. Significant increase were determined with comparing with control (T10) and (T9). Thus, the variations in the contents could be attributed to different doses of mixed bacteria (30 and 30+60 days), by Azolla and Azolla extract foliar spraying.

In general pronounced, increases Fe, Mn, Zn and Cu content were observed with soil treated with Azolla and mixed bacteria which were more effective than Azolla or bacteria alone.

In the presence of T10 and T9 were concentration of soil Fe, Mn, Zn and Cu, increased compared with (T1) and (T2) (control) i.e. from 3.90, 5.69, 1.80, and 0.17 mg kg^{-1} over control treatment (T1) (100% recommended N) 3.01, 3.19, 0.65 and 0.15 mg kg^{-1} soil, respectively.

Barley Yield and yield components

Barley plant height exhibits in Fig.(3) pointed out that any of T8 and T9 treatments had significantly recorded higher values that those recorded by T5 and T4 treatments and control.The corresponding plant height values were 97.19 and 96.00 cm and 91.95 and 93.06 cm, respectively. Soil chemical properties and fertility conditions are reflected on plant growth, which in turn affect their grain and straw yield. As shown in Fig.(3) there was significant increase in plant high with (T10) and (T9) treatments in both seasons being 109.25 and 101.10 cm as compared with all treatments. However, the use of deferent methods of application of Azolla and mixed bacteria in combination mixture, (T7) and (T8) showed increase in plant height as compared with Azolla (fresh or dry) and bacteria (one or two doses) (T5) and (T6) individually.

Number of spikes m^2

Data in Fig.(3) indicated that, the number of spikes m2 was significantly increased with T10, T9, T8 and T7 as compared with control. The interaction between Azolla (fresh or dry) and mixed bacteria (one or two equal doses) attaind the highest number of spikes m2 being 365, 339, 325 and 314 respectively, compared with application of Azolla or mixed bacteria alone. Then, the priority was also noticed for (T10) treatment, rather than the other treatments and control (T1).

Grains and straw yield

Data presented in Fig.(3) demonstrated that, (T10) treatment attained the highest grains and straw yield being 2.99 and 3.12 tons fed^{-1} respectively, followed by (T9) giving (2.79 and 3.11) tons fed^{-1}. All the bio-fertilizers treatments recorded significant increases for grains and straw yield as compared with un-inoculated treatment (control).

1000-grains weight

All bio-fertilizers tested attained significant high values as compared with the control treatment. Data in Fig.(3) showed that, (T9) and (T8) treatments significantly increased the weight of 1000 grains of barley (45.75 and 41.1.9 g respectively), in comparison with control treatments.

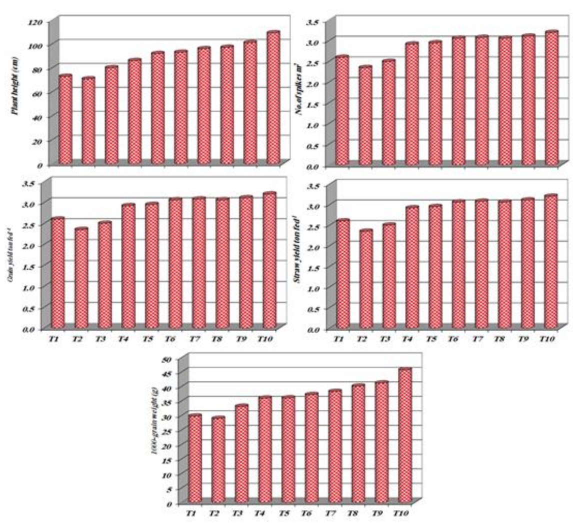


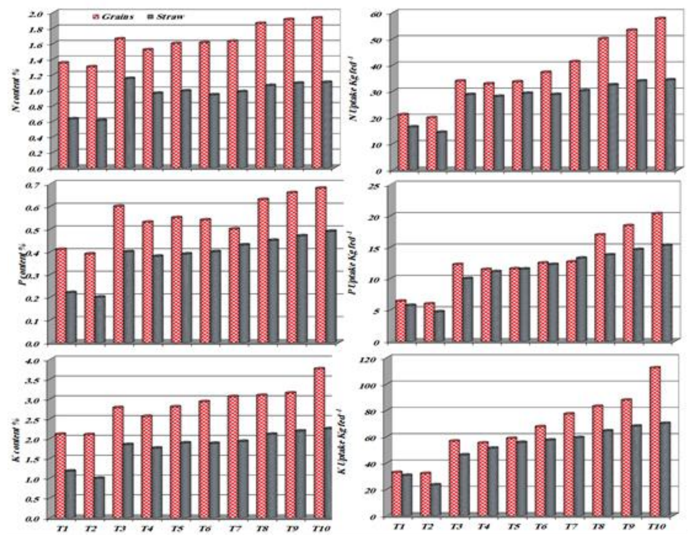
Fig.(3) Barley yield components as affected by mixed bacteria and Azolla .

N, P and k contents and uptake of barley grains and straw

All treatments canting bio-fertilizers caused significant effect on N, P and K uptake (kg fed^{-1}) in both grain and straw of barley during both seasons (Fig.4). N, P and K contents tended to increase with addition of Azolla and mixed bacteria (two doses), However contentes were decreased with the addition of mineral –N (control).

Data in Fig.(4) also shows that, N, P and K content and uptake in straw were affected and could be arrainged as follows: T10, T9, T8, T7, T6, T5, T4, T3 and T1 and T2 for all experimental pilot units.

The relative increments of N, P and K content (%) for straw were 1.10, 0.49 and 2.25 % and uptake were 33.00, 14.70 and 68.11 kg fed $^{-1}$ respectively. (data means two seasons).



Data also showed that application of Azolla or mixed bacteria alone induced non significant effect on the content of N, P, and K in grains and straw. The highest values of N, P and K uptake were 57.71, 20.33 and 112.42, kg fed $^{-1}$ respectively.
Fig. (4) N,P and K content and uptake of grains and straw of barley.

Micro nutrients content and uptake of barely grains and straw plants

The contents of Fe, Mn, Zn and Cu in barley grains and straw were affected by different treatments as presented in Fig.(5) substantial increases in Fe, Mn, Zn and Cu content (mg kg^{-1}) and uptake (g fed^{-1}) were detected with all treatments comprise bio-fertilizers (Azolla and / or bacteria). More pronounced increases were determined compared with control. On the other hand (T10) resulted in significant high value of Fe, Mn, Zn and Cu content (mg kg-1) and uptake (g fed-1) for grains and straw. Thus, content and uptake of Fe, Mn, Zn and Cu in grains reached 203.00. 86.91, 160.55, 7.80 and 606.97, 259.86, 480.04, 23.32 respectively. The content and uptake of Fe, Mn, Zn and Cu in reached 168.00, 93.17, 96.86 and 8.60 mg kg-1 and 504.00, 279.51, 290.58 and 25.80 g fed-1, respectively.

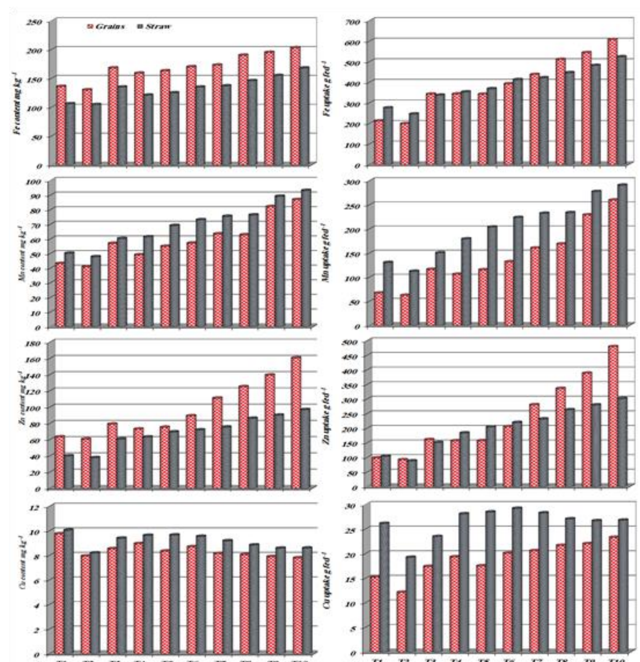


Fig 5. Micro-nutrients content and uptake of barley grains and straw

Discussion

In the area of griculture, Azolla and Cyanobacteria are of importance in view of being used as nitrogen fixing bio-fertilizers (El-Zeky, 2005).

In nature, accdeaminase has been commonly found in soil bacteria that colonize plant roots. Many of these microorganisms were identified by their ability to grow on minimal media containing Accasits as sole nitrogen source in this way, Azospirllum ssp, Azotobacter ssp and diazotrophic bacteria, (Dobereiner and pedrosa. 1987). Azotobacter and Azospirillum were found to produce some or all of the water soluble B-group vitamins (niactin, pantothenic acid, thiamine, riboflavin and biotin) in defined culture media (Revillas, 2000).

In the present study, the use of both Azolla (dry or fresh) and halotolerant mixed bacteria applied by different methods, which are consequently increase the soil biological salt activity and decrese used amounts of N, P, K to 75% of recommended dos, as well as increasing the soil total bacterial and Cyanobacterial count, CO_2 evolution, dehydrogenase and nitrogenase activities (Singh, 2008). This increase of the soil biological activity increased the soil fertility that in turn is reflected positively on the barley yield productivity.

Nitrogen fixing bacteria and Azolla succeeded to improve the soil chemical characters through decreasing both EC and pH compared to control. In this concern using Azolla fresh or dry, generally decreased soil PH and EC therefore the dissolved CO_2 in the soil leading to reduce the soil pH. Also, both Azolla and bacteria have the ability to excrete extracellularly a number of compounds like polysaccharides, peptides, lipids and organic acids, causing decrease of the soil pH (El-Ayouty, 2004).

They also added that, the presence of these materials in Azolla extracts and bacterial cultural filtrate could be adsorb both sodium and magnesium ions upon they get in touch with soil and thus prevent the harm effect of salinity against the cultivated plants.

The experimental soil is generally characterized by high salinity; the character prevailed in such semi-arid regions. In this concern all the applied bio-fertilizer treatments reduced EC according to Molnar and Ordog (2005) who noted that, some plant growth promoting regulators (PGPRs) are found to be released by Cyanobacteria either in free living form and /or in symbioses as in Azolla, these (PGPRs) represent the defense system that encounters the salt stress leading to decrease the soil EC.

Salt affected soils are highly deficient in organic matter and nitrogen. The efficient of nitrogen fertilizers is very poor due to extensive losses through leaching in salt affected soils (Rao and Batra1983). Soil available N, P and K were increased over control treatment as affected by bio-fertilizes. These hidings were observed by Strik and Staden (2003), who noticed that, incorporation of Azolla (fresh or dry) into soil succeeded to increase significantly the soil organic matter, which in turn upon its decomposition by the soil microorganisms could be released macro-and micronutrients into soil, leading to increase the soil available N, P and K.

Using bio-fertilizer bacteria + Azolla spraying (T10 treatment) led to increase significantly barley yield and its components under salt stress condition. This trend was previously confirmed by Abd El-Bake ,(2008) who found that, spraying wheat cultivated under salt stress condition with micro- algae extracts obtained from chlorella sp. led to keep good growth and yield of wheat compared to those received recommended dose of N. In addition, the application of bio-fertilizers extracts significantly increased the contents of the total chlorophyll and antioxidant phenomenon. As well as extracts exhibited strong positive correlation with increase of wheat fresh weighs, grains weight and yield components.

Lillun (2000) reported that, Azolla significantly increased the above ground biomass this increase may be a result of nutrient availability due to Azolla in corporate into soil.

The explained that, bio-fertilizers spray application significantly increased the plant nutrients content and had a positive effect on plant growth, oxidation behavior and activity of antioxidant enzymes in plant affected by salt stress. Furthermore, both bacteria and Azolla extracts are characterized by their cytokines, phytohormones , amino acid, gibberellins and ouxins content that enhance the plant growth and these materials are proved to overcome the adverse effect of salinity (Mussa 2005).

Conclusion

The intensive use of expensive chemical fertilizers in recent years which results in environmental pollution problems has focused the attention of researches on the possibility of using bio-fertilizers as an alternative or complement for chemical fertilizers. The application of 75% of recommended dose of NPK plus bio-fertilizer succeeded to decrease 25 % of recommended dose of N, P and K and increased significantly barley productivity in saline soil as well as decrease the environmental pollution by the extensive use of chemical fertilizers. Thus it may be inferred that Azolla and / or halotolerant bacteria exhibited better nitrogen availability to barley than chemical nitrogen. Since, they improved soil available NPK, Fe, Mn, Zn, Cu, soil biological activity, barley yield, yield components and macro and micro nutrients contents of both grains and straw. However, this study needs to be repeated for other locations using same cereal crop and / or other crops thus to have the condition due to recommendation.

REFERENCES

Abd El-Baky-Hanaa H, Haussein MM and El-Baroty GS. 2008. Algal extracts improve antioxidant defense abilitys and salt tolerance of wheat plant irrigated with sea water. Afr. J. Biochem. Res., 2 (7): 151-164.

Allen MB and Stainer RY. 1968. Elective isolation of blue-green algae from water and soil. J. Gen. Microbiol., 51: 203-209.

Allen OM. 1959. Experiments in Soil Bacteriology. 1^{St} Ed. Burgess publishing Co. Minneapolis, Minnesota. USA.

Awodun MA. 2008. Effect of Azolla (Azolla species) on physiochemical properties of the soil. World J. of Agri. Sciences, 4 (2): 157-160.

Black CA. 1982. Methods of Soil Analysis .Part 2. Chemical and Microbiological properties. Second Edition. Amer. Soc. Of Agron .Madison , Wisconsin, USA .

Bowler C, Van Montagu M and Lnze D. 1992. Superoxide dismutase and stress tolerance. Annu. Fev. Plantphy soil. Plant Mol, Biol., 116: 43:83.

Casida ME, Klein DA and Santor T. 1964. Soil dehydrogenase activity. Soil Science, 98: 371-376.

Dart PJ, Day JM and Harris D. 1972. Assay of nitrogenase activity by acetylene reduction. In Use of Isotopes for Study of Fertilizer Utilization by Legume Crops. pp 85–100. FAO/IAEA, Vienna.

Desikan R, Reynolds A, Hancock JI and Neil SJ. 1998. Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defense gene expression in Arabidopsis suspension cultures. Biochem. J.,330:115-120.

Dobereiner J and Pedrosa FO. 1987. Nitrogen-fixing bacteria in Nonleguminous crop plants. Brock/Springer Series in Contemporary/Bioscience, 155 p.

El-Ayouty YM, Ghazal FM, Hassan AZA and Azza AM. 2004. Effect of algal inoculation and different water holding capacity levels on soils under tomato cultivation condition. J. Agric Sci., Mansoura Univ., 29 (5): 2801-2809.

El-Zeky MM, El-Shahat RM, Metwaly GHS and Aref-Elham M. 2005. Using of cyanobacteria or Azolla as alternative nitrogen source for rice production. J. Agric. Sci., Mansoura Unvi., 30: 5567-5577.

Faten Mohamed M and Sherif AEA. 2011. Isolation and evaluation of some characteristics of halotolerant free N_2-Fixers strains.Egypt J. of Appl.Sci, 26 (7) :256-271.

Faten M, Mohamed. 2011. Use of vinasse (Byproduct of sugar industry) in improving biological properties in salt affected soils. Egypt.J.Biotechnol.,V. 37: 129-144.

Gaur AC, Sadasivan KV, Vimal OP and Mathur RS. 1971. A study of decomposition of organic matter in an alluvial soil, CO_2 evolution mycological and chemical transformation Plant and Soil, 34:17-28.

Gomez KA and Gomez AA. 1984. Statistical procedures for agricultural research. 2nd edition, John Wiley and Sons, New York, 680 pp.13-175.

Jackson ML. 1967. Soil Chemical Analysis, Prentice- Hall India Private Limited, New York.

Jackson ML. 1973. Soil Chemical Analysis Prentic-Hall, Englewood califfs, New Jersey.

Kotpal RL and Bali NP. 2003. Concepts of ecology: Environmental and field biology: visual publishing company India.

Kowalczyk M and Sandberg G. 2001. Quantitative analysis of indole-3-acetic acid metabolites in Arabidopsis. Plant Physiol., 127: 1845–1853

Lillian KN. 2000. The utilization of Azolla filiculoides lan as a bio-Fertilizer under dry land conditions. M.Sc. Thesis, Rhodes University.

Mehdy MC. 1994. Active oxygen species in plant defense against pathogens. Plant physiol., 105: 467-472.

Molnar Z and Ordog V. 2005. The effect of cyanobacterial compounds on the organogenesis of pea cultured in vitro. Acta Biological Szegediensis. 49:37-38

Mussa- El-Shymaa S. 2005. Role of Azolla in different ecosystem. M. Sc. Thesis, Botany Dept., Fac. Sci., (Girls branch), Al-Azher, Universty, Cairo, Egypt.

Piper CS. 1950. Soil and Plant Analysis. Inter. Science Publishers Inc., New York.

Rao DLN and Batra L. 1983. Ammonia volatilization from applied nitrogen in alkali soils. Plant and Soil 70:219-228.

Reda El-Shahat M, Hanaa Abo-Kora A and Khafaga El-Hossiny EF. 2011. Azolla inoculant contribution to improve some soil properties and zea mays yield in saline soil. Egypt J.Biotechnol., 37: 69-81.

Revilla P, Malvar RA, Cartea ME, Butron A and Ordas A. 2000. Inheritance of cold tolerance at emergence and during early season growth in maize. Crop Sci., 40:1579-1585.

Sairam RK and Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci. 86: 407–421.

Sies H. 1991. Oxidative stress: oxidants and antioxidants, academic press, London.

Singh PK, Prakash J, Singh SK and Shukla M. 2008. Cyanophycean algae inhabiting sodic soil exhibit Diverse morphology; An adaptation to high exchangeable sodium. Ecoprint.15: 15-21.

Soltan pour PN. 1991. Determination of nutrient availability element toxicity by AB-DTPA. Soil Test and ICPS Adv. Soil Sci., 16: 165- 190.

Stafener D, Gastaic O, Matkouic B and Varge SZI. 1995. Metolachlor effect on antiodants enzyme activites and pigments content in seeds and yong leaves of wheat (Triticum aistivuml.) Agr. Med. 125: 267-273.

Strik WA and Staden JV. 2003. Occurrence of cytokinin-loke compounds in two aquatic ferns and their exudates. Environ. Exp. Botany. 37: 569-571.