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Co-inoculation effect of *Azospirillum brasilense* and *Bacillus megaterium* with phosphorus on wheat (*Triticum aestivum* L.) growth in calcareous soil

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ABSTRACT

Using microbial bio fertilizer has been widely used in recent years because it improves the plant growth and nutrients uptake A plastic pot experiment was carried out under plastic house at the College of Agricultural Engineering Sciences, University of Sulaimani, Bakrajo, from November 2021 to May 2022 to study the effect of co-inoculation of *bacteria A. brasilense and B. megaterium* bio fertilizer and their interaction on growth responses of wheat (*Triticum aestivumn* L.) at various P levels (0, 40, 80,120, and 160 kg P ha^{-1}). The experiment was performed in a factorial experiment with completed randomized design (CRD) in a silty clay soil with three replications. Before harvesting chlorophyll, intensity was measured, and after that plants were harvested. Results showed that co-inoculation was significantly effect on increasing chlorophyll intensity and plant dry biomass on wheat plant and nutrient uptake. The maximum value was 36.54 spad and 19.82 g pot-^{1} recorded at (160 kg P ha^{-1}) respectively, while the maximum value of wheat shoot N, and Zn nutrients contend was 27.97 g kg^{-1} and 45.44 µg g^{-1} respectively recorded at (160 kg P ha^{-1}), while the maximum value for P, K and Fe was 5.33 g kg^{-1}, 26.43 g kg^{-1} and 301.74 µg g^{-1} recorded at (120 kg P ha^{-1}). But when the soil inoculated with *A. brasilense* significantly increased chlorophyll intensity and plant dry biomass compared with control. The maximum value was 28.50 spad and 19.22 g pot^{-1} recorded at (160 kg P ha^{-1}), while the highest value for wheat shoot N, P, K Fe and Zn nutrients contend were 21.10 g kg^{-1}, 2.80 g kg^{-1}, 15.77 g kg^{-1}, 201.56 µg g^{-1} and 26.45 µg g^{-1} respectively recorded at (160 kg P ha^{-1}), and when the soil inoculated with *B. megaterium* increased chlorophyll intensity and plant dry biomass compared with control. The highest value was 18.50 spad and 16.31 g pot^{-1} recorded at (160 kg P ha^{-1})**.** while the maximum value for wheat shoot N, P, K and Fe nutrients were 20.26 g kg^{-1}, 4.43 g kg^{-1}, 17.83 g kg^{-1} and 276.74 µg g^{-1} respectively recorded at (160 kg P ha^{-1}), while the maximum value for Zn was 32.85 µg g^{-1} recorded at (120 kg P ha^{-1}).

تأثير اللقاح البكتيري الثنائي *Azospirillum brasilense* و *Bacillus megaterium* على استجابة نمونباتات الحنطة(*Triticum aestivumn L*) تحت مستويات مختلفة من الفسفور في التربة الكلسية

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الخلاصة

استخدام لقاح السماد الحيوي الميكروبي في الزراعة المستدامة جذبت اهتمامًا كبيرًا في السنوات الأخيرة لتحسين نمو النبات وامتصاص المغذيات. تم إجراء هذه التجربة في السنادين بلاستيكية في البيت البلاستيكي, كلية علوم الهندسة الزراعية جامعة السليمانية ، بكرجو ، خلال شهر تشرين الثاني2021 لغاية شهر مايس 2022 لدراسة تأثير اللقاح الثنائي البكتيري *A.brasilense* و *B.megaterium* و التداخل بينهما على استجابة نمو نباتات الحنطة (*Triticum aestivumn L*) تحت مستويات مختلفة من الفسفور (0,40,80,120, and 160 kg P ha^{-1}). تم تنفيذ تجربة عاملية مع التصميم العشوائي المكتمل (CRD) في تربة طينية غرينية بثلاث مكررات. وقبل حصاد التجربة تم قياس شدة الكلوروفيل, و بعد ها تم حصاد النباتات وثم تقدير الكتلة الحيوية الجافة للنباتات, و محتوى العناصر الغذائية الكبرى (Nو P، K) والصغرى (Fe وZn). أظهرت النتائج أن التلقيح الثنائي البكتيري كان له تأثير معنوي في نمو الحنطة وامتصاص العناصر الغذائية. وزادت شدة الكلوروفيل والكتلة الحيوية الجافة للنبات مقارنة بغير ملقحة وأعلى قيمة كانت spad 36.5 و 19.82 غرام سندانه-^{1}سجلت عند أعلى مستوى من الفسفور (160 كغم P هكتار$^{-1}$) حسب الترتيب. ووجد اعلى قيمة للعناصر الغذائية N و Zn في الوزن الجاف لنباتات الحنطة كانت 27.90غم كغم$^{-1}$ ، و 45.44 مايكروغرام غم$^{-1}$ حسب الترتيب. سجلت أعلى مستوى من الفسفور (160 كغم P هكتار$^{-1}$) و اعلى قيمة ل K,P و Fe كانت 5.33 غم كغم$^{-1}$ و 26.43 غم كغم$^{-1}$ و 301.74 مايكروغرام غم$^{-1}$ سجلت عند (120 كغم P هكتار$^{-1}$) وعند تلقيح نبات الحنطة باللقاح *A. brasilense* أدت إلى زيادة معنوية في شدة الكلوروفيل والكتلة الحيوية الجافة للنباتات الحنطة مقارنة بالنباتات غير الملقحة. أعلى قيمة كانت spad 28.50 و 19.22 غم سندانه $^{-1}$ تم تسجيله عند أعلى مستوى من فسفور (160 كغم P هكتار$^{-1}$) ، بينما اعلى قيمة لمحتوى العناصر الغذائية N ,P, K, Fe, Zn كانت 21.10 غم كغم$^{-1}$, 2.80 غم كغم$^{-1}$, 15.77 غم كغم$^{-1}$, 201.56 مايكروغرام غم$^{-1}$ و 26.45 مايكروغرام غم$^{-1}$ حسب الترتيب. سجلت في اعلى مستوى الفوسفور (160 كغم P هكتار$^{-1}$), وعند التلقيح التربة بالبكتريا *B. megaterium زادت* شدة الكلوروفيل والكتلة الحيوية الجافة لنباتات الحنطة مقارنة بالنباتات غير الملقحة. وكانت أعلى قيمة spad 18.50 و 16.31 غم سندانه $^{-1}$ حسب الترتيب سجلت عند أعلى مستوى من الفسفور (160 كغم P هكتار$^{-1}$) وجدت أعلى قمة للعناصر الغذاية N ، P ،K ، Fe، في نبات الحنطة كانت (20.66 غم كغم$^{-1}$ ، 4.43 غم كغم$^{-1}$ ، 17.83 غم كغم$^{-1}$ ، 276.74 مايكروغرام غم$^{-1}$ حسب الترتيب سجلت عند اعلى مستوى من الفسفور (160 كغم P هكتار$^{-1}$) بينما اعلى قيمة ل Zn كانت 32.85 مايكروغرام غم$^{-1}$ سجلت عند (120 كغم P هكتار$^{-1}$).

الكلمات المفتاحية: Biofertilizer , *Azospirillum brasilense , Bacillus megaterium ,* الفسفور, الحنطة.

INTRODUCTION

Nitrogen and phosphorus are the most important macro and essential nutrients required by both plants and microorganisms, that playing an important role, metabolic activities and limiting plant growth and yield (Korir et al., 2017). In temperate ecosystems N and P and deficiency limited plants growth and yield. Increasing using chemical fertilizer to increasing the growth and yield of crops by farmers with high energy costs destroying soil health and caused environmental pollution. So, to reduce this problem it can use microbial inoculum biofertilizer, such as using nitrogen-fixing *A.brasilense* andnsolubilizing bacteria *B. megaterium* biofertilizer. Those microbial may play an extremely important role in increasing N , P and other nutrients to the soil, and improve their growth, yield, mineral nutrition, soil fertility, soil health, reducing environmental pollution and eco-friendly fertilizer for increasing agriculture inductivity (Filiz., et al 2021)

Wheat (*Triticum aestivum* L.) is the most essential cereal crops which play a vital role in food nutritional security and is considered the main economically important source of food (Lastochkina et al., 2019) for human and animals. It is the most needful and strategic cereal crop in most countries of the world, and one of the sources of calories and protein. Approximately 85% of the global population depends on wheat (Pathak and Shrivastav, 2015). There are problems in Iraq-Kurdistan reign that there were high amount of $CaCO_3$ in soils which fixes the available phosphors and precipitates it as tri-calcium phosphate, in which soluble nitrogen sources leach readily or loosed by denitrification and volatilization. Since there are no detailed studies carried out on using phosphorus solubilizing bacteria *B. megaterium and* nitrogen-fixing *A. brasilense* inoculum as biofertilizer with wheat plant, especially in the Kurdistan region it is there for to pay attention to such studies in the local area.

MATERIALS AND METHODS

A pot experiment has been conducted from November 2021 to May 2022 in the plastic house, to study the effect of *A.brasilense* and *B. megaterium* biofertilizer and their interaction on the growth response of wheat (*Triticum aestivum L.*) at various P levels (0,40,80,120, and 160 kg P ha^{-1}) in calcareous soil, rock phosphate was used as a source of phosphorus. The soil was collected at 15- 30 cm depth from the agricultural farm of the College of Agricultural engineering Sciences /University of Sulaimani. The soil samples air-dried and sieved to pass through a 4 mm sieve. The soil has been classified as Vertisols according to USA soil taxonomy with silty clay texture (78.80 sand, 523.20 silt, and 398.00 clay g kg^{-1}. The soil has 7.37 pH, 260 g kg^{-1} of $CaCO_3$, 0.09 N g kg^{-1}, 3.10 P mg kg^{-1} of variable P, 31.20 g kg^{-1} of soluble K, 9.30 g kg organic matter and EC (1:1 soil; water) 0.14 dsm^{-1}. The plants were grown in plastic pots containing 5 kg of unsterilized soil was transferred into sterilized plastic post 25 cm diameter by 30 cm depth and five levels of P (0,40,80,120, and 160 kg P ha^{-1}) in the form rock phosphate added to all soil potted inoculated, and non-inoculated. The inoculated pots with *A. brasilense* were achieved by adding 2 ml of inoculum, part of the pots by added 2 ml of *B. megaterium* and 4 ml for interaction between them added to the planting holes in each pot, the sterilized wheat seeds were soaked in a suspension in each bacterium for 30 min. Then seven wheat seeds were planted in each pot’s treatment, and then the seeds were covered with few cm soil. The pots were arranged in plastic house benches in a completed randomized design (CRD) with three replicates per treatment. After germination, the seedlings were thinned to four per pot then the plants were grown under natural light in the plastic house. The pots were watered to maintain 70% of field capacity by a regular weighting of pots, during the study period. Before harvesting chlorophyll intensity SPAD was recorded and after 6 months of growth the plant was harvested and the shoot was separated from the root, and dried at 70 Cº for 72 hours and weighted to determine the plant biomass. The shoot dry weight ground to pass through (0.5 mm) sieve, and some of the macro (N, P, K) and micro (Fe, Zn) nutrients content were determined. The data were statically analysed by using XLSTAT version 12 software.

RESULTS AND DISCUSSION

Chlorophyll intensity (spad)

The effect of N fixing and P solubilizing bacteria and their interaction on chlorophyll intensity of wheat plants show in (Figure 1) which clearly explains a significant effect of co-inoculation increasing leaf chlorophyll intensity of wheat plants and increased with increasing P levels compared with control. The data in the (Figure 1) show that the maximum leaf chlorophyll intensity was observed in dual inoculation of *A. brasilense* with *B. megaterium* was 36.54 spad at (160 kg P ha^{-1}) compared to control which showed least chlorophyll intensity 8.27 spad recorded at lowest P level (zero kg P ha^{-1}). Also the results show that when the soil non-inoculated the maximum value of chlorophyll intensity was 15.57 spad recorded at (160 kg P ha^{-1}) while the minimum value of chlorophyll intensity observed 8.27 spad recorded at (zero kg P ha^{-1}) as shown in (Figure 1). The increasing of chlorophyll intensity by dule inoculation may be due to the ability of *A. brasilense* to increasing nitrogen in the soil by N_2-fixation and increasing more available P in the soil by solubilizing unavailable P to available P in soil by *B. megaterium*. The increasing N and P in the soil enhance ability of plant to uptake more N and P. This will help to increase plant growth and plant biomass which lead to increase in uptake more N which increase total chlorophyll content in wheat plants and increase green colour of plants and contains more N, P, K and S that help to build more chlorophyll rates which gave a significant value of chlorophyll intensity in wheat plants.

The result in (Figure 1) show that inoculation soil with *A. brasilense* affected significantly of increasing chlorophyll intensity of leaf wheat plants and increased with increasing P levels, as compared to control. The maximum value was observed for shoot chlorophyll intensity was 28.50 spad at (160 kg P ha^{-1}) compared to control which showed least leaf chlorophyll intensity 8.27 spad at (zero kg P ha^{-1}) However, in non-inoculated plant with *A. brasilense* ,the maximum value for chlorophyll intensity was 15.57 spad observed at (160 kg P h^{-1}) and the lowest value for chlorophyll intensity was 8.27 spad recorded at (zero kg P ha^{-1})., this result in agreement with (Zare Hoseini et al., 2015).The increasing chlorophyll intensity in wheat plants may be due to the important of bacteria *A. brasilense* which fixed nitrogen, that increase the available nitrogen to wheat plant and plant up take more nitrogen which increase chlorophyll intensity in plant and the beneficial effect of nitrogen on photosynthetic pigments which nitrogen is important and a part of chlorophyll structure and chlorophyll contents are one of the most important criteria to determine the health of the plant, because chlorophyll content are directly related to physiological activist to manufacture plant food (Galindo.,et al 2020)

The result in (Figure 1) show that when the soil inoculated with *B. megaterium* increase chlorophyll intensity on wheat plants but not significantly and chlorophyll intensity increased with increasing P level. The maximum value for chlorophyll intensity recorded at highest P level was 18.50 spad at (160 kg P ha^{-1}) while the minimum value 8.27 spad was obtained from control treatment. However, in non-inoculated plant with *B. megaterium*,the maximum value for chlorophyll intensity was 15.57 spad observed at (160 kg P h^{-1}) and the lowest value for chlorophyll intensity was 8.27 spad recorded at (zero kg P ha^{-1}),the results may be due to the increasing available P in the soil by bacteria *B. megaterium* which dissolve unavailable P to available in the soil which enhance plant growth and increased plant biomass which help plant to uptake more nutrients especially nitrogen which nitrogen fixation and increase chlorophyll intensity (Awasthi et al., 2011).

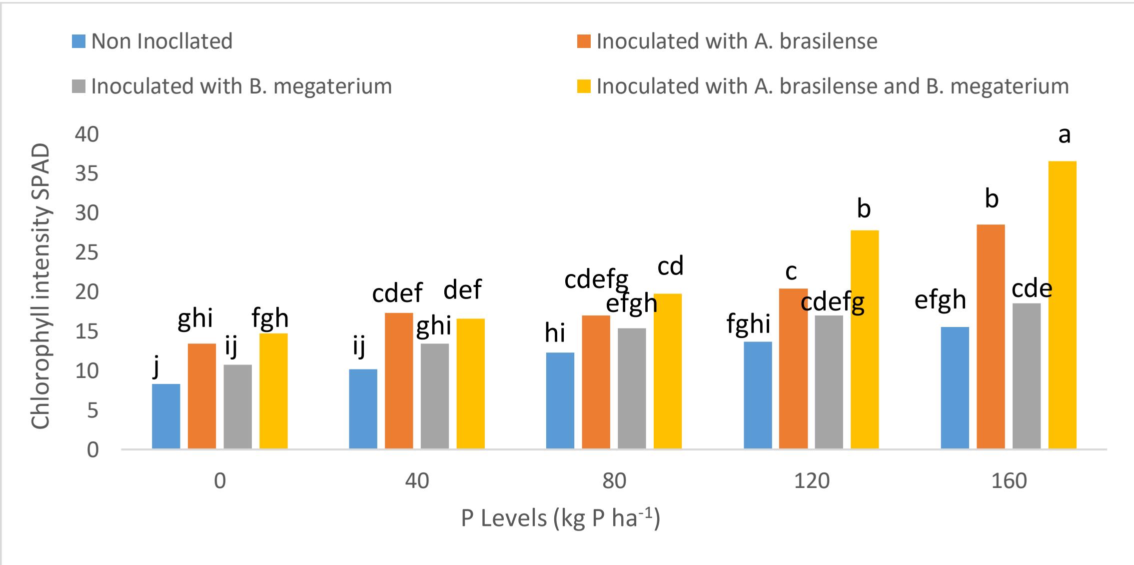
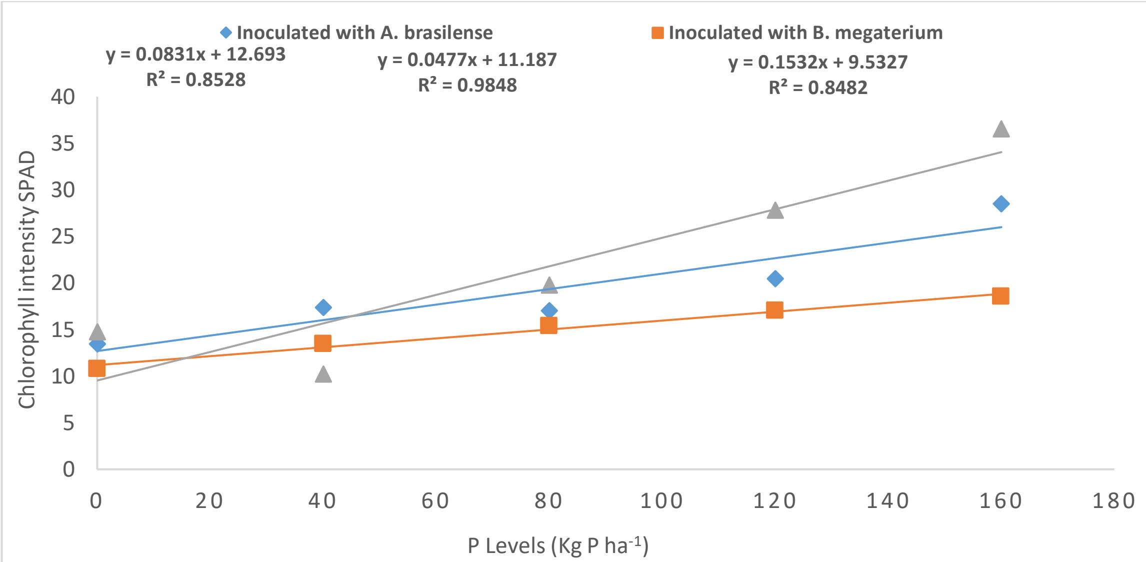


Figure (1): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat Chlorophyll Intensity spad at different phosphorus levels.



Figure(2): The relation between wheat Chlorophyll Intensity spad unit and phosphorus levels for wheat plant inoculated with *A. brasilense, B. megaterium* and their interaction on wheat plant.

Plant Biomass (g)

The result in (Figure3) clearly show that significant influence of co-inoculation of *A. brasilense* with *B. megaterium* on wheat dry biomass at different P levels compared to the control. Wheat plant dry biomass increased significantly by increasing P levels, the maximum value of wheat plant dry biomass was observed in dual inoculation was 19.82 g at (160 kg P ha^{-1}), compared with control which showed least wheat dry biomass 9.89 g recorded at (zero kg P ha^{-1}) This result in agreement with (Pan et al., 2019). The increasing at wheat dry biomass which observed in dual inoculation may be due to the ability of *A. brasilense* to fix-nitrogen and increase available N to plant which enhance plant to uptake more N, and the role of bacteria *B. megaterium* to dissolve rock phosphate in the soil by produce different organic acid which convert unavailable P to available (Rawat et al., 2021) and produce growth promoting by increasing the ability of plant to uptake more P in the soil (Puri et al., 2020). Increase N and P enhance more uptake by wheat plant which increase root and shoot growth result increasing plant biomass.

The results in (Figure 3) show the effect of *A. brasilense* on wheat dry biomass at different P levels. The result show that inoculation wheat plants with *A. brasilense* significantly affected on increase wheat dry biomass and increased by increasing P levels as compared to control treatment. There were significant differences between wheat dry biomass at different P levels. This result is in agreement with the result of (Contreras-Angulo et al., (2019). The maximum value for dry biomass of wheat plant was 19.22 g observed at (160 kg P ha^{-1}), while the minimum value of wheat dry biomass recorded in control treatment was 9.89 g (zero kg P ha^{-1}). The increasing of wheat dry biomass may be due to the important of *A. brasilense* to improve plant growth due N fixation which supply N that important for photosynthetic rate and increasing plant growth due to improving root and shoot dry biomass result increasing plant dry biomass.(Galindoe et al., 2022).

Result in (Figure 3) show that when wheat plants inoculated with P solubilizing bacteria *B. megaterium* was observed significantly increase wheat dry biomass with increasing P levels compared to control. There were significantly differences observed on wheat dry biomass at different

P level. The maximum value for wheat dry biomass observed at (160 kg P ha^{-1}) was 16.31 g, but minimum value was 9.89 g obtained from control treatment. But when the soil was non-inoculated with *B. megaterium* significantly affected on increasing wheat dry biomass at different phosphorus level and wheat plant biomass increased by increasing phosphor levels. The highest value was 15.57 g recorded at (160 kg P ha^{-1}) compared with control which the lowest value 9.89 g recorded at (zero kg P ha^{-1}) The increasing of wheat dry biomass when inoculated wheat plant with *B. megaterium* may be due to the ability at *B. megaterium* to increasing available P in the soil by dissolving unavailable phosphor (rock phosphate) to available P by supply different organic acids and lowering pH of the soil which help to dissolve rock phosphate in the soil which convert unavailable P to available P and help plant to adsorb more P which increase root and shoot biomass of wheat plant result increase wheat dry biomass (Billah et al., 2019).

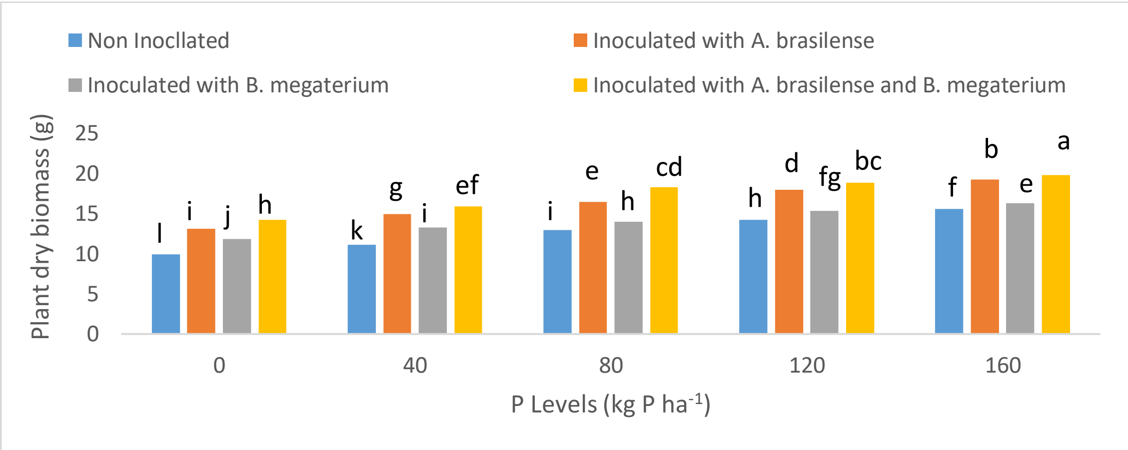


Figure (3): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat dry biomass g at different phosphorus levels

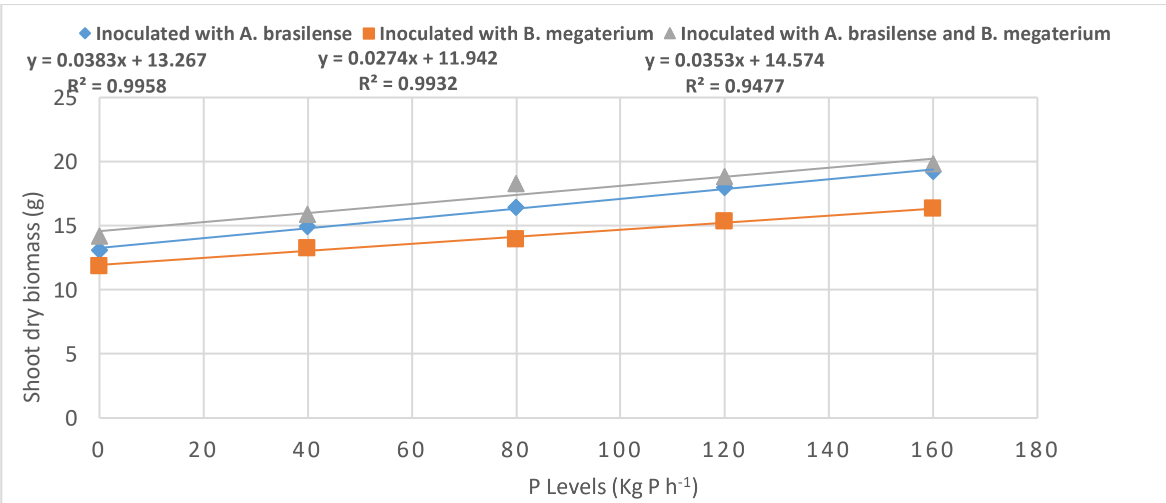


Figure (4): The relation between wheat dry biomass g and phosphorus levels for wheat plant inoculated with *A. brasilense, B. megaterium* and their interaction.

Shoot Nitrogen Content (g kg^{-1})

From (Figure 5) it seems that the effect of co-inoculation wheat plants with *A. brasilense* and *B. megaterium* clearly evidence effect on shoot nitrogen content in plants, N content was significantly increased, compared with control, and N content increased by increasing the rate of applied P. There was a significant differences recorded between co-inoculation plants treatments compared to (control).The maximum value for nitrogen content 27.97 g kg^{-1} was recorded at (160 kg P ha^{-1}) while the lowest value 10.97 g kg^{-1} was recorded at (zero kg P ha^{-1}) in control treatment. However, in non-inoculated plant with *A. brasilense* and *B. megaterium* ,the maximum value for shoot N content was 13.90 g kg^{-1} observed at (160 kg P h^{-1}) and the minimum value for shoot nitrogen content (g kg^{-1}) was 10.97 g kg^{-1} recorded at control (zero kg P ha^{-1}). This result was in agreement with (NAMIL., et al 2017). The increasing of N concentration in wheat plants in co-inoculation may be due to the important of co-inoculation of *A. brasilense* which supply N to the plant by N_2 fixation and *B. megaterium* for caution of P which enhanced plant growth *B. megaterium* can also produce enzymes and organic acids to solubilize rock phosphate which increase more available phosphorus in the soil solution which enhance plant growth result increase the ability of plant to uptake more nitrogen so co-inoculation also contributes to enhanced nutrient uptake by plant this leading to more nitrogen content in plant (NAMLI et al., 2017).

The results in (Figure 5) show the effect of bacteria *A. brasilense* on nitrogen concentration in wheat plant. Plant N content was significant increased compared with (control), and N content increased by increasing application of P levels. Plant N content varied from 21.10 g kg^{-1} observed in highest P level (160 kg P ha^{-1}) compared to (control) was 10.97 g kg^{-1} which recorded at minimum P level (zero kg P ha-1) However, in non-inoculated plant with *A. brasilense* ,the maximum value for shoot nitrogen content g kg^{-1} was 13.90 g kg^{-1} observed at (160 kg P h^{-1}) and the lowest value for shoot N content was 10.97 g kg^{-1} recorded at (zero kg P ha^{-1}).this results was in agreement with the result which found by (Ahmed et al., 2020). The increasing of N content in wheat plant when inoculated with *A. brasilense* may be due to the important of *A. brasilense* in fixing N (Zahid et al., 2015) which supply more available N to soil and uptake by plant . Or This may be due to the activity of *A. brasilense* to production of phytohormones like IAA, Cytokinins, Auxin, Ethylen, Gibberellic acid, and plant growth regulatory substances such polyamines, and osmotic stress in plant which improve plant growth and development, increase plant biomass which enhance plant to uptake more N in the soil and accumulated in plant that increase N content (Ramos-Ibarra et al., 2019)

The result in (Figure 5) illustrates that the inoculated wheat plants with *B. megaterium* increased N content compared with control treatment. The highest value of N content was observed in wheat plant was 20.26 g kg^{-1} at (120 kg P ha^{-1}), and the minimum value of nitrogen content obtained in wheat plants in non-inoculate (control) was 10.97 g kg^{-1} at (zero kg P ha^{-1}). However, in non-inoculated plant with *B. megaterium*, the maximum value for shoot nitrogen content was 13.90 g kg^{-1} observed at (160 kg P h^{-1}) and the lowest value for shoot nitrogen content was 10.97 g kg^{-1} recorded at (zero kg P ha^{-1}).This result was in agreement with the result obtained by (Yadav., 2019). It may be due to the fact that bacteria *B. megaterium* takes the important role in secreting many growth regulators, organic acids that increase the concentration of nutrients in the soil, including N, which positively affected plant growth and increased the ability of wheat plant to uptake more nitrogen (Di et al., 2022) or it may be due to the ability of bacteria *B. megaterium* to excreting different organic acids which dissolve rock phosphate, resulting in raised supply of available P in the soil solution that help in increasing N_2-fixation by *A. brasilense* and improved mineral nutrients uptake, especially available nitrogen by wheat plant leading to increase nitrogen concentration in plant (Mohammed , 2017).

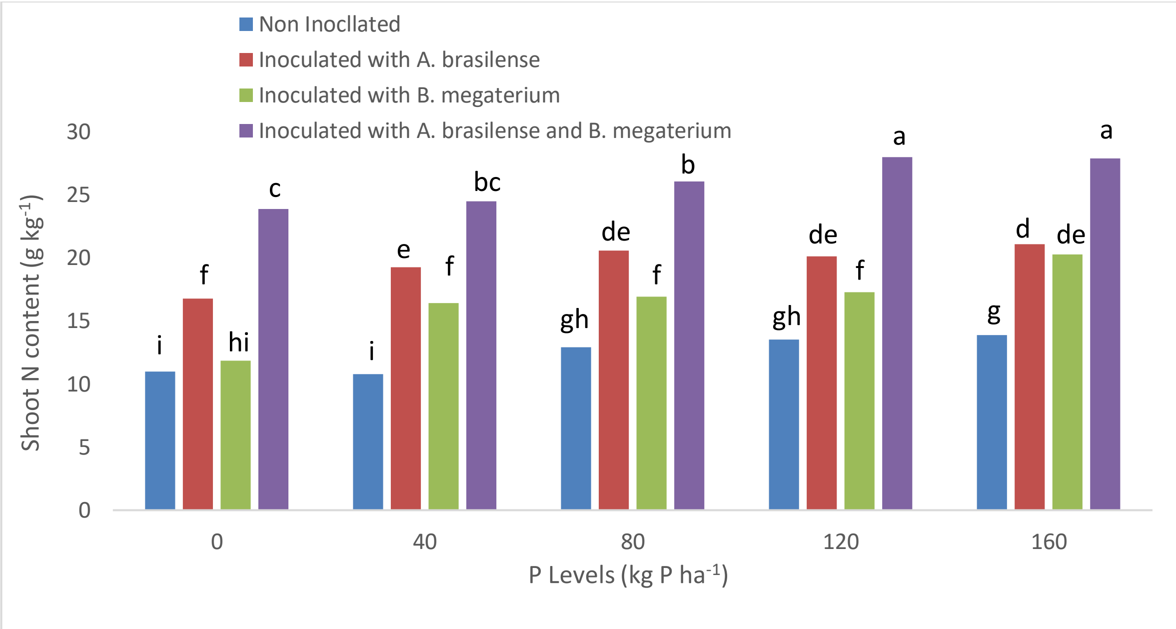


Figure (5): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat shoots N content g kg^{-1} at different phosphorus levels.

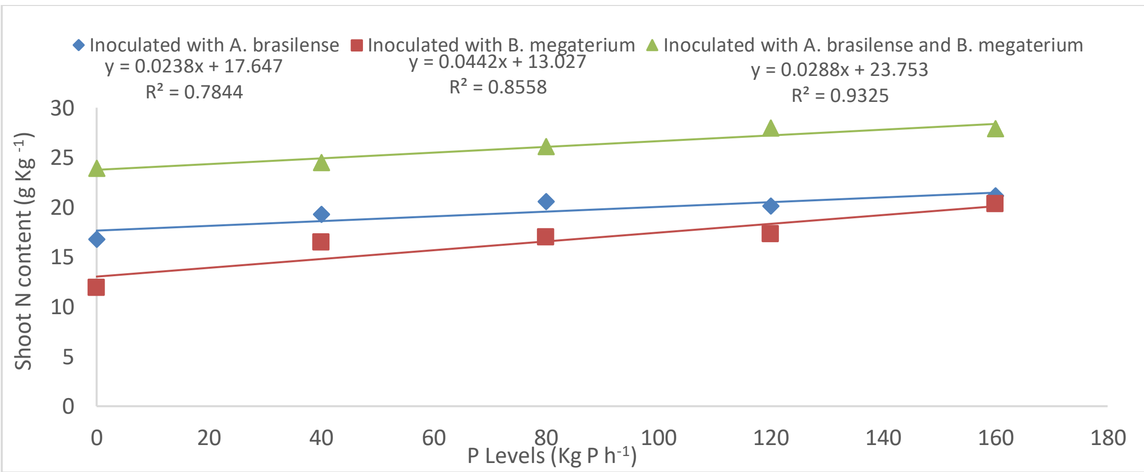


Figure (6): The relation between wheat shoot N content g kg^{-1} and phosphorus levels for wheat plant inoculated with *A. brasilense, B. megaterium* and their interaction on wheat pant.

Shoot Phosphorus Content (g kg^{-1})

The data from (Figure7) indicate that co-inoculation wheat plant with *A. brasilense* and *B. megaterium* significantly increase phosphorus content in plant compared with non-inoculated plant (control) and the amount of phosphorus content in plant increase by increasing the rate of phosphorus levels. The treatment of co-inoculation showed maximum phosphorus content was 5.33 g kg^{-1} recorded at (120 kg P ha^{-1}) and the lowest value was 1.07 g kg^{-1} recorded at (zero kg P ha-1).However , in non-inoculated plant with *A. brasilense* and *B. megaterium*,the maximum the value for shoot phosphorus content was 2.77 g kg^{-1} observed at (160 kg P h^{-1}), and the lowest value for shoot phosphorus content was 1.07 g kg^{-1} recorded at (zero kg P ha^{-1}). This result was in agreement with (Ahmed, 2020).

The increasing of shoot phosphorus content in co-inoculation in wheat plants may be due to the ability of *A. brasilense* to improve plant growth by supply nitrogen due N2-fixation (Ahmed et al., 2020).and important of *B. megaterium* to dissolve rock phosphor by secretion different organic acids and lowering of soil PH which increase more available phosphorus in soil solution and more uptake by plant (Rawat et al., 2021) while nitrogen and phosphorus are two major important macro nutrients essential for plant growth result accumulated more phosphorus in shoot plants. Result in (Figure 7) reveal that inoculated wheat plants with *A. brasilense* affected for increasing phosphors concentration in wheat plants but not significantly at varying rate of phosphors compared to the non-inoculated plants and phosphorus concentration non significantly increase by increasing the rate of phosphorus levels. This result was in agreement with the result which found by (Fattah, 2009) .The maximum value for phosphorus concentration in wheat plant recorded at (160 kg P ha^{-1}) was 2.80 g kg^{-1} and the minimum value was 1.07 g kg^{-1} recorded at (control) (zero kg P ha^{-1}). However, in non-inoculated plant with *A. brasilense* ,the maximum value for shoot phosphorus content g kg^{-1} was 2.77 g kg^{-1} observed at (160 kg P h^{-1}) and the lowest value for shoot phosphorus content was 1.07 g kg^{-1} recorded at (zero kg P ha^{-1}).The increasing of shoot phosphorus content in wheat plant which inoculated with *A. brasilense* may be due to the important of phosphorus in the soil which help N2-fixation and increase available nitrogen, to plant while nitrogen and phosphors important nutrients essentials for plant growth high biomass and more uptake phosphorus in the soil by plants resulting more phosphorus concentration in plant (Andersen and Andersen, 2006).

The obtained results_in (Figure 7) showed that the phosphorus concentration significantly increased on wheat plants when inoculated with *B. megaterium* compared with control treatment, the phosphorus content in plant increased significantly by increasing the rate of phosphorus application. This result was in agreement with the result which found by (Mahammed, 2017;Ahmed, 2020) and (Rawat et al., 2021). The heights value for P concentration in wheat plant when inoculated with *B. megaterium* was 4.43 g kg^{-1} recorded at (160 kg P ha^{-1}) while the lowest value was 1.07 g kg^{-1} recorded at (zero kg P ha-1).However, in non-inoculated plant with *B. megaterium*, the maximum value for shoot phosphorus content was 2.77 g kg^{-1} observed at (160 kg P h^{-1}) and the minimum value for shoot phosphorus content was 1.07 g kg^{-1} recorded at (zero kg P ha^{-1}). The increasing of shoot phosphorus content in wheat plants when inoculate with *B. megaterium* may be due to *B. megaterium* perform a significant role in soil by their metabolic activates and is a remarkable part of integrated nutrient management in the soil, as they improved the plant nutrient acquisition from the soil (Rajwar et al., 2018). Or maybe due to the ability of *B. megaterium* to produce growth promoting like, Cytokinin's, Auxin, Gibberellic acid, which promote plant growth, and increased the ability of plant to uptake more phosphorus in the soil result increase phosphorus in the plant. (Puri et al., 2020).Or it may be due to the bacteria *B. megaterium* have the ability to produce different organic acids like oxalic acid, malic acid, propionic acid, gluconic acid, lactic acid, tartaric acid, citric acid, and lactic acid (Do Carmo et al., 2019, Chawngthu et al., 2020). These exuded acids, which solubilize inorganic P by chelation of cation bound to P, reducing soil pH, complexation with metal ions bound to phosphorus, and challenging phosphorus for adsorption site (Kishore et al., 2015).

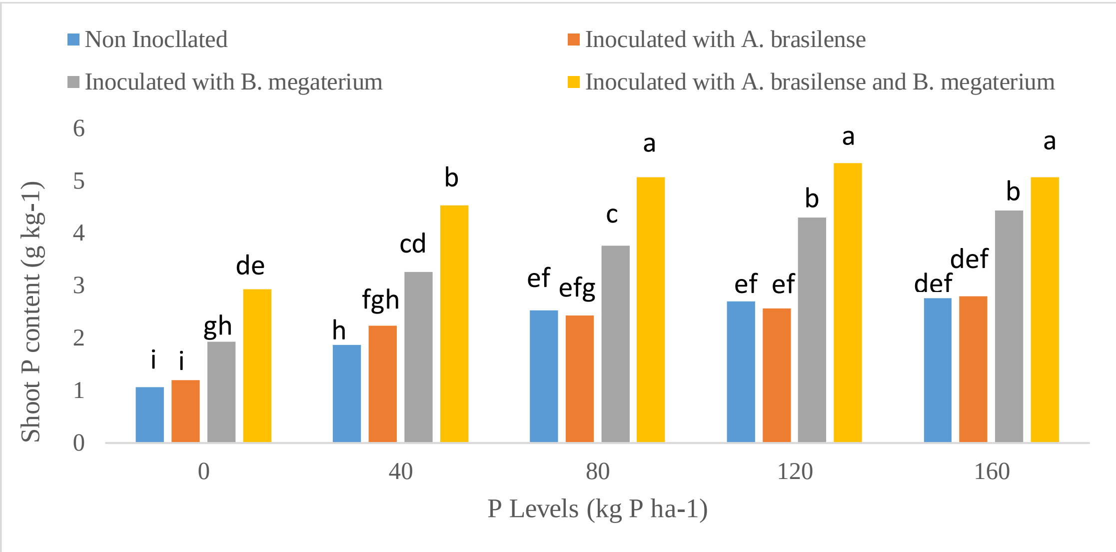
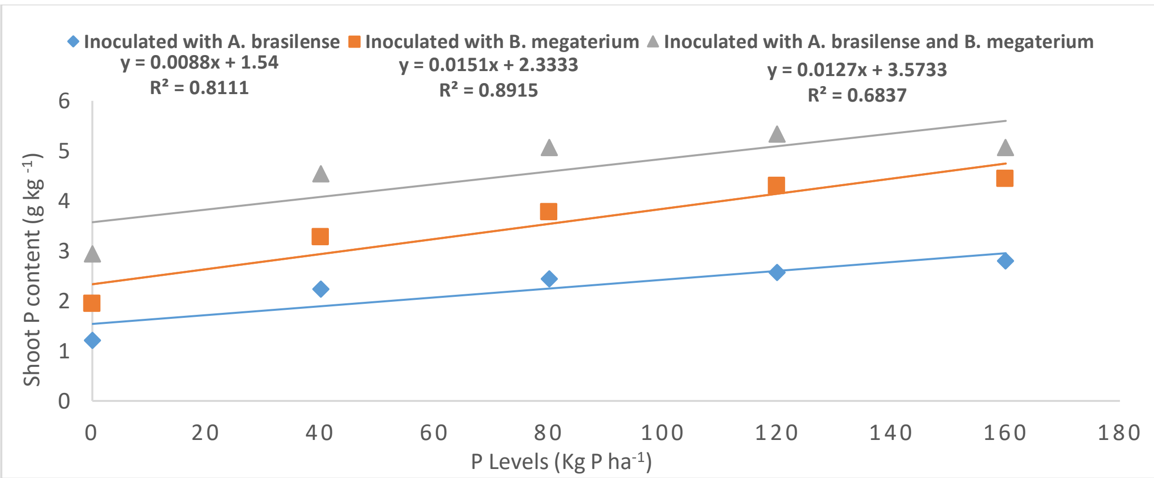


Figure (7): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat shoots P content g kg^{-1} at different phosphorus levels.



Figure(8): The relation between wheat shoot P content g kg^{-1} and phosphorus levels for wheat plant inoculated with *A. baselines* , *B. megaterium* and their interaction on wheat plant

Potassium content (g kg^{-1})

Results given in (figure 9) showed that co-inoculation wheat plants with *A.brasilense* and *B. megaterium* significantly increased K content on wheat plants compared with (control) and there are significantly differences recorded of K contend at various P levels between co-inoculated treatment and other single inoculated treatment. This results in agreement with the result of (Ahmed, 2020 .The highest value of K shoot content was 26.43 g kg^{-1} recorded at (120 kg P ha^{-1}) while the lowest value was 11.87 g kg^{-1} recorded at (zero kg P ha^{-1}).The results showed that co-inoculation caused a positive promoting effect to all treatments on K content on wheat plants this may be due to the important of N and P which supply by co-inoculation while nitrogen and phosphorus are the two major elements which supply to the soil by nitrogen fixation and by dissolving rock phosphate which improve plant growth and increase activity of wheat plants and increased biomass result increasing the ability of wheat plant to uptake more K and accumulated on shoot plant (Fattah, 2009). The obtained data from (Figure 9) demonstrated that inoculation wheat plants with bacteria *A. brasilense* significantly affected on increased K content on wheat plants at different P levels and it increased by increasing the rate at phosphors level. the result show that there was no significant difference of K content between treatments at different P levels compared to control treatment, from the data revealed in (Figure 9) the highest value for K content was 15.77 g kg^{-1} obtained at highest P level (zero kg P ha^{-1}) when compared to control treatment 11.87 g kg^{-1} obtained at (160 kg P ha^{-1}).In this experiment it was observed that when the soil non-inoculated the highest value for K content in wheat plant 13.23 g kg^{-1} recorded at (160 kg P ha^{-1}) compared with control treatment 11.87 g kg^{-1}, and K content increased by increasing P levels but not significantly this result agreement with the result discovered by (Fattah, 2009). The increasing of K content in wheat plants when inoculated with *A. brasilense* may be due to the potassium solubilizing microbes could be dissolve potassium from insoluble forms and converted to available form which directly uptake by plants producing different organic acids (Teotia et al., 2016) or may due to the role of bacteria *A. brasilense* to supply available nitrogen to the soil by N_2-fixation and uptake by plants and nitrogen which enhance plant growth and high plant biomass which increase the ability of plant to absorber mineral nutrition such K and accumulated in shoot plant.(Besen et al., 2020).

The data from (Figure 9) inoculation at wheat plant with bacteria *B. megaterium* significantly increase the concentration of K in wheat plants compared to control of different P levels only in lowest P level (zero kg P ha^{-1}) which are no significantly, the result show that concentrate of K in wheat plant increased with increasing P levels. This results an agreement with the result which found by (Fattah., 2009).The highest K concentration in wheat plants was 17.83 g kg^{-1} recorded at highest P level (160 kg P ha^{-1}) compared with control treatment was 11.87 g kg^{-1} recorded at (zero kg P ha^{-1}). This results in agreement with the result which found by (Mohammed, 2017). the increasing K content in wheat plants when inoculated with *B. megaterium* may be due to the ability of bacteria *B. megaterium* to increase P in the soil by dissolving rock phosphate which increase plant growth and nutrient uptake significantly in wheat crop or accumulated in plant and may be due to the of potassium solubilizing bacteria activity to dissolve an available K to available and up take by plant and increasing K and P in the soil increased plant growth and biomass which plant able to uptake more K in the soil , and accumulated in plant (Rajawat et al., 2019)

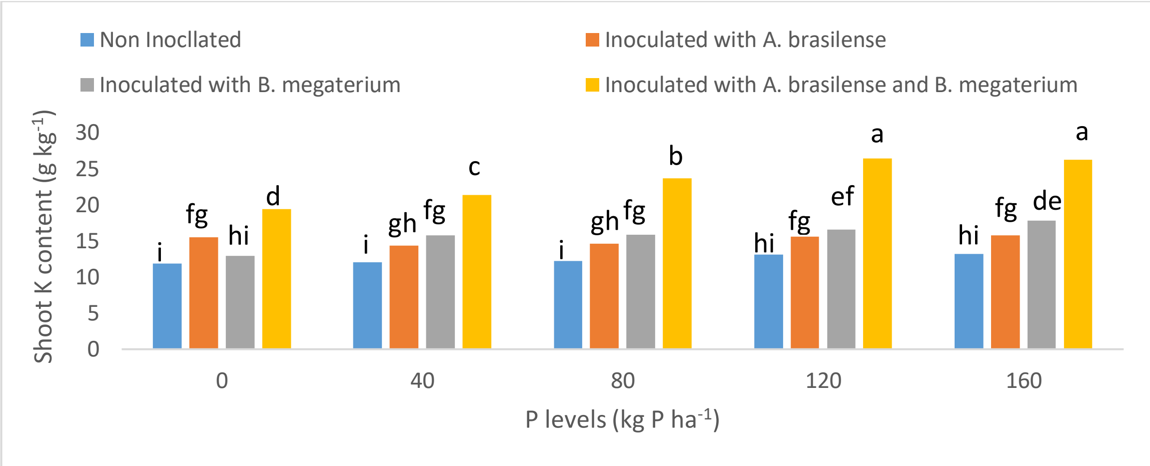


Figure (9): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat potassium content at different phosphors levels.

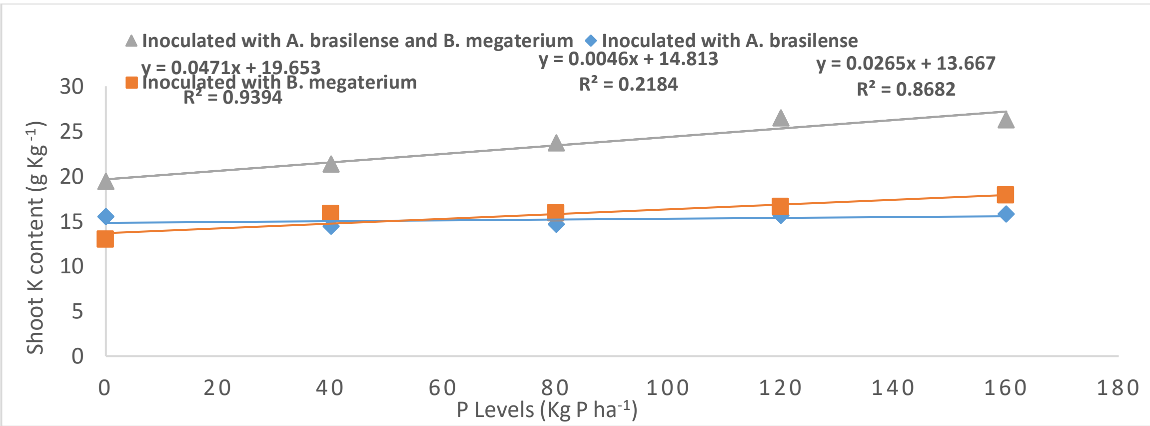


Figure (10): The relation between wheat potassium content g kg-1 and phosphor levels for wheat plant inoculated with *A. brasilense, B. megaterium.*

Zinc Content (µg g^{-1})

The result in present study in (Figure 11) clearly indicates that co-inoculation wheat plants with *A. brasilense* and *B. megaterium* was significantly increase zinc µg g^{-1} concentration over all treatments at different phosphorus levels and increased by increasing the rate of phosphors application. In co-inoculation treatment the maximum value obtained was 45.44 µg g^{-1} at (160 kg P ha^{-1}) and the lowest value 14.53 µg g^{-1} recorded in control treatment at (zero kg P ha^{-1}), but in non-inoculated treatments maximum value for Zn concentration was 23.52 µg g^{-1} recorded at (160 kg P ha^{-1}) compared with control was 14.53 µg g^{-1} recorded at (zero kg P ha^{-1}). The increasing of zinc concentration in co-inoculated treatments may be due to the role of bacteria *A. brasilense* to fix nitrogen and supply available nitrogen which absorb by wheat plant. (Ahmed, 2020) or may be due to the role at *B. megaterium* dissolve an-available P in the soil and make it available by supply various organic acid, and lowering soil pH and biochemical phosphate mineralization (Chen et al., 2021), result nitrogen and phosphors are two major macro essential nutrients for improving plant growth that increase the uptake of nitrogen and phosphorous by wheat plant which enhanced the ability of wheat plant to uptake more nutrients especially Zn and accumulated in shoot plant (Salomi et al., 2012). The obtained result in (Figure 11) show that the zinc content on shoot wheat plant, increased when inoculated with *A. brasilense* at different phosphors levels but not significantly, and the Zn content increase by increasing the rate of phosphorus application. The maximum value of Zn content 26.45 µg g^{-1} was obtained from (160 kg P ha^{-1}), while the lowest Zn content in wheat plant 14.53 µg g^{-1} was obtained from control at (zero kg P ha^{-1}), but when the soil non-inoculated with *A. brasilense* the maximum value for Zn content was 23.52 µg g^{-1} recorded at (160 kg P ha^{-1}), compared to control treatment 14.53 µg g^{-1} was recorded at (zero kg P ha^{-1}). This results in agreement with the result which found by (Ahmed , 2020). The increasing of wheat Zn content may be due to important of bacteria *A. brasilense* to fix nitrogen which caused increase of availability nitrogen to the plant which enhances plant growth and plant biomass which uptake more Zn in the soil and accumulated in shoot plant (Fattah, 2009). And by production of phytohormones like IAA, cytokinin, auxin and gibberellin and plant growth regulatory which enhance plant growth and increase the mineral uptake like Zn and accumulation in shoot plant (Moreira et al., 2022)

Results in present study show in (Figure 11) clearly indicates that inoculated wheat plant with bacteria *B. megaterium* significantly affected on increasing Zn concentration at varied phosphorus levels and Zn content increased by increasing the rate of phosphorus levels, the maximum Zn content was 32.29 µg g^{-1} obtained in (160 kg P ha^{-1}) as compared to control treatment was 14.53 µg g^{-1} recorded at (zero kg P ha^{-1}) but in non-inoculated plants the maximum value of Zn content was 23.52 µg g^{-1} recorded at (160 kg P ha^{-1}) and the minimum value was 14.53 µg g^{-1} recorded in control treatment at (zero kg P ha^{-1}). The increasing of Zn concentration in wheat plants may be due to the ability of bacteria *B. megaterium* to dissolve rock phosphate by secreting enzymes and different organic acids which convert fixed Zn into available for plant resulting in elevated a concentration of Zn in the plant (Mohammed, 2017) and (Sammauria et al., 2020) or may be due to the supply different organic acids by bacteria *B. megaterium* which decreased soil pH which raised the availability of Zn in the soil and more uptake by plant (Khan et al., 2019) and (Mohammed, 2017).

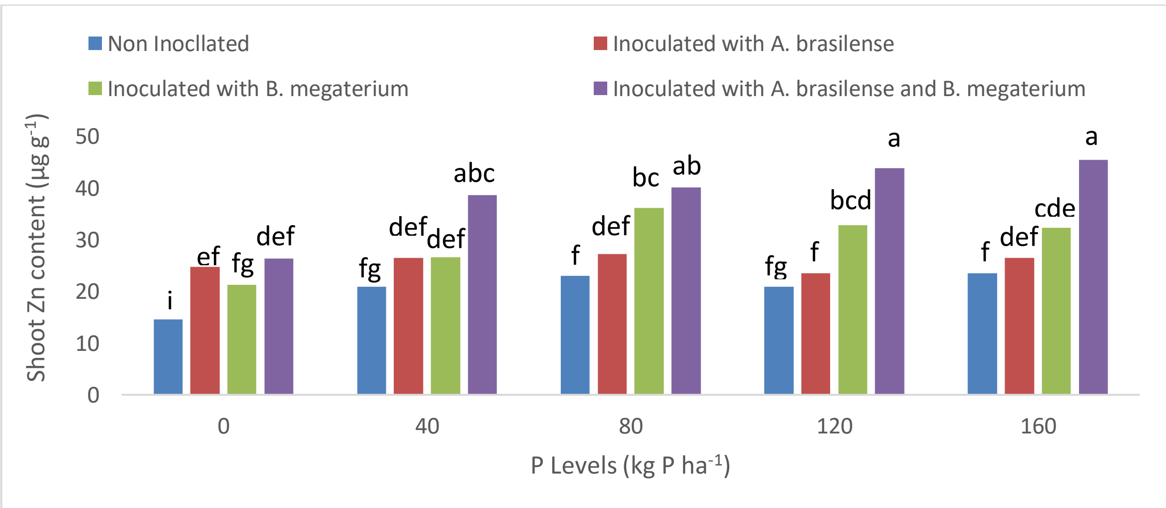


Figure (11): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat zinc content at different phosphorus levels.

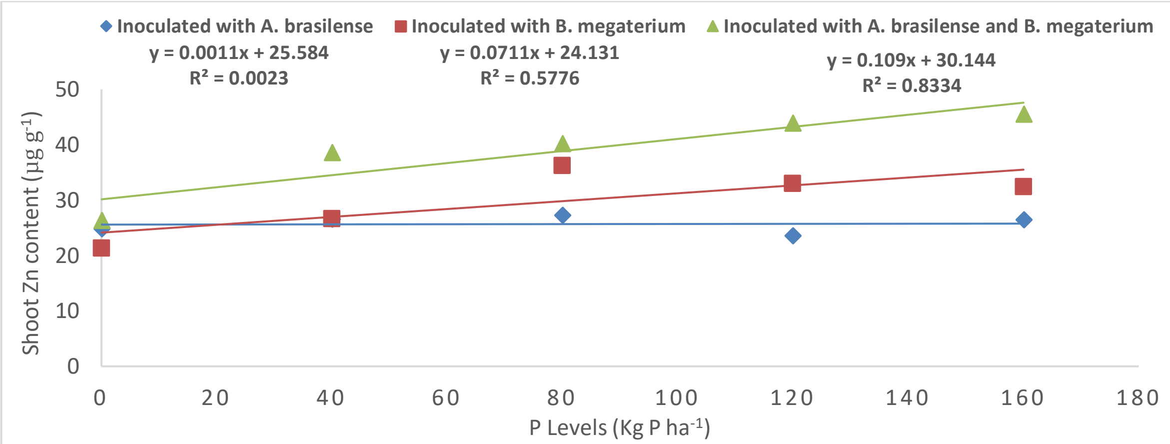


Figure (12): The relation between wheat zinc content (µg g^{-1}) and phosphorus levels for wheat plant inoculated with *A. brasilense, B. megaterium* and their interaction on wheat plant.

Shoot Iron Content (µg g-1)

The results in (Figure 13) show that there was a significant effect of co-inoculation plants with *A. brasilense* and *B. megaterium* on Fe concentration in wheat shoot plants at various P levels compared with control treatment, and Fe content increased by increasing p levels, and the result indicate that there are non-significantly differences recorded between treatments at various P levels. The maximum value for Fe content in wheat plants was 301.74 µg g^{-1} recorded at (120 kg P ha^{-1}) compared with the control, the minimum value was 164.35 µg g^{-1}) recorded at (zero kg P ha^{-1}). This results in agreement with the result which found by (Song et al., 2021)). But when the soil non-inoculated the maximum Fe content recorded at (160 kg P ha^{-1}) was 175.97 µg g^{-1} while the lowest value 164.35 µg g^{-1} was recorded at control treatment (zero kg P ha^{-1}). The increasing of Fe content in co-inoculate treatments may be due to the ability of *A. brasilense* to increase available N to plants by nitrogen fixation and role of *B. megaterium* to increase available P in the soil due to production different organic acid and acid phosphatase play a major role of mineralization of rock phosphate in the soil, so nitrogen and phosphor the two macro nutrients essential which promote plan growth. Result increase the ability at wheat plant to uptake more macro and micro nutrients especially Fe and accumulated in shoot plants (Shah ea al., 2022) The results of the inoculation are shown in(Figure 13) according to the results, which observed the concentration of Fe content on wheat plants increased at different phosphors levels when wheat plants inoculated with *A. brasilense* increased Fe content compared to non-inoculated plants (control). Results also indicated that there were no significantly differences on wheat plant Fe content response when wheat plants inoculated with *A. brasilense*. this results was in agreement with the result which found by (Housh et al., 2021).The highest value for Fe content on wheat plants 201.56 µg g^{-1} was recorded at (160 kg P ha^{-1}) compared to control treatment 164.35 µg g^{-1} was recorded at (zero kg P ha^{-1}). The increasing of Fe concentration in wheat plants were inoculated with *A. brasilense* may be due to the important of Fe in the structure of nitrogenise enzyme that necessary for N fixation which supply available N to the soil, lead to increase plant growth and uptake more nutrients such Fe and increased in shoot plants. Or it may be due to production of the phytohormones and plant growth regulation substance by bacteria *A. brasilense* which help plant growth and increase Fe uptake and accumulation in plant (Housh et al., 2021). The data presented in (Figure 13) showed that when the soil inoculated with *B. megaterium* the concentration of Fe in shoot plants significantly increase at different phosphors levels and Fe content significantly increased by increasing P levels but non-significantly increase in minimum P levels (zero kg P ha^{-1}). the maximum value of Fe content obtained in maximum phosphorus level was 276.74 µg g^{-1} compared with control treatment 164.35 µg g^{-1} at (zero kg P ha^{-1}). This results agreement with the result which found by (Fattah, 2009) the increasing of Fe content in wheat plant when inoculated with *B. megaterium* may be due to the role and ability of bacteria to dissolve rock phosphate and convert unavailable P to available P in the soil or by mineralization of P and become available in the soil which increase the amount of available P uptake by the plants which increase Plant growth results increase ability of Plants to up take more Fe in the soil (Etesami et al., 2021).

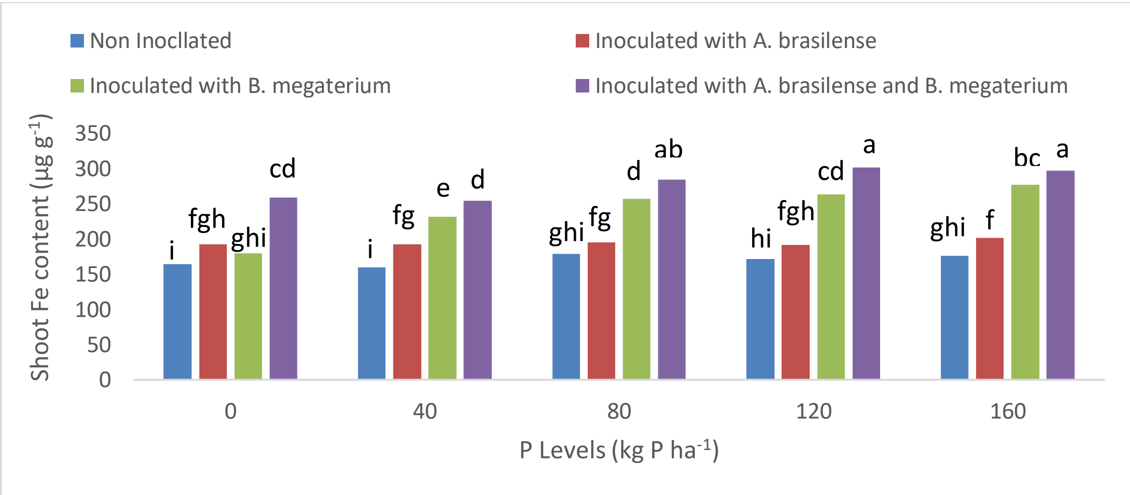


Figure (13): Effect of *A. brasilense and B. megaterium* inoculated and non-inoculated and their interaction on wheat shoot iron content µg g^{-1} at different phosphorus levels.

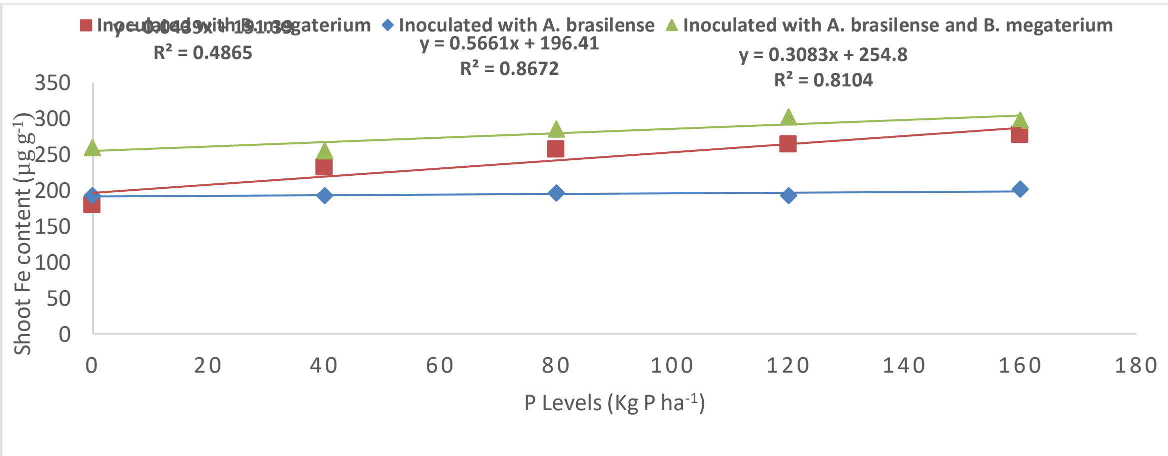


Figure (14): The relation between shoot iron content (µg g^{-1}) and phosphorus levels for wheat plant inoculated with *A. brasilense, B. megaterium* and their interaction on wheat plant.

CONCLUSIONS

Using microbial inoculum may play an extremely important role in increasing nutrients to the soil, and improve their growth, yield, mineral nutrition, , reducing environmental pollution and eco-friendly fertilizer for increasing agriculture inductivity. According to the results of this study the following conclusions can be made. Inoculation wheat plant with *A. brasilense* presented the positive effects on plant growth and nutrient uptake. Application of PSB *B. megaterium* by inoculating in soil appears to be efficient to convert the insoluble P compounds to plant available and co-inoculation wheat plant with *A. brasilense* and *B. megaterium* had greater effect in enhancing plant growth and nutrient uptake compared to *A. brasilense*, *B. megaterium* inoculation and control.

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