Plant Archives Vol. 19 No. 2, 2019 pp. 3227-3230 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

INTERACTION EFFECT OF PHOSPHOBACTERIA AND AMF (*GLOMUS MOSSEAE)* AT THE BARLEY RHIZOSPHERE WITH ROCK PHOSPHATE NANOPARTICLES

Basim K. Hasan[1], Mahmood T. Abed[2] and Ali A.S. Hawal[3]

[1]College of Agriculture and Marshes, University of Thi–Qar, Iraq.
[2]College of Agriculture, University of Al-Muthanna, Iraq.
[3]Directorate of Agriculture of Al-Muthanna, Iraq.

Abstract

The field experiment was conducted in Nasiriyah city during the season (2016-2017) to study the effect of possible benefits of the co-inoculation of phosphate soluble bacteria. The inoculation was consisted of *(P. fluorescence*), (*B. megaterium)* and *Arbuscular mycorrhizal* fungi in the presence of rock phosphate nanoparticles (3 kg ha^{-1}). The design of experiments was according to the (RCBD) with three replicates. The results showed that all the treatments were statistically significant for all the parameters of the barley plant as compared to control treatment (T_0). The highest value of studied parameters was in the treatment of co-inoculation between all bacteria and fungi (T_3), which gave a significant increase in the rate of plant height, No. of tillers, length of the ear, No. of ear, No. of grains per ear, weight of grains per ear, grain yield and biological yield, respectively, (52.33 cm), (333.25 m^2), (10.92 cm), (330.60 m^2), (35), (330.60 g), (6.39 t ha^{-1}) and (16.95 t ha^{-1}). The use of Phosphate solubilizing bacteria enhanced colonies rate and spores number of mycorrhizal fungi at the barley rhizosphere. This helps in solubility of the mineral phosphate, contributes to the biogeochemical phosphorus cycling, and provides nutrients to the plant, which leads to increase productivity.

Key words : Nanoparticles, Phosphate, Co-inoculation, Rhizosphere, Biofertilizers.

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in Iraq. It ranks as the second after wheat in terms of planting and production this is due to the ability of the plant to cope with the water stress and salinity. Chemical fertilizers have played positive role in promoting plant yields such as cereal crops in order to feed the demand of the rising population of the world, however, the fertilizer cost and pollutant environment issues have reduced the use of mineral fertilizers which led to decrease yield. Biofertilizers can be used to reduce the cost of agricultural production and environmental pollution (Qureshi *et al.,* 2009; Al dulaimi, 2014). The use of biological inoculants whether bacterial or fungal have increased significantly in recent years comparing to the chemical fertilizers, which is very expensive and needs a lot of efforts to be used in planting (Al Khafaji, 2018). Free living bacteria promotes plant growth and reduces diseases caused from soil-borne plant pathogens. Beneficial free living bacteria found in the rhizosphere of roots of many different plants, some of these bacteria belong to the genera *Pseudomonas* and *Bacillus* (Kaushal *et al.,* 2013). The soil environment surrounding plant roots is the zone of biological activity. Many researchers have indicated that microorganisms are capable on converting non-available phosphorus into available phosphorus for plant absorption as well as nitrogen fixation. These organisms which are present in the soil rhizosphere formation of complex compounds with calcium, which produces hydrogen ion. Hydrogen ion leads to reduction of pH and this increases of phosphorus solubility in the soil rhizosphere. Microorganisms can reduce pH by forming CO_2 during analyzing organic residues in the soil, thus dissolving insoluble phosphate compounds (Kumari *et al.,* 2008; Nehwan *et al.,* 2010). Mycorrhizae is association of plant roots and the fungi, the associations that AMF form with plant roots called symbiotic associations because they are usually beneficial to both plant and fungi ((Evelin *et al.,* 2009). In exchange for carbohydrates produced by the plant through photosynthesis, the fungi help the host plant take up water and soil nutrients such as P, Cu, and Zn; co-inoculation of Phosphobacteria and Micorrhiza fungi has been demonstrated effective in promoting plant growth (Felici *et al.,* 2008). Nanoparticles of rock phosphate have better mobility in soils than the granular size; the application of nano rock phosphate as fertilizer would be a good for soil fertility and productivity as well as good reducer to the environmental hazards (Arifin *et al.,* 2017). The present study has conducted to determine the Phosphate solubility potential of *Pseudomonas fluorescence, Bacillus megaterium* and AMF (*Glomus mosseae*) at the barley rhizosphere with nanoparticles of rock phosphate in order to determine single and co-inoculation effect of them on the growth and yield of barley.

Materials and Methods

Microbial Culture

P. fluorescence and B. megaterium were obtained of the Marshes research center Thi-Qar University. Bacterial inoculum prepared by growing *P. fluorescence and B. megaterium* in 25 ml Luria Bertani broth, and log-phase culture centrifuged at 12,000 g for 10 min. For remove mineral phosphate present in medium the; pellet washed three times with sterile 0.85% sodium chloride. The bacterial pellet re-suspended in 25 ml of 0.85% sodium chloride was used as inoculum. The arbuscular mycorrhizal fungi (*Glomus mosseae*) was obtained from the laboratory of biofertilizers/ Agricultural Research Center - Baghdad, which consists of (infected roots + spores + soil), the detection of the presence of spores were by method of wet sieving and detecting (Gerdmann and Nicolson, 1963).

Soil analysis

The soil samples from depth (0–30 cm) were collected, air dried and analyzed for physical, chemical and biological properties as explained in table 1.

Treatments

Four treatments with three replicates were designed in randomized complete block design (RCBD). The first treatment was (T_0) seed without treatment (Control), second being (T_1) treated seed with *Pseudomonas fluorescens* + nanoparticles of rock phosphate, third (T_2) treated seed with *Bacillus megaterium* + nanoparticles of rock phosphate and forth (T_3) is combination of all bacteria with Arbuscular mycorrhizal fungi (*Glomus mosseae*) + nanoparticles of rock phosphate.

Table 1: Chemical, Physical and Biological Properties of Soil Samples before Sowing.

Parameters	Unit	Amount
pH		7.58
ECe	$ds.m^{-1}$	4.3
O.M	$g.kg^{-1}$	7.1
$CaCO_3$	$g.kg^{-1}$	158.7
Available nitrogen	$ml.kg^{-1}$	26.01
Available phosphorus	$ml.kg^{-1}$	15.10
Available potassium	$ml.kg^{-1}$	170.0
CEC	$cmol\ kg^{-1}$	16.02
Soil texture		Silty
Total number of bacteria	$cell.g^{-1}$ dry soil	3.3×10^6
Total number of fungi	$cells.g^{-1}$ dry soil	0.7×10^3

Detection of the ability of microbial inoculants to solubilize Phosphate

The ability of the microbial strains to solubilize phosphate were tested by calculating phosphate solubilizing index (PSI) according to the method described by (Nautiyal, 1999) as shown in the table 2 using the formula:

$$PSI = \frac{\text{Zone diameter} + \text{Colony diameter}}{\text{Colony diameter}}$$

Table 2: Phosphate solubilizing index of microbial inoculants.

Microbial Strains	PSI (mm)
Pseudomonas fluorescens	1.89
Bacillus megaterium	2.36
AMF *(Glomus mosseae)*	3.16
P. fluorescens + *B.megaterium* + AMF (*Glomus mosseae)*	3.96

Seed inoculation

Barley seeds (Abba 99) treated with the bacterial inoculants by mixed 50 ml of the microbial cultures for each inoculant and under the conditions of sterilization. Barley seeds were coated with slurry of the respective broth according to the treatments. The mycorrhizal fungi inoculant added to the soil by put 5 g in depth 5 cm under the soil surface with the addition of 3 kg / ha^{-1} of nano rock phosphate for all treatment.

Statistical analysis

The data obtained during the study analyzed statistically according to randomized complete block design (RCBD).

Results and Discussion

Effect of different treatments on growth parameters

The growth of barley crop was measured in terms

Table 3: Effect of different Biofertilizers growth parameters.

Treatments	Plant height (cm)	Number of Tillers m^2	Length of the ear (cm)
T_0 (Control)	39.69	292.75	9.21
T_1 (*Pseudomonas fluorescens*)	44.40	310.50	9.49
T_2 (*Bacillus megaterium*)	44.04	315.41	9.72
T_3 (*P. fluorescens* + *B. megaterium* + AMF (*Glomus mosseae*)	52.33	333.25	10.92
LSD	3.38	5.69	0.81

of plant height (cm), number of tillers (m^2) and length of the ear (cm) as reported in table 3 showed increased significantly with Single and co-inoculation compared to control.

Single inoculation with *Pseudomonas fluorescens* (T_1) and *Bacillus megaterium* (T_2) at 120 days after sowing gave significant increase of plant height rate reached (44.40 cm) and (44.04 cm) respectively compared to control (T_0) (39.7 cm). Maximum plant height of barley was observed with (T_3) co-inoculation with *Pseudomonas fluorescens, Bacillus megaterium* and AMF (*Glomus mosseae*) (52.33 cm). The use of biological inoculants had significant influence on number of tillers. Among the applied treatments, (T_3) co-inoculation resulted in significantly higher number of tillers (333.25 m^2) than control and other treatments in comparison. However, Single inoculation with *Bacillus megaterium* (T_2) gave slightly better number of tillers (315.41 m^2) as compared to *Pseudomonas fluorescens* (T_1) (310.50 m^2), single inoculation with both treatment T_1 and T_2 gave better results as compared to control (T_0) (292.75 m^2). The use of bio fertilizers had significant effect on length of the ear with (T_3) co-inoculation recorded significantly maximum length of the ear (10.92cm), followed by T_2 *Bacillus megaterium* (9.72 cm), T_1 *Pseudomonas fluorescens* (9.49 cm) and (T_0) control recorded the lowest length of the ear (9.21cm). The highest value in growth characteristics of barley may be due to the interaction between bacterial and fungal inoculants which play a positive role in alleviating inhibitory products, providing nutrients and in stimulating each other via biochemical activities that may increase beneficial attributes of their physiology, in addition to the fact that Biofertilizers enhanced nutrients uptake especially phosphorus and nitrogen. (Panhwar *et al.,* 2011) who reported that the use of phosphate-solubilizing bacteria as inoculants increased phosphorous uptake in plants and improved growth parameters of aerobic rice. Mishra *et al.,* (2010) obtained similar results in a previous study, that *Bacillus* sp. and *Pseudomonas fluoresens* have been shown influence on plant height up to 50.4 cm and 51.5 cm respectively as compared to control 48cm. Similarly, (Al dulaimi, 2014) who reported that application of bio inoculant with bacillus showed significant increase of plant growth parameters of barley with rock phosphate.

Effect of different treatments on yield parameters

The yield parameters *viz.* number of ear m^2, number of grains per ear, weight of grains per ear (g), grain yield(t/ha) and biological yield (t/ha) as reported in Table 4 found to be significantly higher with the treatment of co-inoculation (T_3) *Pseudomonas fluorescens, Bacillus megaterium* and AMF *Glomus mosseae* of wheat.

The highest number of ear was superior in treatment T_3 than other treatments and control with mean of T_3 (330.60m^2), T_2 (304.72m^2), T_1 (301.27m^2), T_0 (280.50m^2) respectively. The results showed that the treatment of co-inoculation (T_3) gave maximum number of grains per ear (35) followed by (T_2) *Bacillus megaterium* (22), (T_1) *Pseudomonas fluorescens* (19) and minimum seeds was recorded in (T_0) control (18). The maximum weight of grains per ear (g) was again observed with (T_3) co-inoculation (3.17g) followed by (T_2) *Bacillus megaterium* (2.68g), (T_1) *Pseudomonas fluorescens* (2.08g) and minimum weight was recorded in (T_0) control (1.69g). The data presented in table 4 depicts that treatment co-inoculation (T_3) recorded significantly maximum grain yield (6.39) t/ha followed by T_2 *Bacillus megaterium* (5.12) t/ha, T_1 *Pseudomonas fluorescens* (5.07) t/ha and minimum seeds was recorded in T_0 control (4.38) t/ha. The data presented in table 4 revealed that the treatment (T_1) Pseudomonas fluorescens was statistically at par with the treatment (T_2) *Bacillus megaterium*. However, treatment (T_3) *Pseudomonas fluorescens, Bacillus megaterium* and AMF (*Glomus mosseae*) recorded significantly higher biological yield (16.95) t/ha and (T_0) control recorded less biological yield (14.22) t/ha. The increase in yield parameters may be due to the positive role of bacterial and fungal fertilization in increasing soil fertility and plant growth promoting by increasing the essential nutrients, especially solubilize phosphorus at the soil rhizosphere by transformation of phosphorus into available form for plant uptake as well as improving physical soil properties. The current study is consistent with (Datta *et al.,* 2011) who reported that the treatment of barley plant with Biofertilizers individually and in combination gives superior results in terms of number of grains per ear and grain weight as compared to control. Liu and Lal, (2014) who indicated that application of tiny rock phosphate as a phosphorus fertilizer for soybean (Glycine max) increased the growth rate and seed yield by 32.6% and 20.4% respectively compared to those of soybeans treated with a regular P fertilizer. (Kanchana

Table 4: Effect of different biofertilizers yield parameters.

Biological Yield t/ha	Grain yield t/ha	Weight of grains per ear (g)	No. of grains per ear	No. of ear m^2	Treatments
14.22	4.38	1.69	18	280.50	T_0 (Control)
15.50	5.07	2.08	19	301.27	T_1 (*Pseudomonas fluorescens*)
15.92	5.12	2.68	22	304.72	T_2 (*Bacillus megaterium*)
16.95	6.39	3.17	35	330.60	T_3 (*P. fluorescens* + *B. megaterium* + AMF (*Glomus mosseae*)
1.21	0.12	1.50	5.85	4.45	LSD

et al., 2014) who indicated that the cause in increased yield parameters such as No. of grains per plant and grain weight per plant in barley plant due to interaction effect of microbial inoculants. Billah and Bano, (2014) who reported that the use of PGPR inoculation with rock phosphate on growth and yield of wheat increased the plant height, phosphorus uptake, grain yield, and seed phosphorus content over untreated control.

Conclusions

The application of phosphobacteria and AMF with nano rock phosphate increased nutrients uptake especially phosphorus and improved growth and yield of barley. The application of rock phosphate nanoparticles with microbial inoculation can be a suitable alternative to chemical phosphate fertilizer.

References

Al-Duliami, A.M. Sabri (2014). Evaluation of the efficiency of some Bacillus spp. Isolates In phosphate solubility under different salinity levels, growth and yield of barley. Master thesis, College of Agriculture - University of Baghdad.

Al-Khafaji, M.H. (2018). Effect of Rhizobia and Mycorrhiza inoculants and concentrations of vitamin B-Complex on the growth and yield of *Vicia faba* L. PhD thesis, College of Agriculture-Al-Muthanna University.

Arifin, M., N. Nurlaeny, R. Devnita, B.N. Fitriatin, A. Sandrawati and Y. Supriatna (2017). The Variable Charge of Andisols as Affected by Nanoparticles of Rock Phosphate and Phosphate Solubilizing Bacteria. The 1st International Conference and Exhibition on Powder Technology Indonesia, 030033-1- 030033-7.

Billah, M. and A. Bano (2014). Role of plant growth promoting rhizobacteria in modulating the efficiency of poultry litter composting with rock phosphate and its effect on growth and yield of wheat. Waste Management & Research, 1-10.

Datta, M., R. Patil, C. Sengupta, M.K. Pandit and S. Banerjee (2011). Plant growth promoting rhizobacteria enhance growth and yield of chilli (*Capsicum annum* L.) under field conditions. *Australian Journal of Crop Science*, **5(5):** 531-536.

Evelin, H., R. Kapoor and B. Giri (2009). Arbuscularmycorrhizal fungi in alleviation of salt stress. *Ann. Bot.*, **104(7):** 1263-1280.

Felici, C., L. Vettori, E. Giraldi, L.M.C. Forino, A. Toffanon, A.M. Tagliasacchi and M. Nuti (2008). Single and co-inoculation of *Bacillus subtilis* and *Azospirillum brasilense* on *Lycopersicon esculentum*: effects on plant growth and rhizosphere microbial community. *Applied Soil Ecology*. **40:** 260-270.

Gerdmann, J.W. and T.H. Nicolson (1963). Spores of mycorrhizal Endogene species extracted from soil by wet sieving and decanting. *Trans. Brit. Mycol. Soc.*, **46:** 234-244.

Kanchana, D., M. Jayanthi, G. Usharani, P. Saranraj and B. Sujita (2014). Interaction effect of combined inoculation of PGPR on growth and yield parameters of chillivar K1 (*Capsicum annum* L.). *International Journal of Microbiology Research,* **5(3):**144-151.

Kaushal, S., A. Karnwal and R. Yuvneet (2013). Potential Plant Growth-Promoting Activity of Rhizobacteria *Pseudomonas sp* in *Oryza sativa. Journal of Natural Productand Plant Resource*, **3(4):** 38-50.

Kumari, A., K.K. Kapoor, B.S. Kundu and R.K. Mehta (2008). Identification of organic acids prodicedduring rice straw decomposition and their rolin rock phosphate solubilization. *Plant Soil Environment*, **54(2):** 71-77.

Liu, R. and R. Lal (2014). Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (Glycine max). *Scientific Reports*, **4 :** 5686.

Mishra, R.K., O. Prakas, M. Alam and A. Dikshit (2010). Influence of plant growth promoting rhizobacteria (PGPR) the productivity of *Pelargonium Graveolens* L. herit. *Recent Research in Science and Technology,* **2(5):**53-57.

Nautiyal, C.S. (1999). An efficient microbiological growth medium for screening Phosphate solubilizing microorganisms. *Federation of European Microbiological Societies Microbiology Letters,* **170(1):** 265-270.

Nehwani, V., P. Doshi, T. Saha and S. Rajkumar (2010). Isolation and characterization of a fungi isolated for phosphate solubilization and plant growth promoting activity. *Journal of Yeast and fungi Research*, **1(1):** 9-14.

Panhwar, Q.A., O. Radziah, A.R. Zaharah, M. Sariah and I.M. Razi (2011). Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice. *Journal of Environmental Biology*, **32:** 607-612.

Qureshi, M. A., M.J. Ahmad, M. Naveed, A. Iqbal, N. Akhtar and K.H. Niazi (2009). Co-inoculation with *Mesorhizobiumcicer* and *Azotobacterchroococcum* for improving growth, nodulation and yield of chickpea (*Cicer arietinum). Soil and Environment*, **28(2):**124- 129.