Effect of different bio-fertilizers on growth of wheat

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تأثير الأسمدة الحيوية المختلفة على نمو القمح

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الملخص:

أجريت الدراسة بمحطة الأبحاث والتجارب/كلية الزراعة/جامعة طرابلس (أكتوبر-2021). تمت زراعة النباتات في أصص، وتم تعبئة الأصص سعة 2 لتر بتربة من الحقل، وزرعت ثلاث بذور قمح في كل أصيص، ثم تم تخفيفها إلى نبات واحد لكل أصيص بعد الإنبات. هدفت هذه الدراسة إلى معرفة تأثير العديد من أنواع الكائنات الحية الدقيقة بالتربة، مثل الفطريات (*Mycorrhiza sp*) والبكتيريا (*Bacillus sp*) كأسمدة حيوية، على نمو محصول القمح. كانت هناك أربع معاملات في هذه الدراسة؛ (التحكم: بدون الأسمدة الحيوية، فطريات المايكورايزا كسماد حيوي، بكتيريا البسلس كسماد حيوي، خليط من فطريات المايكورايزا * بكتيريا البسلس، كمزيج من الأسمدة الحيوية)، تم تكرار كل معاملة ثلاث مرات. كانت استجابة النباتات للأسمدة الحيوية (فطريات المايكورايزا * بكتيريا البسلس) مختلفة لكل سماد حيوي. حيث تشكل بعض هذه الكائنات الدقيقة علاقة تكافلية مع النباتات، مثل (فطريات المايكورايزا)، وكذلك (بكتيريا البسلس) التي تعيش حرة في التربة أي غير تكافلية. لهذه الأسباب، أظهرت النتائج اختلافات معنوية قوية في معظم الصفات المختبرة في هذه الدراسة. كان هذا التأثير أقوى بشكل ملحوظ عند استخدام الفطريات والبكتيريا معًا. مقارنة بإضافة الأسمدة الحيوية بشكل منفصل لمعظم الصفات المدروسة.

الكلمات المفتاحية: *فطريات المايكورايزا، بكتيريا البسلس، الأسمدة الحيوية.*

Abstract:

In a study conducted at the Research and experiment stations/at the College of Agriculture/University of Tripoli, (October – 2021). Plants were grown in pots, two-litre pots were filled with soil from the field, and three seeds of wheat were planted in each pot, then thinned to one plant per pot after germination. The aim of this study is to investigate the effect of several species of microorganisms, such as fungi (*Mycorrhiza sp*.), and bacteria (*Bacillus sp*.), as a biofertilizer, on the growth of the wheat crop. There were four treatments in this study; (Control: without-bio fertilizers, whit Mycorrhizal Fungi as a biofertilizer, Bacillus bacteria as a biofertilizer, a mix of Mycorrhizal Fungi * Bacillus bacteria, as a mixture of bio fertilizers), each treatment combination was replicated in three blocks. Plants' responsiveness to bio fertilizers (Bacillus bacteria & Mycorrhizal Fungi) was different for each biofertilizer. Where some of these micro-organisms form a symbiotic relationship with plants, such as fungi (*Mycorrhiza sp*.) and bacteria (*Bacillus sp*.) which are free-living or non-symbiotic. For these reasons, the results showed strong significant differences in most of the traits tested in this study. This effect was significantly stronger when fungi and bacteria were used together. Compared to adding bio-fertilizers separately, for the majority of the parameters which studied.

Keyword: *Mycorrhizal Fungi, Bacillus bacteria, bio-fertilizers.*

Introduction:

Wheat crop is one of the most important strategic cereal crops cultivated in Libya and the world, due to the dependence of the majority of the population on its products for their food, and for the energy, it provides necessary for the continuation of life. Wheat crop is among the top cereals to provide food. In the mid-term (2050). (Sharma et al., 2015), explained that the requirement for wheat would increase by up to 840 million tons from its current production rate of 642 million tons. The production is declining due to various factors like drought stress, late sowing, low-quality seeds, climate variability, and insect pests, but using of fertilizers is also important for wheat production for food security and the environment (Hochman and Horan 2018; Rahman *et al*., 2018). Accordingly, (Feng et al. 2016) explained that the current demand for cereal production and the gradual depletion of the soil make it increasingly necessary to apply more fertilizers, mainly nitrogenous ones. High quantities of nitrogen (N) per hectare need to be applied to soil to produce optimum wheat grain yields (Żuk-Gołaszewska *et al*., 2016). Improving nutrient use efficiency in wheat is important to increase the yield and reduce chemical fertilizers, keep the clean environment and reduce production costs. For that, agricultural operations have been used the diagnose nutritional problems, assess fertilizer recommendations (e.g. type of fertilizer, methods of add, rates of add and time of add), and determine total nutrients.

One possibility to increase soil fertility is by adding bio-fertilizers, as an alternative to chemical fertilizers (Wu *et al*., 2005). Bio-fertilizers are a mixture containing microorganisms that are able to supply the plant with required nutrients from natural sources and reduce reliance on chemical fertilizers. Accordingly, these fertilizers are able to release nutrients constantly in quantities sufficient to cover plants' needs (Ratti *et al*., 2001) as well as to help the plants to absorb nutrients through the overlap with the root zone (Mishra and Dash, 2014).

Where bio-fertilizer is able to supply the plant with required nutrients from natural sources and at the same time reducing reliance on different chemical fertilizers. Accordingly, these fertilizers are able to release nutrients constantly in quantities sufficient to cover plants' needs. It also helps the plants to absorb nutrients through the overlap with the root zone (Mishra and Dash, 2014). According to Ratti *et al*., (2001) effects of phosphate solubilizing bacteria, nitrogen-fixing fixing bacteria and arbuscular mycorrhizal fungi were found that all microbes inoculated together help in the uptake of tricalcium phosphate in a low phosphate soil. In addition, some of these micro-organisms form a symbiotic relationship with plants, such as mycorrhizal fungi and the bacteria (*Bacillus sp*.) which are free-living or non-symbiotic.

The arbuscular mycorrhizal fungi (AMF) could be used as a bio control agent against white root rot, and their presence in soils has been used as an indicator of fruit tree tolerance to this soil-borne plant pathogen (Oliveira Soares, 2014).

However, mycorrhizal symbiosis regulates the expression of plant genes that are involved in the biosynthesis of proline. The regulation of these genes allows mycorrhizal plants to maintain a better water status in their tissues (Porcel *et al*., 2011).

Mycorrhizal fungi colonization can also protect plants against the deleterious effects of water stress by improving water relations to plants, nutrient content and chlorophyll content of the plants (Asrar *et al*., 2012). Richardson (2001) explained that phosphate solubilizing bacteria (PSB) play a key role in phosphorus movement in soil and the availability of phosphate to plants.

Microbial biomass assimilates soluble phosphorus and thus prevents it from adsorption or perhaps fixation (Khan and Joergesen 2009). The phosphate solubilization microorganisms (PSB) and plant growth-promoting rhizobacteria (PGPR) with each other could greatly reduce phosphorus fertilizer application by 50% without any significant reduction of crop yield (Yazdani *et al*., 2009). It has been suggested that bacteria are more effective in phosphorus solubilization than fungi (Alam *et al*., 2002). The use of phosphate-solubilizing microorganisms (PSMs) can increase crop yields by up to 70 per cent (Verma, 1993). Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance plant growth by improving biological nitrogen fixation (Kucey *et al*., 1989; Ponmurugan and Gopi, 2006). Jilani *et al*., (2007) suggested that integration of half a dose of NP fertilizer with biofertilizer gives crop yield as with the full rate of fertilizer, and also through reducing the use of chemical fertilizers the production cost is minimised and the net return maximised. Several mechanisms have been proposed to explain phosphorus solubilization by phosphate solubilizing rhizobacteria (PSRB). They are associated with the release of organic and inorganic acids and the excretion of protons that accompany the NH^{4+} assimilation (Illmer *et al*., 1995). In addition, (Kim *et al*., 1997) expounded that mechanisms involved in the microbial solubilization of phosphorus include the production of organic acids and the release of protons into the soil solution. Furthermore, it has been suggested that the release of phosphatase enzymes to mineralise organic phosphorus compounds may be another mechanism involved (Stevenson, 1986).

In addition, microbial biomass is important for maintaining both inorganic and organic phosphorus in soil solution (Seeling and Zososki 1993). Additionally, the turnover of biomass represents an important potential supply of phosphorus to plants (Oberson and Joner 2005). However, the cycling of phosphorus in the microbial biomass and its subsequent release is critical to the phosphorus cycle in soil and is represents an important pathway for the movement of phosphorus from soil solution into plant-available forms (Magid *et al*., 1996; Oberson *et al*., 2001). Microbial biomass may also serve to protect orthophosphate from becoming unavailable in the soil due to various physicochemical reactions (Magid *et al*., 1996; Oberson *et al*., 2001).

This study aims to investigate the effect of several species of microorganisms, such as fungi (*Mycorrhiza sp*.)* and bacteria (*Bacillus sp*.)** as a bio fertilizer, on the growth of the wheat crop, with 45 kg P h^{-1} of phosphorus (rock phosphate as a source of phosphor fertilizer).

***** Mycorrhizal fungi as a biofertilizer: (The Nutrient Company (TNC) Ltd., Rochdale, Greater Manchester, UK), **TNC Mycorr Multi**: contains 15 different species of mycorrhizal fungi. It also contains natural bio-stimulants such as humic acid and derivatives of natural marine algae.

****** Bacteria (*Bacillus*) as a biofertilizer: (The Nutrient Company (TNC) Ltd., Rochdale, Greater Manchester, UK), **TNC Bactorr**^{S13} is a 100% natural product containing 13 different species of Bacillus (active bacteria) friendly bacteria which are known to be beneficial to plants, and also packed with other natural substances such as humate (Humic acid), as well as cytokins, auxins and giberelins along with amino acids and trace elements all derived from natural marine algae.

Materials and methods:

Plants were grown in pots, at the Research and experiment stations / College of Agriculture / University of Tripoli, (October – 2021). Two-litre pots were filled with soil from the field, and three seeds were planted in each pot, then thinned to one plant per pot after germination. There were four treatments in this experiment; (Control: without-bio fertilizers, whit Mycorrhizal Fungi as a bio fertilizer, Bacillus bacteria as a bio fertilizer, a mix of Mycorrhizal Fungi * Bacillus bacteria, as a mixture of bio fertilizers), each treatment combination was replicated in three blocks. In addition, used mycorrhizal fungi (15 species, including types which are known to associate with wheat), it has been used 3 g per pot (Mycorr Multi as a source of mycorrhizal fungi) per pot, and 3 g per pot, inoculation (Bactorr^{S13} as a source of Bacillus bacteria). Also, the other elements necessary, including nitrogen was added in the form of ammonium nitrate (33% N), potassium was added in the form of potassium sulphate (48% K_2O), magnesium and calcium were added in the form of Dolomite (30.4% CaO & 21.7% MgO & 47.9% CO_2) and microelements were added in the form of fritted trace elements (B, Zn, K, Fe, Mn, Cu, Mo)).

Data analysis:

Data were analysed using Complete Randomized Design (CRD) in GenStat (Release 19.3.0.9425; VSN International, Nottingham, UK) to estimate the sources of variation. Significant consideration was taken when the probability value is 0.05 or less.

Results:

Length of plant (cm) & Length of spike (cm) & Number of spikes fertile

The results of this study showed that bio-fertilizer had no significant effect on the length of the plant (cm), the length of the spike (cm) and the number of spikes fertile.

Number of seeds in the spike

The number of seeds in the spike showed a strong interaction between the Mycorrhizal Fungi and the Bacillus bacteria (P=0.009) (Figure 1). Where the largest number of seeds in the spike were obtained from using the Mycorrhizal with the Bacillus, it's statistically different from the addition of bacteria and fungi individually and the control.

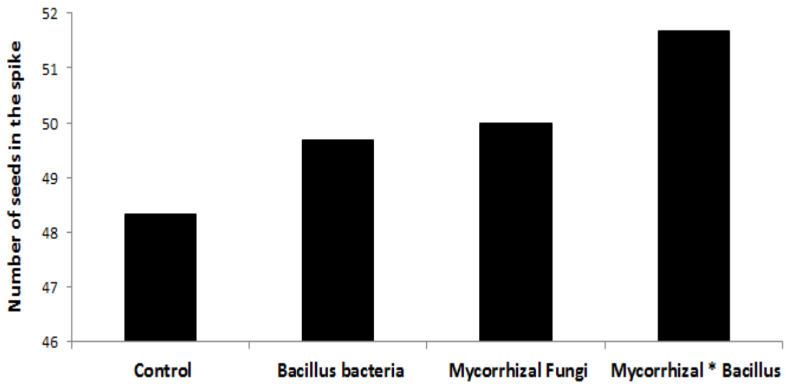

Figure 1: The effect of the interaction of the bio fertilizer on the number of seeds in the spike (L.S.D =1.49).

Weight of 1000 seeds (g)

The results showed that the weight of 1000 seeds (g) differed significantly with the different mixtures of bio fertilizers (P<0.001) (Figure 2). The highest mean value in the Weight of 1000 seeds (61 g) was obtained by using (Mycorrhizal * Bacillus), which is significantly different from (58.67 & 59 g) from (Bacillus bacteria) and (Mycorrhizal Fungi) respectively. Also, there were significant differences between these values with the control treatment.

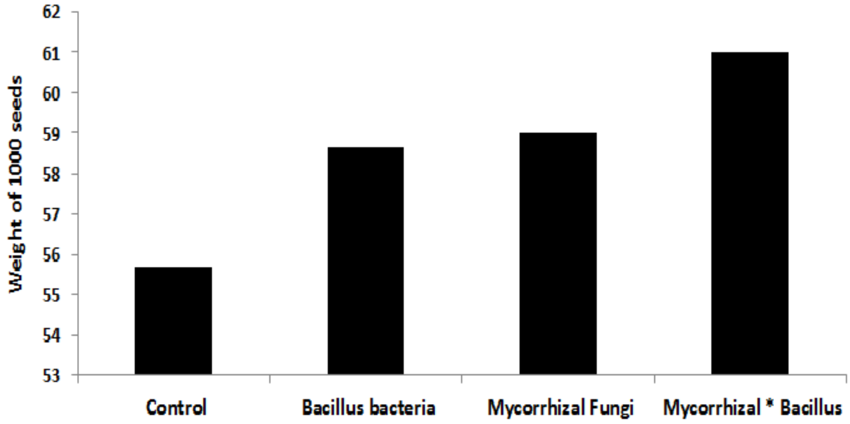

Figure 2: The effect of the interaction of the bio fertilizer on the Weight of 1000 seeds (g) (L.S.D =1.33)

Weight of dry matter in the plant (hay) (g)

The Weight of dry matter in the plant (hay) (g) differed significantly with the different mixtures of bio fertilizers (P<0.001) (Figure 3). The highest mean value in the Weight of dry matter in the plant (18.19 g) was obtained by using (Mycorrhizal * Bacillus), which is significantly different from (17.31 & 17.5 g) from (Bacillus bacteria & Mycorrhizal Fungi) respectively. Also, there were significant differences between these values with the control treatment (13.74 g).

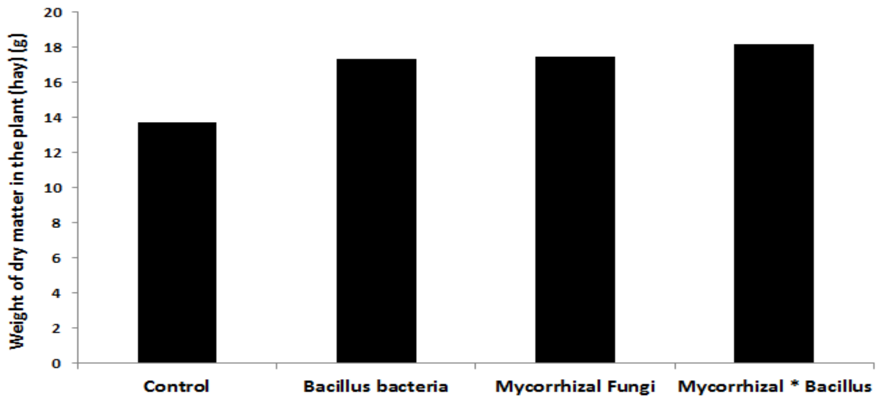

Figure 3: The effect of the interaction of the bio fertilizer on the Weight of dry matter in the plant (g) (L.S.D =0.48)

Weight of seeds in spike (g)

The Weight of seeds in spike (g) differed significantly with the different mixtures of bio fertilizers (P<0.001) (Figure 4). The highest mean value in the Weight of seeds in spike (3.14 g) was obtained by using (Mycorrhizal * Bacillus), which is significantly different from (2.82 & 2.91 g) from (Bacillus & Mycorrhizal) respectively. Also, there were significant differences between these values with the control treatment (2.56 g).

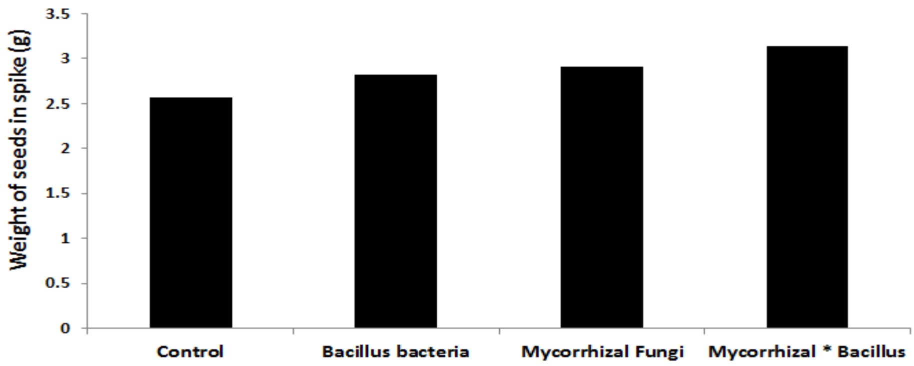

Figure 4: The effect of the interaction of the bio fertilizer on the Weight of dry matter in the plant (g) (L.S.D =0.16)

Weight of seeds in plant (g)

The Weight of seeds in the plant (g) differed significantly with the different mixtures of bio fertilizers ($P<0.001$) (Figure 5). The highest mean value in the Weight of seeds in the plant (21.30 g) was obtained by using (Mycorrhizal * Bacillus), which is significantly different from (18.20 & 18.97 g) from (Bacillus bacteria & Mycorrhizal Fungi) respectively. Also, there were significant differences between these values with the control treatment (14.20 g).

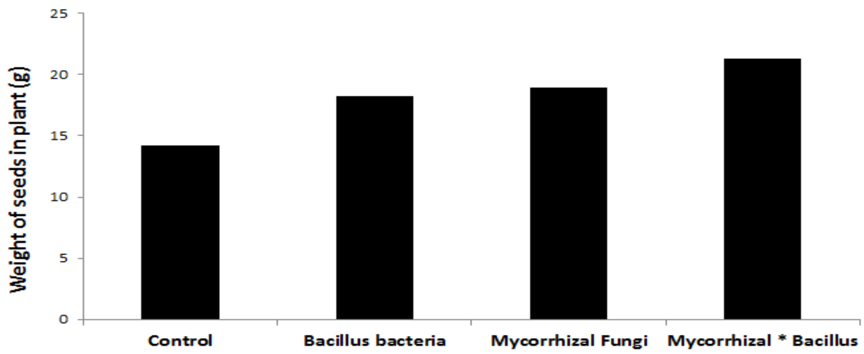

Figure 5: The effect of the interaction of the bio fertilizer on the Weight of seeds in plant (g) (L.S.D =1.96)

Discussion:

Plants' responsiveness to bio fertilizers (Bacillus bacteria & Mycorrhizal Fungi) was different for each type. Where some of these micro-organisms form a symbiotic relationship with plants, such as fungi (*Mycorrhiza sp.*) and bacteria (*Bacillus sp.*) which are free-living or non-symbiotic. For these reasons, the results showed strong significant differences in most of the traits tested in this study. This effect was significantly stronger when fungi and bacteria were used together. It can be the contribution of bio fertilizer to improved plant growth through the increase in the availability of soluble phosphate by the production of organic acids and the release of protons to the soil solution (Kim *et al*., 1997; Illmer *et al*., 1995) or the release of phosphatase enzymes (Stevenson 1986) or improving nitrogen fixation (Kucey *et al*., 1989; Ponmurugan and Gopi, 2006).

Conclusion:

According to the results of this study, it can be said that microorganisms; especially fungi (*Mycorrhiza sp*.) and bacteria (*Bacillus sp*.), that live free in the soil as a bio-fertilizers, also, as an alternative to chemical fertilizers had an important impact on the growth of wheat. Also, these experiments should be repeated in the field with isolated active bacteria from the same area of the experiment and study the effect of these bacteria on the yield with cost estimation.

Reference

Alam, S., Khalil, S., Ayub, N., & Rashid, M. (2002). In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. *Int J Agric Biol*, *4*(4), 454-458.

Asrar, A. A., Abdel-Fattah, G. M., & Elhindi, K. M. (2012). Improving growth, flower yield, and water relations of snapdragon (Antirhinum majus L.) plants grown under well-watered and water-stress conditions using arbuscular mycorrhizal fungi. *Photosynthetica*, *50*(2), 305-316.

Feng, J., Li, F., Deng, A., Feng, X., Fang, F., & Zhang, W. (2016). Integrated assessment of the impact of enhanced-efficiency nitrogen fertilizer on N2O emission and crop yield. *Agriculture*, *Ecosystems & Environment*, *231*, 218-228.

Hochman Z, Horan H (2018). Causes of wheat yield gaps and opportunities to advance the water-limited yield frontier in Australia. Field Crop Res 228:20–30.

Illmer, P., Barbato, A., & Schinner, F. (1995). Solubilization of hardly-soluble AlPO4 with P-solubilizing microorganisms. *Soil Biology and Biochemistry*, *27*(3), 265-270.

Jilani, G., Akram, A., Ali, R. M., Hafeez, F. Y., Shamsi, I. H., Chaudhry, A. N., & Chaudhry, A. G. (2007). Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. *Annals of Microbiology*, *57*(2), 177-184.

Khan, K. S. and R. G. Joergensen. (2009). Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Bioresour. Technol.100:303-309.

Kim, K., McDonald, G. and Jordan, D. (1997). Solubilization of hydroxyapatite by Enterobacter agglomerans and cloned Escherichia coli in culture medium. Biology and Fertility of Soils, 24(4), pp.347-352.

Kucey, R. M. N., H. H. Janzen and M. E. Legget. (1989). Microbial mediated increases in plant available phosphorus. Adv. Agron. 42:199 - 228.

Magid J, Tiessen H, Condron LM (1996). Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. In: Piccolo A (ed) Humic substances in terrestrial ecosystems. Elsevier Science, Amsterdam, pp 429–466.

Mishra, P. and Dash, D. (2014). Rejuvenation of Biofertilizer for Sustainable Agriculture and Economic Development. The Journal of Sustainable Development, 11 (1), pp. 41-61.

Oberson A, Friesen DK, Rao IM, Bühler S, Frossard E (2001). Phosphorus transformations in an oxisol under contrasting land-use systems: The role of the microbial biomass. Plant Soil 237:197–210

Oberson A, Joner EJ (2005) Microbial turnover of phosphorus in soil. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. CABI, Wallingford, UK, pp 133–164.

Oliveira Soares, W. (2014). Impact of the Arbuscular Mycorrhizal Fungi and Bacteria on Biocontrol of White Root Rot in Fruit Seedlings. Journal of Plant physiology & pathology, 02(01).

Ponmurugan, P. and C. Gopi. (2006). Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J. Agron. 5:600-604.

Porcel, R., Aroca, R. and Ruiz-Lozano, J. (2011). Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agronomy for Sustainable Development, 32(1), pp.181-200.

Rahman MHU, Ahmad A, Wang X, Wajid A, Nasim W, Hussain M *et al*., (2018). Multi-model projections of future climate and climate change impacts uncertainty assessment for cotton production in Pakistan. Agri For Meteorol 253:94–113.

Ratti, N., Kumar, S., Verma, H.N. & Gautams, S.P. (2001). Improvement in bioavailability of tricalcium phosphate to Cymbopogon martini var. motia by rhizobacteria, AMF and azospirillum inoculation. Microbiology Research, 156: 145-149.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol. 28:897-906.

Seeling, B. and Zasoski, R. (1993). Microbial effects in maintaining organic and inorganic solution phosphorus concentrations in a grassland topsoil. Plant Soil, 148(2), pp.277-284.

Sharma I, Tyagi BS, Singh G, Venkatesh K, Gupta OP (2015) Enhancing wheat production-A global perspective. Indian J Agric Sci 85:3–13.

Stevenson, F. J. (1986). Cycles of soil. New York: John Wiley and Sons. 380 p.

Verma, L. N. (1993). Bio fertilizer in agriculture. In: P. K. Thampan (ed.) Organics in soil health and crop production. Peekay Tree Crops Development Foundation, Cochin, India. pp. 152-183.

Wu, S., Cao, Z., Li, Z., Cheung, K. and Wong, M. (2005). Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma, 125(1-2), pp.155-166.

Yazdani M., M. A. Bahmanyar, H. Pirdashti and M. A. Esmaili. (2009). Effect of Phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR). on yield and yield components of Corn (Zea mays L.). Proc. World Acad. Science, Eng. Technol. 37:90-92.

Żuk-Gołaszewska, K., Żuk-Gołaszewska, K., Żerańska, A., Krukowska, A. and Bojarczuk, J., (2016). Biofortification of the nutritional value of foods from the grain of Triticum durum Desf. by an agrotechnical method: A scientific review. Journal of Elementology, (3/2016).