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RESEARCH PAPER

The combined Application of Iron and Phosphate Solubilizing Bacteria to enhance Wheat (Triticum aestivum L.) growth and yield

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A B S T R A C T:

This study was conducted to evaluate the efficiency of prepared iron and phosphate biofertilizers in improving wheat growth and yield. To isolate iron and phosphate solubilizing bacteria, different soil samples were collected from Erbil governorate, a total of eighteen iron solubilizing bacteria were selected on modified agar medium and all isolates belonged to Pseudomonas fluorescense. Twenty two isolates of phosphate solubilizing bacteria were recognized and they referred to Pseudomonas putida, Pseudomonas fluorescense and Bacillus megaterium using microscopical, cultural, physiological and molecular tests. According to iron and phosphate solubilizing efficiency test, the most efficient isolate of iron solubilizing was (Pfl3) and P-solubilizing bacteria were (Ppu2, Bm14), and they were selected for biofertilizers preparation. Local and imported iron and phosphate biofertilizers were applied in pot experiment. Most biofertilizers were significantly increased growth, yield and yield components of wheat plants. Maximum shoot length, root length, tillers number/5,1000 seed weight, spike number/5 and yield (99.60cm, 58.95cm, 22, 38.75g. 20, 4.12 ton/ha respectively) were recorded by incoculation with P. fluorescense + P. putida +B. megatrium significantly higher than uninoculated control and treatments with imported biofertilizer. It can be concluded that local iron and phosphate solubilizing bacterial isolates can be used for Fe biofertilizers and P biofertilizers preparation.

KEY WORDS: *Wheat, Biofertilizer, Ps.Putida, P. fluorescense , B. megatrium*.
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1.INTRODUCTION :

Phosphate (P) and iron (Fe) are two essential nutrients for the survival and development of all living organisms (Salgueiro et al., 2000). These two mineral elements are relatively inaccessible to plants and crops because of their low solubility and relative immobilization in the agricultural soils (Lopez et al., 2000; Hirsch et al., 2006). Crops are therefore subjected to P and Fe deficiencies, which can adversely impact multiple metabolic processes in cells. Nevertheless, plants have evolved a number of strategies to cope with low P and Fe availabilities (Smith and Read, 2008). In recent decades, the effects of P and Fe deficiencies on crop yield and quality have become a global concern due to the issues of food availability and malnutrition (Neset and Cordell, 2012).

Some rhizospheric bacteria are able to colonize plant roots, and these bacteria are capable of solubilizing inorganic phosphate, iron and potassium through the production of organic acids such as gluconic acid and ketogluconic acid, also esterase type enzymes are known to be involved in releasing phosphorus and iron from organic compounds. These rhizospheric bacteria require iron for the chelation process and form siderophore complex. Phosphate solubilization and transport of ferric iron by siderophore released from PGPR increases the accessibility of different types of nutrients in the rhizosphere (Jetiyanon, 2015). Besides these mechanisms the production of chelating substances, H2S, mineral acids, CO2, siderophores, biologically active substances like indole acetic acids, gibberllines and cytokinins are also correlated with P and Fe solubilization. Among rhizobacteria: Bacillus and Pseudomonas are considered to be one of the most promising groups of plant growth promoting rhizobacteria involved

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in P and Fe solubilization, synthesis phytohormones and promote plant growth. Some Pseudomonas strains have the ability to synthesize phytohormones, iron chelators, solubilize soil low availability compounds, toxic substances, and cope with and catabolize environmental pollutants, making them promising candidates for agriculture and soil treatment (Saharan, and Nehra, 2011). Some Pseudomonas strains have a set of genes that code for rhizosphere colonization and plant growth determinants; these strains can solubilize inorganic phosphate via the prod. Extracted organic acids (Lamizadeh, et al. (2016)

The current study aimed to isolate and characterize iron and phosphate solubilizing Pseudomonas fluerescense, Pseudomonas putida and Bacillus megaterium from Erbil soil and to evaluate the ability of them in phosphate and iron solubilization, and use them as biofertilizer with wheat crop.

2.MATERIALS AND METHODS

2.1. Soil Sample Collections

Sampling was conducted from different geographical locations in Erbil governorate/Kurdistan region-Iraq included: (Grdarasha, Khabat, Jamadubze, Mala-Omer, Zargazawy, Bawakhalan, Choman, Harer, Makhmoor, Qushtapa, Dibaga, Spilk and Warte on October 2020. The soils were brought to Microbiology laboratory, College of Agriculture Engineering Science, University of Salahaddin-Erbil Samples were collected in sterilized bags and kept at 4°C and processed a few days after sampling.

2.2. Isolations and Identifications of Bacterial strains

For isolation of iron and phosphate solubilizing bacteria, serial dilutions were carried out for soil samples and cultured onto plate of Biotite containing King's B medium agar (Proteose peptone 20.0, Biotite 2, Dipotassium hydrogen phosphate 1.50, Magnesium sulphate. heptahydrate 1.50, Agar 20.0 and Final pH 7.2±0.2 all in gm / Liter) and Pikovskaya (Yeast extract 1.000, Mannitol 10.000, Dipotassium phosphate 0.500, Magnesium sulphate 0.200, Sodium chloride 0.100, Calcium carbonate and 1.000 Agar 15.000 all in gm / Liter) agar medium (Johnsen and Nielsen, 1999) and (Melvin Joe et al., 2018), respectively, following the protocols of Anderson and Pascual, 2000. After 75hr. incubation at 28°C, microscopical, morphological, biochemical and physiological tests were conducted according to Bergey's Manual for Determinative Bacteriology. included (cell shape, gram stain, Colony morphology, color, size, spore formation, arrangement, motility, colony characters, flagellum observation, aerobic test, pigmentation, oxidase and catalase test, Gelatin liquefaction, starch hydrolysis, glucose fermentation, arginine dihydrolase, levan production, nitrate reduction, utilization of trehalose and tryptophane, growth at 4˚C and 41˚C, and different sugar utilization (Loekas et al., 2011) and (Gulez et al., 2014). Additionally, all isolates were identified by using API 20e depending to API 20e cod book and then the results were interpreted through API20e online cod book application system. Moreover, studied bacteria were identified to species level by using VITEK 2 technique according to Biomerieux-diagnostics protocol. Molecular Identification of each strain was based on the partial sequence of 16S rDNA. DNA was extracted and amplified with universal bacterial primers: F (5'-AGAGTTTGATCCTGGCTCAG -3') and R (5'- ACGGCTACCTTGTTACGACTT - 3'). PCR was performed using Lucigen EconoTaq Plus Green 2X master mix (Lucigen Corp.) with cycling parameters: initial denaturation at 95°C for 5 min; 31 cycles of 94°C for 1 min, 57°C for 45 sec, 70°C for 2 min; and a final extension at 70°C for 10 min. All sequences were blasted against the type strains in the ribosomal database project to identify bacterial taxa of each isolate. Obtained sequences were aligned with reference RNA sequences from National Center for Biotechnology Information database (http://blast.ncbi.nlm.nih.gov) using the nucleotide basic local alignment and search tool (BLAST).

2.3. Preservations of Isolated Bacteria

The purified colonies of isolated bacteria were preserved at −75 °C with 25% glycerol and at 4 °C on agar slants for further study (Delves et al., 1996).

2.4- Phosphate solubilizing assay

Phosphate solubilization activity of all isolates was investigated using the modified Pikovskaya's agar medium. The studied isolates were spot-inoculated onto the surface of the agar medium. Clearance zone around the bacterial colony indicates phosphate solubilization. The presence of clearing zone around the bacterial growth as the indicator of P solubilization was noted after seven days of incubation.

2.5- Iron solubilizing efficiency test

Iron solubilization was manifested by the formation of clearing zones on basal medium supplement with insoluble iron source. To solubilize Magnetite Fe_2O_4, the bacterial culture discs were transferred to dishes containing the basal medium (NaCl, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; KH_2PO_4 (3 mM P); Fe_2O_4 0.5 mg; $MnSO_4 \cdot H_2O$, 1.56 mg; $ZnSO_4 \cdot 7H_2O$, 1.40 mg; vitamin B12, 2 µg; N sources nitrate+ammonium. Inorganic iron sources were washed three times to remove any soluble Fe described by Reyes et al. (1999) and modified by Gadagi and Sa (2002). Bromocresol green (BCG) was used as a pH indicator as follows: 5 mL of a BCG stock solution (0.5% BCG in 70% ethanol, adjusted to pH 6.5 with 1 N KOH) was added to 1 L of Reyes medium. Dishes were incubated in a bacteriological incubator at 30ºC for 15 days. The Evaluation of $Fe(OH)_2$ solubilization in solid medium occurred through the 15th day of incubation. The diameters of the solubilization zones (clear zone) around the bacterial colonies were measured after 7days, the solubilization Index was calculated using the following formula (Berraqueiro et al., 1976):

SI = Halo diameter /Colony diameter

2.6. Preparation of Biofertilizer

Depending on phosphate and iron solubilizing assay tests, the most efficient isolates in iron and phosphate soilubilization were selected for biofertilizer preparation. Biofertilizers was prepared by addition of 1ml of fresh broth culture with (35*107cell/ml) to 100g carrier (%20 compost + %20 charcoal + %4 biochar + %20 CaCO3+ %20 clay +%10 sand +%1 gum+ % 5 pillite). Different types of biofertilizer were prepared.

2.7. Pot Experiment

The pot experiment were conducted in Khabat institute field, which is far about 35 km to the west of Erbil city, during December 26, 2020 to June 20, 2021. The pots (45cm height, 30cm diameters) were filled with unsterilized soil. Some of the chemical and physical characteristics were shown in the Table (1).

Table (1) Some physical and chemical properties of the studied soils.

PH	EC	O.M	N	Available (p)	K^+	Ca^{+2}
	$ds.m^-$	%	%	$mg.g^{-1}$	$Mmolie.L^{-1}$	
7.5	1.3	1.1	0.27	3.76	0.22	4.12
Depth		PSD%		Soil texture		
0-30 cm	Sand	6		Silty clay		
	Silt	50				
	clay	44				

Growth promoting effects of prepared biofertilizers were studied on wheat plant. The surface sterilized seeds of wheat were inoculated with prepared biofertilizer (one gram freshly biofertilizer per 100g seed) before sowing (Milani and Anthofer, 2008). There were 11 treatments with four replications included: non inoculated control (without biofertilizer), *Pseudomonas fluerescense* biofertilizer, *Pseudomonas putida* biofertilizer, *Bacillus megatrium* biofertilizer, *Pseudomonas fluerescense* + *Pseudomonas putida* biofertilizer , *Pseudomonas fluerescense* + *Bacillus .megatrium* biofertilizer, *Pseudomonas putida* + *B.megatrium* biofertilizer, (*Pseudomonas fluerescense* + *Pseudomonas putida* + *B.megatrium)* biofertilizer, with three treatment of two type of industrial imported biofertilizer phosphate biofertilizer and iron biofertilizer with mixed of them .

The experiment was set up in randomized complete block design (RCBD) using four replicates per treatment. Data was recorded for dry weight of shoots (the shoots were dried at 70°C for 72hr to calculate the dry matter, shoot

length, number of seeds/plant, root length, 1000 seed weight and total yield.

2.8. Statistical Analysis

In all cases, Duncan's H.S.D. multiple range tests were applied for comparing between mean of treatments by using SPSS 16.0 (Casanova et al., 2004).

3.RESULTS AND DISCUSSION

3.1. Isolation and Identification of Iron and Phosphate Solubilizing bacteria

Samples collections were performed to isolate Phosphate and iron Solubilizing bacteria from different locations in Erbil city. Among thirty eight soil samples, only eighteen isolates were selected on biotite containing King's B medium based on their similarities in morphological and biochemical tests. All isolates negative to gram nature, rod shaped, greenish yellow colony, motile, aerobic, non-spore former, Isolates were biochemically characterized for their ability to produce catalase, gelatinase, arginine dihydrolase, oxidase, starch and urea hydrolysis, and had ability to utilize trehalose , levan production from sucrose, nitrate reduction, and growth at 4°C and 41°C . All these strains have been reported to use galactose, dextrose, citrate and mannose but showed varying degree of utilization towards other carbon sources such as xylose,lactose, fructose, melibiose, glycerol, Larabinose, ribose, D-arabinose, xylitol, malonate, sorbose, sorbitol, trehalose, mannitol, and glucosamine. These strains did not utilize maltose, sucrose, inositol, cellobiose, and rhamnose. According to the results, all isolates revealed extensive phenotypic characterization with *P. fluorescense* (Patel et al., 2013).

A total of twenty two isolates were recognized on PVK agar medium and thirteen of them characterized by were reacted negatively to gram staining, short rods, had flagella and did not create endospores, reduced nitrates, motile, did not liquefy gelatin, positive to oxidase and catalase test, produced HCN, could not hydrolysis starch, they hydrolyzed urea, unable to utilize trehalose, could not grow at 41ºC but grew at 4ºC. Results of morphological, physiological and biochemical characterization indicated that all thirteen isolates belonged to the genus *Pseudomonas putida* according to methods described in Bergey's Manual of Determinative Bacteriology. Only three *Ps. fluerescense* isolates were identified on PVK medium, while the other seven isolates on PVK medium desicribed by gram positive, motile, spore former, aerobic, with irregular and yellow colonies, positive reaction for oxidase, catalase, and hydrolysis of starch and gelatin, they could use arabinose, fructose, galactose, raffinose, mannitol, ribose and xylose, but they could not utilize rhamnose and mannose. Also they were able to grow at 4°C and 45°C. Depending on above characteristics, these five isolates were recognized as *B. megaterium*. Identification procedure of isolates by 16S rRNA gene sequencing revealed that Fluorescent pseudomonas isolates were *P. fluorescense* and *P. putida* were with 99% similarity. It means that all selected fluorescent pseudomonads belong to the above species (Fig 1).

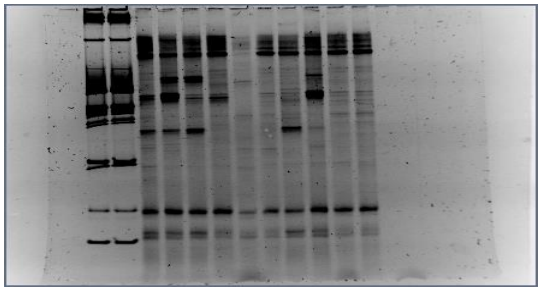

Figure (1) PCR results of some fluorescent pseudomonas isolates. 1 kb ladder band (the primer), the identified bands are p. fluorescense and p.putida .

3.2- Phosphate solubilizing activities

All isolated PSB strains were tested for P-solubilizing efficiency on solid PVK media. Production of clear halo zone on agar plates indicating P-solubilizing activity. The results (Table 2) showed that all isolates were able to solubilize P on PVK media plates. The highest P-solubilizing activity (76.2%) was found in Ppu2 treatment followed by Ppu8 (68.9%), while isolate Pfl21 recorded minimum solubilization index (21.3%), which differed significantly from other isolates. These results may be attributed

to the ability of *Ps. putida* to produce higher amount of organic acids which led to increase the available phosphorus compared with other tested bacterial strain. This result is in agreement with Castro et al., 2009 who observed the role of *Pseudomonas putida* to solubilize fixed phosphorus to available phosphorus. It is generally accepted that the mechanism of mineral phosphate solubilization is associated with the release of low molecular weight organic acids, which, through their hydroxyl and carboxyl groups, chelate the cations bound to phosphate, thereby converting it into soluble forms (Editha et al., 2017).

Table (2): Estimation of phosphate solubilization efficiency of isolated bacterial strains using pikovskaya's medium

Isolate code	Phosphate solubilization (clear zone (%)
Ppu1	38.6^{f}
Ppu2	76.2^{a}
Ppu3	45.4^{e}
Ppu4	28.3^{h}
Ppu5	46.4^{e}
Ppu6	28.1^{i}
Ppu7	31.0^{h}
Ppu8	68.9^{b}
Ppu9	61.1^{c}
Ppu10	35.5^{g}
Ppu11	55.6^{d}
Ppu12	37.4^{f}
Ppu13	66.3^{bc}
Bm14	47.4^{e}
Bm15	38.2^{f}
Bm16	28.9^{i}
Bm17	26.0^{jij}
Bm18	36.7^{g}
Bm19	27.2^{i}
Pfl20	32.5^{h}
Pfl21	21.3^{k}
Pfl22	24.6^{j}

3.3- Iron solubilizing activities:

The results of Iron solubilizing efficiency test indicated that all P. fluorescense isolates had ability to dissolve iron from insoluble Magnetite Fe_2O_4 in modified basal medium**.** The maximum iron solubilization was recorded by isolate Pfl3 (41.92%) and significantly differ from other treatments, whereas Pfl16 was released the lowest amount (9.80%) of dissolved iron. The iron-dissolving ability of each isolate of microorganisms different. This specificity can be attributed to the different nature or concentration of metabolic products of each microorganism, or both of the factors .The action of the same microorganism on different minerals is dependent on the chemical and crystallographic characteristics of the mineral.

Table (3): Estimation of iron solubilization efficiency of isolated bacterial strains using king's B medium

Isolate code	Iron solubilization (clear zone (%)
Pfl1	15.13^{i}
Pfl2	18.27^{h}
Pfl3	41.92^{a}
Pfl4	23.05^{ef}
Pfl5	20.32^{fgh}
Pfl6	26.53^{e}
Pfl7	15.91^{i}
Pfl8	38.68^{b}
Pfl9	24.58^{i}
Pfl10	29.76^{d}
Pfl11	22.30^{fg}
Pfl12	21.98^{f}
Pfl13	19.43^{gh}
Pfl14	33.87^{c}
Pfl15	10.32^{j}
Pfl16	9.80^{j}
Pfl17	14.70^{i}
Pfl18	21.80^{fg}

3.5. Pot Experiment

3.5.1 Plant Growth

The result showed significant response of bio-fertilizers on growth and productivity of wheat (Table 4, 5). Combined application of bio-fertilizers caused considerable increase in plant length over all the treatments. Tillers number enhanced significantly due to application of biofertilizer either alone or in combination. Greater tillering was noticed when the crop received combined (T7) treatment than other treatments. Similar trend of results was also observed in case of yield components of wheat is spike number and 1000 grain weight (g) increased significantly when the crop received biofertilizers either alone or combined. Accordingly, the highest grain yields were recorded when the crop received combined bio-fertilizers. From the above results it may be stated that the use of iron and phosphate bio-fertilizers are beneficial in improving the growth and productivity of wheat. Corroborative findings have been reported by Sharma and Singh (2008) for wheat grown. As can be noticed in table (4), all the treatments improved root length over non-inoculated treatment. A significant variation in root length was observed in response to different bacterial inoculums. Co-inoculation

with *P. flourecense + p. putida +B. megatrium* (T7) produced the highest root length 58.95 cm; it means that this inoculation treatment caused 53% increase in root length in comparing with non-inoculated treatment (control). *P. flourecense* in combination with *P. putida and B. megatrium* was observed that the most efficient inoculums for enhancement of root length. Better root development may be attributed to synergistic relationship of the inoculated bacteria for enhancing root length; *pseudomonas flourecense* (T1) was the least effective inoculums but still it produced 6.8% longer roots as compared to the non-inoculated treatment (control).

Seed germination, root length, shoot length, fresh weight, dry weight, and vigor index were significantly increased by T. viride and P. fluorescense (Shanmugaiah et al., 2009). Similar to these results, increasing of plant root length by phosphate solubilizing strain alone also recorded by (Chaiharn and Lumyong, 2011, Bakhshandeh et al., 2017). The strains of P- solubilizing bacteria causes increase in root length and growth regulators production which are causing increase in nutrient and water absorption by plants or host plants(Gupta et al., 2002, Barea et al., 2005). Healthy roots help the plant for absorption of soil water and uptake of major plant nutrients as well as results in good yield. The increase in plant root size and number or depletion zone of infected roots are leading to increase in nutrient absorption and water stress of plants, which in turn could be the major factors improving plant growth (Bai et al., 2003).

The results also indicated that the inoculated plants with different inoculums showed shoot height ranging from (76.15cm) up to (99.60cm). All produced biofertilizer exhibited significant increase in shoot height of wheat over control, the maximum increase (99.60cm) was shown by co-inoculation of Ps.putida+ B.megatrium+Ps fluorescense, this increase may be attributed to the beneficial impact of bacterial inoculums in stimulating plant growth and increasing nutrient uptake, while the plants which treated by pseudomonas fluorescense alone was report the lowest value of shoot length (81.65cm).

Table 4: Effect of single and co-inoculation of (P. fluorescense, B. megatrium, P. Putida, iron biofertilizer, phosphor's biofertilizer) biofertilizer on the growth of wheat (Triticum aestivum spp) plants.

Treatments	Shoot height (cm)	Root length (cm)	Tillers number/5
T1	81.65^{c}	38.00^{f}	18.75^{cd}
T2	85.05^{c}	52.15^{d}	17.00 ^{de}
T3	85.15^{c}	52.50^{d}	18.75 ^{cd}
T4	84.45^{cd}	50.50^{e}	19.75 ^{bc}
T5	83.00^{de}	53.90^{c}	20.00 ^{b}
T6	89.75^{b}	53.35^{c}	20.25 ^{b}
T7	99.60^{a}	58.95^{a}	22.00^{a}
T8	84.90^{cd}	55.00^{c}	18.75 ^{cd}
T9	86.45^{b}	55.75^{c}	17.75 ^{de}
T10	86.5500^{ab}	56.10^{ab}	20.00 ^{b}
T11	76.15^{d}	36.45^{g}	16.75^{d}

***Similar letter or letters means non-significant difference.**

T1=P. Florence's T2= B.Megatrium

T3=P. Putida),T4=P. Flourence + putida,T5=P. Flourence+B.Megatrium,T6=P.putida+B.megatrium,T7=P.Flourence+P.putida+B.megatrium, T8=Iron biofertilizer,T9=Phosphorus biofertilizer, T10=iron+phosphorus biofertilize, T11=Control

Data regarding to tillers number as shown in table (4) revealed that application of prepared biofertilizer caused significant increase in tiller number compared with control treatment. The maximum tillers number/5 plant are (22) was recorded in *P. fluorescense + P.putida with B.megatrium* followed by *P.putida +B.megatrium* was (20.25) treatments.

The treatment in *P. fluorescense + P.putida with B.megatrium* combinations caused 31.34% increase in tillers. Here, control and *B.megatrium* showed nearly similar results (16.75 and 17) tillers. Tillering enhanced significantly due to application of biofertilizers either alone or in combination. Similar trend was found in yield components of wheat like 1000 grain weight (g) increased significantly when the crop received biofertilizers either alone or combined. (Singh and Prasad, 2011)

The combined application of biofertilizer with *Azospirrillum, Azotobacter* significantly increases the spikes, no of tillers, grain weight and spike per plants of wheat (Chauhan et al., 2011).

The seed yield values provided in table (5) showed that all the tested isolates had the capability to increase the seed yield of wheat significantly as compared to control treatment. The highest seed yield (4.12 ton/hectare) was obtained in pots which received *P. fluorescense* + *P.putida* +*B. megatrium* inoculation that were noticeably greater than any other treatments. While the control showed the lowest value (2.20 ton/hectare) . Inoculation with either microbe enhanced the yield in wheat but their interactive effect was more prominent. This is in agreement with the report of similar increase in plant seed yields due to inoculation of PSB strains were observed by (Pereira and Castro, 2014, Sarker et al., 2014, Sindhu et al., 2010).

These results are agreed and supported by several researchers Ali and Khandan (2013), Heidaryan and Feilinezhad (2015), Verma, and Sharma (2011)]. They indicated that in some species and strains of Bacillus, Pseudomonas. Several local contributions were achieved; out of them some were practically related to response of wheat to biofertilizers. (Majed, et al (2017) who reported that biofertilizers alone or in combination significantly increased yield of wheat and improve soil characteristics.

According to 1000 seed weight the results also indicated that the inoculated plants with different inoculums showed 1000 seeds weight ranging from (33.64gm) up to (38.75gm). All produced biofertilizer exhibited significant increase in 1000 seed weight of wheat over control, the maximum increase (38.75gm) was shown by co-inoculation of *Ps.putida+ Pse . fluorescense with B.megaterium*, this increase may be attributed to the beneficial impact of bacterial inoculums in stimulating plant growth and increasing nutrient uptake, while the plants which treated by iron biofertilizer alone was report the lowest value of 1000 seed weight (34.37gm). Carlier et al. (2008) observed a significant increase in several parameters of wheat crop under field conditions.

Growth promoting rhizobacteria have shown positive effect in many annual crops including wheat, maize and lettuce. PGPR influence plant growth in different ways by producing phytohormones like IAA and gibberellins, siderophore production, phosphate solubilization, synthesis of antibiotics, enzymes and/or antifungal compounds (Bharathi et al., 2004).

Table 5: Effect of single and co-inoculation of (*P. fluorescense, B. megatrium, P. Putida, iron biofertilizer, phosphor's biofertilizer*) biofertilizer on the 1000 Seed weight, Spike number /5 plant) and Yield (ton/hectare) of wheat (*Triticum aestivum spp*) plants.

Treatments	1000 Seed weight	Spike number /5 plant)	Yield (ton/hectare)
T1	35.25^{bc}	18.75^{ab}	3.35^{bc}
T2	34.65^{c}	16.00^{bc}	2.78^{cde}
T3	34.75^{c}	18.00^{abc}	2.80^{cde}
T4	36.00^{abc}	18.75^{ab}	3.00^{c}
T5	34.75^{c}	18.75^{ab}	2.82^{cde}
T6	35.75^{bc}	19.50^{ab}	3.25^{bc}
T7	38.75^{a}	20.00^{a}	4.12^{a}
T8	34.37^{c}	17.75^{abc}	2.99^{cd}
T9	35.62^{abc}	16.25^{bc}	2.94^{cd}
T10	35.25bc	18.00^{abc}	3.92^{b}
T11	33.64^{c}	15.00^{c}	2.20^{e}

***Similar letter or letters means non-significant difference.**

T1=Ps. Florence's T2= B.Megatrium

T3=Ps.Putida,T4=Ps.Flourence+ps.putida, T5=Ps.Flourence+B.Megatrium,T6=Ps.putida+ B.megatrium,T7=Ps.Flourence+Ps.putida+ B.megatrium,T8=iron biofertilizer

T9=Phosphorus biofertilizer, T10=Iron+phosphorus biofertilizer,

T11=Control

4.CONCLUSIONS

This study was conducted to study the effect of prepared biofertilizers from local bacterial strain and imported synthetic biofertilizer on wheat growth and yield. Results suggested that prepared inoculums have a positive effect on wheat plant growth when used as a single or combined inoculation. Specifically, inoculation increased several growth parameters (shoot height, root length, number of spike, tiller number, 1000 seed weight and total yield); the inoculation causes improved growth parameter compared to the non-

inoculated treatments. Combination Pseudomonas fluorescence, Pseudomonas putida and in combination with Bacillus megatrium was found highly effective and significant enhancement in the growth and yield of wheat. Based on our results, local isolates of bacteria can be used as biofertilizer to enhance soil fertility and plant growth.

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