SYNERGISTIC EFFECT OF *BACILLUS MUCILAGINOSUS* AND *PSEUDOMONAS FLUORESCENS* ON THE AVAILABILITY OF SOIL POTASSIUM, GROWTH AND YIELD OF *CUCURBITA PEPO* L.

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ABSTRACT

A field experiment was carried out at the field of Agricultural Research and Experiments Station during the season 2021-2022 in order to study the synergestic effect and compatible between *Bacillus mucilaginosus* and *Pseudomonas fluorescens* when they added as combination on potassium availability in the soil and on some plant growth characteristics and yield of *Cucurbita pepo* L. The experiment included four treatments B0, control , B1 addition of *Bacillus mucilaginosus* inoculum, B2 addition *Pseudomonas fluorescens* inoculum and B1 B2 addition of acombination of B1 and B2 inoculum . The experiment was conducted by using randomised complete blocks design in three replicates . The results showed that the co- inoculation with *Bacillus mucilaginous* and *Pseudomonas fluorescens* significant effect on quantity of soluble,exchangeable potassium as well as the plant height, dry weight of shoots, percentage of potassium in the shoot and in the fruits and total yield as compared with individual inoculation for any bacteria B1 or B2 which refers to positive synergestic effect. B1B2 treatment achieved the highest mean of soluble, exchangeable potassium which amounted to(0.163,0.856) cmol kg^{-1} soil respectively with an increase of (21.642 %, 15.676%) and (33.606%, 22.636%) of what was achieved by treatment (B1, B2) for the mean of soluble, exchangeable potassium respectively . B1B2 treatment achieved the lowest mean of(non-exchangeable and mineral) potassium which amounted to (35.280) cmol kg^{-1} soil.

Keywords: biological weathering; biofertilizer ;biotite and muscovite ;rhizobacteria, sustainability, climate action

***Part of MS.c. thesis of the 1st author.**

التأثير التآزري لبكتريا و*Bacillus mucilaginosus* و *Pseudomonas fluorescens* في جاهزية البوتاسيوم في التربة ونمو وحاصل قرع الكوسة *Cucurbita pepo L.*

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المستخلص

اجريت تجربة حقلية في محطة الأبحاث والتجارب الزراعية للموسم 2021-2022- بهدف دراسة التأثير التآزري والتوافق بين أثنين من بكتريا (PGPR) *Bacillus mucilaginosus* و*Pseudomonas fluorescens* عند إضافتها بصورة توليفة في جاهزية البوتاسيوم في التربة ,وفي بعض صفات النمو وحاصل قرع الكوسة *Cucurbita pepo* L. وتضمنت التجربة أربع معاملات B0 هي (معاملة المقارنة) وB1 (معاملة إضافة لقاح بكتريا (*Bacillus mucilaginosus*) و B2 (معاملة اضافة لقاح بكتريا *Pseudomonas fluorescens*) وB2 B1 أضافة لقاح البكتريا بصورة توليفة, أُجريت التجربة على وفق تصميم القطاعات العشوائية الكاملة وبثلاثة مكررات. أظهرت النتائج أن التلقيح المشترك ببكتريا *Bacillus mucilaginosus* و *Pseudomonas fluorescens* مع إضافة 50% من التوصية السمادية لسماد البوتاسيوم المعتمدة لنبات قرع الكوسة أدى الى تفوق معنوي في كمية البوتاسيوم الذائب والمتبادل, فضلاً عن إرتفاع النبات ,والوزن الجاف للمجموع الخضري ومحتوى المجموع الخضري والثمار من البوتاسيوم ,والحاصل الكلي مقارنة بالتلقيح الفردي لأي من البكتريا B1 أوB2 ,مما يدل على التأثير التآزري الأيجابي .إذ حققت معاملة B1B2 أعلى معدل للبوتاسيوم الذائب والمتبادل بلغ (0.163 , 0.856) سنتمول كغم$^{-1}$تربة على التتابع, وبنسبة زيادة بلغت (15.676, 21.642) % و(22.636,33.606) % عن ماحققته معاملة (B1, B2) لمعدلات البوتاسيوم الذائب والمتبادل على التتابع. كما حققت معاملة B1B2 أقل معدل للبوتاسيوم (غير المتبادل والمعدني) بلغ 35.280 سنتمول كغم$^{-1}$تربة .

الكلمات المفتاحية: التجوية الإحيائية، الأسمدة الحيوية، البايوتيت والمسكوفيت، البكتريا الجذرية، استدامة، العمل المناخي

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INTRODUCTION

Bofertilizers demonstrated their validity in Iraq in sustainable approach by enhancing soil ecosystem and various plant yield (1, 2, 10). In fact; the release of cations especially potassium from minerals occurs slowly through weathering or by microorganisms including bacteria. Bacteria play an important role in the biogeochemical cycle of potassium (4, 6, 23). *Bacillus mucilaginosus* and *Pseudomonas fluorescens* are silicate-solubilizing bacteria (SSBs) that have an active role in soil by dissolving insoluble forms of silicates and can also dissolve potassium-bearing minerals which increase soil fertility and improve plant growth (14). These bacteria have multiple mechanisms in increasing potassium availability, including direct solubilization (production of strong organic acids such as oxalic, tartaric, citric acids), which is the most important mechanism for solubilization potassium-bearing minerals, especially muscovite and biotite, and an indirect solubilization (chelation of cations bound to potassium silicate, exchange or complexation reactions, and metal complexing ligands), secretion of polysaccharides (capsular exopolysaccharides) and biofilms formation on the surfaces of minerals(23). *Bacillus mucilaginosus and Pseudomonas flourescens* are among the Plant growth promoting rhizobacteria (PGPRs.) that contribute to increasing the productivity of agricultural crops through various mechanisms including nutrients optimization and plant protection (7). *Bacillus mucilaginosus* has the ability to fix atmospheric nitrogen (22,9,24)this may be related to the fact that Bradyrhizobium sp. It is a symbiotic nitrogen-fixing genus in legumes that is closer to *Bacillus mucilaginosus* than other genera; these bacteria also have a role in promoting plant growth as a result of their ability to produce the hormone IAA and siderophore compounds (8) .The main objective of this research is to study the effect of co-inoculation of *Bacillus mucilaginosus* and *Pseudomonas flourescens* and to verify its effectiveness in releasing potassium and enhancing the growth and production of *Cucurbita pepo* L., as well as its ability to reduce the use of chemical fertilizer.

MATERIALS AND METHODS

Bacteria were isolated and identified from soil samples and were diagnosed based on cultural, microscopical and biochemical tests, then these isolates were grown in nutrient broth and kept at 4 °C until the bacterial inoculum was prepared.

Antagonism Test

The antagonism test was carried out between the two bacterial isolates *Bacillus mucilaginosus* and *Pseudomonas flourescens in vitro*, as they were grown on sterilized nutrient agar medium in an orthogonal manner, and incubated at a temperature of 28 °C for 5 days to observe the growth and the results showed that there is no antagonism between these two bacterial isolates.

Bacterial inoculum preparation

One liter of nutrient broth medium was prepared and placed in a conical flask, sterilized by autoclave at a temperature of 121°C and a pressure of 15 lb in^{-2} for 20 minutes, cooled and then 1 ml of the bacterial inoculum was added using a sterile digital pipette, and inocubated at a temperature of 28°C for 72 hours. The density of bacterial inoculum of the two bacterial isolates (combination) was estimated by serial dilution method and it was 10^8 cfu ml^{-1}.

Study Factor

The experiment included one factor, which is biofertilizers, as following:

1- Without biofertilizer, which was symbolize by B0.

2- Addition of *Bacillus mucilaginosus* inoculum and symbolized by the symbol B1.

3- Addition of *Pseudomonas fluorescens* inoculum and symbolized by B2.

4- Addition of a combination of *Bacillus mucilaginosus* and *Pseudomonase fluorescens*, symbolized by B1B2.

Field Experiment

Cucurbita pepo L. seeds were used, type FATIN F1, produced by the Dutch Atlantis Seeds Company, with a purity of 99 % and a germination rate of 90 %, seeds were treated with the anti-fungal substance Thiram. The seeds were planted in cork germination dishes with a capacity of 200 holes, which were filled with sterilized peatmoss to carry the inoculum later. The planting took place in one of the private nurseries / east of the canal area on August 23, 2021 for the Autumn season, and after the emergence of the seedlings and the appearance of the three true leaves, that is, after the passage of 7 days after planting, the seedlings were transferred to the field, as the roots of the seedlings were immersed in 20 % gum Arabic solution to ensure the adhesion of the bacterial inoculum which used as a biofertilizer. then planted in the hollow of the distance between one hole and another 40 cm. The complete fertilizer recommendation of nitrogen and phosphorous at a rate of (200,44) kg ha^{-1} was added. Urea fertilizer was added as a source of nitrogen (46 % N) after a period of planting and in two batches, the first two weeks after planting the seedlings to maintain the efficiency of the bio-fertilizer and the second at the beginning of the flowering stage. Triple Super Phosphate TSP (21% P) fertilizer was added as a source of phosphorous at once before planting, while half of the potassium fertilizer (K_2SO_4) recommendation was added in an average of 83 kg ha^{-1} at once after two weeks of planting seedlings. Experiment achieved according to Randomized Complete Blocks Design (R.C.B.D) in three replications (12 experimental units), as one sector contains 4 treatments that were randomly distributed to the experimental units in each sector.Determination of water –soluble potassium extracted by shaking soil samples with distilled water using a soil solution ratio of 1:1 (21). Determination of the exchangeable potassium extracted with neutral 1*N* NH_4OAc solution (21). Determination of The total potassium by the method as described by (11).The non-exchangeable and mineral potassium calculated according to the mathematical formula proposed by(18) and as follows: Non-exchangable and mineral potassium = Total potassium- (exchangeable potassium + soluble potassium). Potassium in the extracts determined by using a flame photometer . The important physicochemical properties of the soil before planting are presented in Table 1. The statistical program Genstat V. 12.1 was used in data analysis for the experiment and the means were compared using the Least Significant Difference (LSD) test at a significance level of 0.05.

RESULTS AND DISCUSSION

Effect on Potassium availability

The result in Table 2. showed that biofertilizer combination of the two bacterial isolates *Bacillus mucilaginosus* and *Pseudomonas fluorescens* was the best compared with other treatments, as it recorded a significant increase in all parameters compared to the use of each bacterial isolate alone, Table 2 showed that adding B1B2 bacterial biofertilizer supplemented with 50 % of the recommended potassium mineral fertilizer for *Cucurbita pepo* L. was more effective in dissolving potassium compounds and gave the highest mean of soluble potassium which was amounted to 0.163 cmol kg^{-1} compared to the non-addition treatment that gave 0.063 cmol kg^{-1} and treatment B1, which achieved 0.134 cmol kg^{-1}, and treatment B2, which achieved 0.122 cmol kg^{-1}, with increasing percentages of 158.730 %, 21.642 % and 33.606%, respectively. The results also showed the superiority of the treatment of adding biofertilizer in the form of a combination B1B2, which achieved the highest mean of exchangeable potassium in the rhizosphere soil, which amounted to 0.856 cmol kg^{-1} soil, compared to the treatment of no addition of biofertilizer B0, which gave a lowest mean of exchangeable potassium, which amounted to 0.563 cmol kg^{-1} soil and the treatment of adding biofertilizer in the form of bacteria B1, which recorded an quantity of exchangeable potassium amounted to 0.740 cmol kg^{-1} soil, and the treatment of adding biofertilizer in the form of bacteria B2, which achieved a quantity of exchangeable potassium amounted to 0.698 cmol kg^{-1} soil, with an increase rate of 52.043%, 15.676% and 22.636 %. It was noted from the results that the quantity of (non-exchangeable and mineral) potassium amounted to 35.280 cmol kg^{-1} soil when adding biofertilizer in the form of combination B1B2 compared to the treatment of no addition of biofertilizer B0 which gave amount of(non-exchangeable and mineral) potassium amounted to 35.680 $cmolkg^{-1}$ soil and the treatment B1, which recorded an quantity of (exchangeable and mineral) potassium amounted to 35.430 $cmolkg^{-1}$ soil, and the treatment of B2, which achieved a quantity of(

exchangeable and mineral) potassium amounted to 35. 480 cmolkg^{-1} soil,.The treatment of adding biofertilizer in the form of a combination (B1B2) achieved the highest mean of available potassium of 1.023 cmol kg^{-1} soil, compared with the treatment (B0), which gave the lowest average of available potassium of 0.626 cmol kg^{-1} soil, and treatment B1 that achieved an amount of the available potassium amounted to 0.873 cmol kg^{-1} soil and the treatment B2, which achieved 0.820 cmol kg^{-1} soil, , with an increase rate of 63.418%, 17.182% and 24.756 % respectively The dynamics of potassium in the soil indicated that the inoculation with the bacterial combination led to a significant release of potassium, and greater quantities of soluble and exchangeable potassium were retained, and a significant correlation was observed between plant growth and yield of plant and potassium release, therefore the synergistic effect between these bacterial isolates could be an alternative and applicable technique for application to dissolve potassium compounds and use them efficiently as a source of fertilizer and maintain soil sustainability and crop productivity. The increase in the amount of soluble potassium in the soil may be attributed to the presence of 2:1 minerals in the soil, which serves as a renewable reservoir for potassium, increases the availability of potassium in the soil, and that the addition of *Bacillus mucilaginosus* bacteria and *Pseudomonas fluorescens* liberated potassium as a result of their compatibility and performance in a synergistic manner, this led to an increase in the amount of secreted organic acids and an increase in the efficiency of biofertilizer in weathering potassium-bearing minerals and releasing potassium, in addition to being a PGPR that works to produce materials that increasing secretions of organic acids, especially citric acid and oxalic acid from the root, which have a role in dissolving potassium compounds in potassium-bearing minerals (2).Fertilization processes using biofertilizer in combination led to an increase in the concentration of exchangeable potassium in the soil and this increase is natural as a result of the effectiveness of KSB and the potassium liberated was higher than what was consumed by the plant, part of the liberated potassium adsorbed on the surfaces of the colloids (3). The decrease in the amount of (non-exchangeable and mineral) potassium for the treatment of the fertilizer combination can be attributed to the fact that possibility of increase in weathering is due to a synergistic effect, which could result from three hypothetical process: 1- The fragmentation of the mineral caused by root activity increases the direct positive effect of the bacteria on mineral weathering by increasing the reactive surfaces. 2- The root exudates indirectly provide the substrates required for the production of weathering metabolites by the bacteria or 3- The production of growth phytohormones by the bacteria, in addition to weathering agents, Stimulates root development and modifies root physiology and root exudation which improves weathering and nutrient uptake(4) . The liberated potassium in the soil, part of it increases the concentration of soluble potassium in the soil solution and the adsorbed potassium on the exchange sites, (15) which leads to the significant increase in the amount of available potassium in the rhizosphere soil for the treatment of the bacterial fertilizer combination, this increase is logical because the available potassium is the sum of the two forms (soluble and exchangeable) and because of the increase in these forms as a result of adding biofertilizer, the amount of available potassium will increase. Through the results obtained, it is clear that there is an increase in available potassium in the specified period when adding KSB in the form of a combination loaded on peat moss and enriched by addition 50 % of the recommended potassium mineral fertilizer for *Cucurbita pepo* L. The increase in the amount of available potassium took the following sequence:

B1B2>B1>B2>control

It is also clear from the results that there is a movement and transfer of potassium from one form to another in the rhizosphere soil. The results agree with (4), as there was an increase in available potassium up to 60 days of cultivation.

Table 1. Some physicochemical properties of the soil before planting

characteristics	values
pH	7.17
EC (ds m^{-1})	1.5
ECe (cmol kg^{-1} soil)	26.4
Mechanical analysis	
Silt (gm Kg^{-1} soil)	264
Clay (gm Kg^{-1} soil)	300
Sand (gm Kg^{-1} soil)	436
Textural class	Clay loam
Different pools of potassium	
Soluble potassium(cmol kg^{-1} soil)	0.078
Exchangeable Potassium (cmol kg^{-1} soil)	0.606
Non-exchangeable and mineral Potassium (cmol kg^{-1} soil)	35.618
Total Potassium(cmol kg^{-1}soil)	36.302

Table 2. Effect of biofertilizer treatments on potassium forms in soil

Treatment	Soluble Potassium cmolkg^{-1}	Exchangeable Potassium cmolkg^{-1}	Availabe potassium cmolkg^{-1}	Non-exchangeable and mineral Potassium cmolkg-1
B0	0.063	0.563	0.626	35.680
B1	0.134	0.740	0.873	35.430
B2	0.122	0.698	0.820	35.480
B1B2	0.163	0.856	1.023	35.280
L.S.D$_{0.05}$	0.001	0.0013	0.0020	0.0020

Effect on plant growth parameters

It is clear from the results of Table 3 that the addition of biofertilizer in the form of a combination B1B2 achieved the highest percentage of potassium in the shoot and in the fruits, the highest mean in plant height, the highest mean in the dry weight of the shoot, and the highest mean of the total yield amounted to (3.319 %, 2.957 %, 81,160 cm, 0.392 kg plant^{-1} and 10.168 Mg ha^{-1}) respectively, compared to the control treatment that achieved the lowest mean of (2.410%, 0.881%, 66.530 cm, 0.185 kg plant^{-1} and 6.268 Mg ha-1) respectively , and treatment B1 which achieved (2.767 %, 1.435 %, 74.130 cm, 0.243 kg plant^{-1} and 8.478 Mg ha^{-1}) respectively, and treatment B2 which achieved (3.010 %, 2.270 %, 77.770 cm, 0.288 kg plant^{-1} and 9.365 Mg ha^{-1}) respectively. The addition of biofertilizer alone enhanced the growth parameters of *Cucurbita pepo* L. such as plant height, dry weight of the shoot and the total yield, meaning that there is a significant effect of the biofertilizer in all the above-mentioned characters and in a positive way. Plant height is one of the characteristics of vegetative growth that depends on the division and elongation of cells. The high uptake of nitrogen, phosphorous and potassium leads to the promotion of plant growth as it is one of the main nutrients the plant needs for growth and production. The increase in nitrogen uptake by the plant is a result of inoculation with *Bacillus mucilaginosus*, which has the ability to fix atmospheric nitrogen (22,9,24). These bacteria also have a role in promoting plant growth as a result of their ability to produce the hormone IAA and siderophore compounds (8). Therefore, the abundance and availability of nutrients and their absorption, especially nitrogen, which has an effect in the center of the chlorophyll molecule leads to an increase in the rate of carbonate metabolism, whose effect is reflected in Improving the shoot and then increasing the height of the plant, and nitrogen has a role in increasing the size of the root system, which helps in absorbing the nutrients that the plant needs for growth and development, which increases the number of fruits and the rate of weight of the fruit, which leads to an increase in yield. In addition to the role of *Bacillus mucilaginosus* in increasing availability of potassium in the soil (8) and therefore adequate potassium supply enhances the photosynthesis for the primary role of potassium in controlling stomata opening, allowing adequate water and gas flow, improving nutrient supply and increasing mineral content in the plant (percentage of potassium in the shoot and fruits) (13). Thus, increasing the efficiency of the photosynthesis leads to an improvement in the shoot and an increase in the root system, which increases the efficiency of the absorption of water and nutrients, which is reflected in the increase in the dry weight of the plant. As for *Pseudomonas fluorescens*, it increases the availability of nutrients in the soil such as nitrogen, phosphorous, potassium and micro-nutrients, thus increasing the plant's absorption of nutrients that enter into biological and physiological processes (stimulating cell division and elongation), which promotes vegetative growth (plant height) and plant biomass, increase the dry weight of the shoot, enrich soil health and enhance crop production (2). Inoculation with this bacteria increased the number of fruits of *Cucubita pepo* L. by more than 400%, which led to an increase in yield (20). These bacteria have the ability to produce ACC-deaminase, which is known for its effect in reducing ethylene synthesis, which leads to improve root growth of plants along with IAA (12) as well as its production of antibiotics, maintaining the integrity of the roots and creating a clean environment free of diseases(3), which It is positively reflected on the total yield (5,19). However, the B1B2 biofertilizer combination treatment outperformed all treatments, and the reason for this is that in co-inculation, bacteria interact synergistically and complement each other, which increases the efficiency of inculation and leads to an improvement in plant growth (16). With PGPRs, it is represented by preparing macro and micro nutrients in addition to regulating the levels of hormones responsible for plant growth such as IAA, which increases plant growth by increasing the surface area and length of plant roots, which leads to increase absorption of water and nutrients from the soil and leads to improve vegetative growth through Increasing the division and elongation of cells, which leads to an increase in plant height and an increase in the dry weight of the shoot (6,15). The co-inoculation with KSB bacteria and PSB bacteria which dissolves phosphorous and potassium compounds leads to an improvement in the availability of potassium and phosphorus in the soil through the production of organic acids and other chemicals. Therefore, the increase in the absorption of nitrogen, phosphorous and potassium promoted plant growth by increasing the height of the plant and the dry weight of the shoot, in addition to increasing the total yield (8).

Table 3. The effect of adding the bio-fertilizer mixture on the growth and production of *Cucurbita pepo* L.

Treatment	Potassium% in the shoot	potassium% in fruits	Plant height cm	Dry weight of shoot kg plant^{-1}	Total yield Mg ha^{-1}
B0	2.410	0.881	66.530	0.185	6.268
B1	2.767	1.435	74.130	0.243	8.478
B2	3.010	2.270	77.770	0.288	9.365
B1B2	3.319	2.957	81.160	0.392	10.168
L.S.$_{D0.05}$	0.004	0.005	0.168	0.002	0.004

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