EFFECT OF BIOFERTILIZERS ON SOYBEAN GROWTH AND THEIR KEY PEST DENSITIES AS ALTERNATIVE APPROACH TOWARD SUSTAINABLE AGRICULTURE PRODUCTION

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ABSTRACT

Soybean (*Glycine max* L.) is one of the most important crops belong to the leguminous family native to East Asia. Chemical fertilizers are widely used to increase production in quantity and quality. As a result of the harmful effects caused by chemical fertilizers, it become necessary to find alternative solutions as sustainable approach toward supply plant by elements needs such as biofertilizers. In this study, *Bradyrhizobium japonicum* alone or in combination of microorganism inoculation included (*B. japonicum*, *Bacillus subtilis*, *Glomus mosseae*, and *Anabaena azollae*) were tested toward soybean growth parameters under open field condition. Results showed that there is a significant differences in terms of plant height, vegetative and dry grain of soybean and other parameters with combined inoculation and *B. japonicum* alone in compare with control treatments. Moreover, key pest larval density were also affected by treatments such as *Popillia japonica*, *Spodoptera littoralis* and *Agrotis ipsilon*. The results suggest the use of specific combination of microorganism is recommended as alternative approaches toward sustainable agriculture production of soybean crop.

Keyword: Bacteria; Mycorrhiza, economic pest, population density,

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ثأثير الأسمدة الحيوية على نمو محصول فول الصويا .Glycine max L وتأثيراتها على كثافة بعض الآفات الرئيسية كنهج بديل نحو الإنتاج الزراعي المستدام

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المستخلص

محصول فول الصويا (.L) *Glycine max* من أهم المحاصيل التي تنتمي إلى الفصيلة البقولية. تستخدم الأسمدة الكيميائية على نطاق واسع لزيادة الإنتاج من حيث الكمية والنوعية. نتيجة للآثار الضارة التي تسببها الأسمدة الكيميائية، أصبح من الضروري إيجاد حلول بديلة لتزويد النبات باحتياجاته من العناصر مثل الأسمدة الحيوية. في هذه الدراسة، تم اختبار عزلة بكتيرية من *B. japonicum* بمفرده أو مع التلقيح بالكائنات الحية الدقيقة (*B. japonicum* ، *B. subtilis*، *G. mosseae*، و*A. azollae*) تجاه محصول فول الصويا. أظهرت النتائج وجود فروق معنوية في ارتفاع النبات والحبوب الجافة و الوزن الخضري. علاوة على ذلك، قلت كثافة يرقات الآفات الرئيسية لل*P. japonica* و*S. littoralis* و *A. ipsilon.* بعد المعاملات قيد الدراسة. تشير النتائج إلى أن استخدام مجموعة محددة من الكائنات الحية الدقيقة يمكن ان يكون كنهج بديل نحو الإنتاج الزراعي المستدام لمحصول فول الصويا.

الكلمات المفتاحية: بكتريا، مايكورايزا، افات اقتصادية، الكثافة السكانية

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INTRODUCTION

Soybean (*Glycine max*) is one of the most important crops in the world due to its high oil and protein content reach to18 % and 40% respectively. In addition, their use in many industries, such as providing food, fodder, fuel, and some medical uses (8, 47). Moreover, soybean contribution toward soil fertility, leading to high productivity, and profitability; and, thus, is rightly referred to as the miracle crop. In the growth cycle, plants needs many nutritional elements in different proportions that must be present in the soil to achieve optimal plant growth that is reflected in the quantity and quality of production (9, 59). However, plants may face many problems during their growth stages due to biotic or abiotic factor including a lack of some elements necessary for growth, which may negatively affect the development of all growth stages (14). In response to luck of nutrients elements, many farmers are using chemical fertilizers extensively to increase their production. On the other hand, the intense use of chemical fertilizers is accompanied by many harmful effects on human health and environment. Hence there is an important need for safe and sustainable alternative (26). Many studies have been conducted for exploration the effects of biofertilization by microorganism and their influence on crop production (1, 16, 18, 19, 25). Microorganism interaction with host plant are well known to improve the absorption of many elements such as phosphorus, potassium, nitrogen and even microelements. In the same context, biofertilizers can also be useful for crop protection from many pests, diseases, and others, thus reducing the use of pesticides application (14). Many specific plants of the leguminous family are characterized by their symbiotic relationship with rhizobia bacteria that have grate benefits in fixation of nitrogen in the soil, which may provide part or all of the plant's needs (60, 61). In the rhizosphere, there are several group of bacteria called rhizobacteria which induce many positive effects on plant growth (plant growth promoting rhizobacteria). These organisms belong to several genera such as *Azotobacter sp., Bacillus sp., Rhizobium sp., Bradyrhizobium sp. Streptomyces sp. Azospirillum*, and *Trichoderma sp.* (10, 61). Moreover, some species of Streptomyces are known as plant growth-promoting and biocontrol agents because of their potential to produce a wide range of secondary metabolism such as alkaloids, vitamins, enzymes, plant growth factors, and enzyme inhibitors (15, 38). Furthermore, rhizobium inoculation is used as agronomic practice to ensuring for supply of adequate nitrogen for legumes crops. In specific, inoculating soybeans with a *Bradyrhizobium* strain is essential for supplying crop nitrogen demand, which may reduce the need for conventional of nitrogen fertilizers and thus reduces the cost of production (61). Moreover, Phosphate-solubilizing microorganisms produce several organic acids such as oxalate, lactate, acetate, gluconate, tartrate citrate and succinate that have several positive effects on plant production for example, phosphate-solubilizing bacteria have led to increase wheat yield under different experiments (29, 30, 34, 36, 45, 52). Other studied indicated that the use of different combinations of microorganisms also has great benefits on the crop, for example using of phosphate solubilizing bacteria with one strain of rhizobial bacteria on the pea plant led to increase of grain yield and grain protein level significantly compared with control treatment (5,9, 31). Cyanobacteria are emerging as microorganism used for sustainable agricultural development for provide plant with elements needs (6, 20, 27, 54). For example, Diazotrophes are cyanobacteria, it is used in the manufacturing of environmentally friendly fertilizers, and it can also compensate for nitrogen deficiency in plants, as well as improve soil aeration, water retention capacity, and provide vitamin B12 (22, 55). The most efficient nitrogen-fixing cyanobacteria are *A. azollae A. variabilis*, *Nostoc linkia*, *Aulosira fertilisima*, *Calothrix sp*., *Tolypothrix sp*., and *Scytonema sp*. which present in the rice and legume crop cultivation area (46). On the other hand, Soybean crop is attacked by a number of pests that have significant economic impacts. For example *P. japonica* (49), *S. littoralis* (21) and *A. ipsilon* (13). Many studies have shown that biofertilizer give the plants a great ability to tolerant of the threat of pests. Therefore, this study was aimed to evaluate some of microorganism such as (*Bradyrhizobium japonicum*, *Bacillus subtilis*, *Glomus mosseae,* and *Anabaena azollae*) in combination or alone toward soybean crop and test their effectiveness on the growth characteristic and productivity, also to monitoring of some key pest larvae densities under open field conditions.

MATERIALS AND METHODS

Microorganisms used in this study are *B. japonicum*, *B. subtilis* and *G. mosseae* were obtained from laboratories of the department of biotechnology, ministry of science and *A. azollae* was obtained from plant protection directorate, ministry of agriculture. Microorganisms were isolated previously and confirmed by National Center for Biotechnology Information (NCBI).

Preparation of *B. japonicum* inoculation

A concentration 10^9 cells/ml of *B. japonicum* isolation was prepared according to the procedure that described by Alkurtany (3). After that, 1 kg of soybean seeds free of insect and fungal infections were prepared and sterilized. The seeds were soaked with the inoculation solution for 1 hours. The seeds were left to dry and prepared for sawing.

Preparation of inoculation combination treatments: The combination of the following isolation was prepared to the procedure that described by Alkurtany (3) with some modification using (*B. japonicum*, *B. subtilis* and *A. azollae)* at 10^9 cell/ml concentration of each in addition to the mycorrhizal fungi *G. mosseae* at 50 cell/ml. After that 1 Kg of soybean seeds were soaked for two hours at room temperature, then left to dry.

Preparation of control Treatments

For control treatments, a conventional fertilizer containing nitrogen (N), phosphorus (P) and potassium (K), was used with an application rate N 20, P 20 K 20 kg/ha before seed sowing. Another application of chemical fertilizer was applied in mid of season with N 12 , P 36 , K 12 kg/ha. Final application also was applied before the maturation stage with N 12 , P 12 , K 36 kg/ha.

Field evaluation: The field study was carried out at the Dibbana research station located in south of Baghdad from June – October 2023. Seeds were then sowing in rows (5 m the length of each row), 5 rows with *B. japonicum* treatments alone and other 5 rows for inoculation with (*B. japonicum*, *B. subtilis* and *G. mosseae, A. azollae* with *B. japonicum*) treatments. Moreover, other 5 rows for control treatments. 3seeds were added per each hole and 15 cm the distance between each. A drip irrigation system (T-tape) was used. An analysis of soil properties was performed before and after planting. The experiment was designed according to Randomized Complete Block Design (RCBD), with three replications of the treatment in addition to three replications of the control treatment. The following of some growth factors such as: plant height, vegetative weight of plant and weight of grain was evaluated.

Determination of some compounds contents

To determine the content of soybeans of phenolic compounds, as well as the percentage of oils, carbohydrates and protein in the grain, samples were collected and then sent to the central laboratories- University of Baghdad for conducting the required analyses.

Monitoring of pest larvae density

To determine the density of *Popillia japonica* larvae which was already collected from soil Soil samples of 1 kg were collected for each experimental unit (each experimental unit had 33 seedlings) at a depth of 20 cm in the early morning on a weekly basis, then placed in special bags. As for the *Spodoptera littoralis*, *Agrotis ipsilon* larvae, they were collected from soybean leaves. The samples were transferred to the laboratory for examination. The larval presence mean was calculated. The larval were already described by Iraqi Natural History Museum.

Data analysis: The data were statistically analyzed by using SPSS package with ANOVA- one way. Analysis of variance technique was employed to test the overall significance of the data, while the least significance difference (LSD) test at P = 0.05 was used to compare the differences among treatment means.

RESULTS AND DISCUSSION

Soil properties: The analysis of the soil properties before and after planting with *B. japonicum* are show in the table (1).The quantity of ready nitrogen was increased at the end of the season. In addition to the quantity of phosphorus was also increased slightly. Moreover, potassium was also increased significantly at the end of the season. While, with the combination inoculation of (*B. japonicum, B. subtilis, G. mosseae,* and *A. azollae*) table (2), the results of ready nitrogen, phosphors, potassium and soil acidity was also increased in the end of the season more than treatments with *Bradyrhizobium* alone.

Table 1. Analysis of soil properties before and after treatments with (*B. japonicum*) only (mean +SE).

No.	soil properties mg/kg	Before planting	Mid of season	End of season
1	N	56(2.3)	77(4.1)	87.5(3.6)
2	P	17.6 (1.4)	21(2.6)	20 (1.8)
3	K	253(15.2)	285(16.5)	332 (13.8)
4	pH	7.3 (0.6)	5.99 (0.2)	6.1(0.3)

Table 2. Analysis of soil properties before and after combined treatments with (*B. japonicum, B. subtilis, G. mosseae,* and *A. azollae*) (mean +SE).

No.	soil properties mg/kg	Before planting	Mid of season	End of season
1	N	56(4.6)	59.5 (7.8)	105(6.7)
2	P	17.6 (2.5)	15.8 (2.7)	45(3.6)
3	K	253(11.5)	264(16.3)	532(15.6)
4	pH	7.3 (0.8)	6.6 (0.4)	6.3 (0.2)

Plant height: The effects of microbial inoculants on plant height of soybean are show in table (3). A significant differences were observed between treatments after 3 months of planting (p = 0.001). The highest growth rate in plant height was 57. 3 cm recorded with the combination of the inoculants (*B. japonicum, B. subtilis, G. mosseae,* and *A. azollae*), while the less height was 41.06 cm observed with control treatments. However, with *B. japonicum* the height plant was 47 cm.

Table3. Effects of biofertilizer on height of soybean plant (P < 0.05).

Tretments	Mean	Std. Error	LSD differences
Control	41.0	0.63	a
Bradyrhizobium	47	2.30	b
Combination of biofertilizer	57.3	0.72	c
LSD	0.0006		
f - value	32.46		

Vegetative weight: The effects of microbial inoculants on plant vegetative weight on soybean are show in table (4). Application of the inoculums were showed a significance differences between the treatments after 3 months (p = 0.001). The highest average of vegetative weight in soybean plant with (*B. japonicum, B. subtilis, G. mosseae,* and *A. azollae*) treatment was 149.2 g, while the less weight observed with control treatments was 49.8 g. Furthermore the treatments with *B. japonicum* was 95.6 g.

Table 4. Effects of biofertilizer on vegetative weight of soybean plant (P < 0.05).

Treatments	Mean	Std. Error	LSD differences
Control	15	2.0	a
Bradyrhizobium	51.3	13.8	b
Combination of biofertilizer	108.3	5.3	c
LSD	0.001		
F-value	29.482		

Weight of 100 g soybean grain.

The effect of microbial inoculants on the weight of dried soybean grain are showed in table (5). A significance differences were observed between the treatments (p= 0.001). The highest weight was 19.40 g observed with the combination of the inoculants (*B. japonicum, B. subtilis, G. mosseae,* and *A. azollae*). As well as, with control treatments the weight of soybean grain was 13.5 g. While with *B. japonicum* treatments the grin weight was 15.6 g.

Table 5. Effects of biofertilizer on weight of soybean seeds (P < 0.05).

Tretments	Mean	Std. Error	LSD differences
control	13.15	0.28	a
Bradyrhizobium	15.63	0.84	b
Combination of biofertilizer	19.40	0.22	c
LSD	0.001		
F-value	32.460		

Determination of some compounds contents

The results of the analysis of phenolic compounds in table (6) indicated that there were clear significant differences between the treatments. The highest amount of phenolic was in the combination of biofertilizer treatment, while the amount of phenolic compounds decreased in the bradyrhizobium treatment. Also, the control treatment contained the least amount of phenolic compounds compared to the other treatments.

Furthermore, the results of the oil analysis also indicated that there were significant differences between the treatments (P < 0.05) table (7), as the amount of oils was the highest in the combination of biofertilizer treatment, where it was 23%, then it decreased in the bradyrhizobium treatment alone, where it was 20%, then it also decreased in the control treatment, where it was 15%. Moreover, there were also significant differences in carbohydrate compounds (P < 0.05), which were higher in combination of biofertilizer treatment where it was 35% compared to the bradyrhizobium and control treatments where it was 30% and 31 % respectively table (7). Analysis of the amount of protein also indicated that there were significant differences between the treatments, as the amount was highest in the combination of biofertilizer treatment, where it was 38%, while it was lowest in the bradyrhizobium treatment alone, where it was 29%. It also decreased a lot in the control treatment, as it was 14% table (7).

Table 6. Soybean content of phenolic compounds after treatments by *B. Japonicum* alone and in combination inoculants. Different letter indicate a significant difeances (p < 0.05), one way anova followed by LSD test.

Treatments	Phenolic compounds	Std. Error	LSD differences
Control	244.17	54.22	a
bradyrhizobium	761.07	76.45	b
combination of Biofertilizer	958.07	111.76	c
LSD	0.01		

Table 7. Soybean grain content of oil, carbohydrate and protein after treatments by *B. Japonicum* alone and in combination inoculants. Different letter indicate a significant difeances (p < 0.05), one way anova followed by LSD test.

Treatments	Oil %	Carbohydrate %	Protein %
Control	15.51 (a)	31.63 (a)	13.91 (a)
bradyrhizobium	20.57 (b)	31.8 (a)	29.64 (b)
combination of Biofertilizer	22.25 (c)	34.63 (b)	38.3 (c)
LSD	0.003		

Monitoring of pest larvae density

The results of monitoring the density of *Popillia japonica* larvae indicated that the density was lower in the combination of biofertilizer and bradyrhizobium treatment compared to the control treatment table (8). Its highest activity was in the seventh week of planting, while its lowest activity was in the first and thirteenth weeks of planting.

Table 8. Means density of *Popillia japonica* larvae during soybean growing season after treatments with treatments by *B. Japonicum* alone and in combination inoculants

Weeks	Control	Bradyrhizobium	Combination of biofertilizer
1	0	0	0
2	1	1.7	1
3	2.67	1.5	1
4	3.67	2	1.33
5	3.67	1.5	1
6	3	1.5	0.67
7	4.33	1	0
8	4	0.6	0
9	3	1	0.33
10	3.33	1	0
11	4.33	1	0
12	2	0.3	0.33
13	0.67	0	0

Moreover, the results of monitoring the density of *spodoptera littoralis* larvae indicated that the density was lower in the combination of biofertilizer and bradyrhizobium treatment compared to the control treatment table (9). Its highest activity was in thirteenth weeks of planting, while its lowest activity was in the first of planting.

Table 9. Means density of *spodoptera littoralis* larvae during soybean growing season after treatments with treatments by *B. Japonicum* alone and in combination inoculants

Weeks	Control	Bradyrhizobium	Combination of biofertilizer
1	0	0	0
2	1	0	0
3	1.33	1	0
4	2	1.3	1
5	2	1	1.67
6	4	2	0.67
7	4	2	0
8	3.33	1.7	0
9	3.33	1.9	0
10	6	2	1
11	5.33	1	0
12	7	2	0
13	7	0.6	0

Furthermore, the results of monitoring the density of *Agrotis ipsilon* larvae indicated that the density was lower in the combination of biofertilizer and bradyrhizobium treatment compared to the control treatment. (Table 10). Its highest activity was in third weeks of planting, while its lowest activity was in the twelfth week of planting.

Table 10. Means density of *Agrotis ipsilon* larvae during soybean growing season after treatments with treatments by *B. Japonicum* alone and in combination inoculants

Weeks	Control	Bradyrhizobium	Combination of biofertilizer
1	1.67	1	0.33
2	1.4	1.2	1
3	2.33	1.9	1
4	2	1	0.33
5	2	1.5	0
6	1.33	1.2	0
7	1.33	1	0.33
8	0.67	0.5	0
9	1	0.6	0.67
10	0.33	0.2	0
11	0.67	0.1	0
12	0	0	0
13	0	0	0

Biofertilization is considered one of the most important and safe alternatives to chemical fertilizers (7, 24, 28). Biofertilization would provide many benefits to the plants in terms of promotion of plant growth, plant protection and increasing the yield (2). Results of the present study indicated that the use combined inoculation of (*B. japonicum*, *B. subtilis*, *G. mosseae*, and *A. azollae*) on soybean plants led to an increase in the plant productivity compare with *B. japonicum* alone and control treatments. Several studies have been demonstrated that the effects of *Bradyrhizobium sp.* and *Azotobacter sp.* alone or in combination inoculant led to increase of nodules number in several crops such as Mungbean varieties due to their ability to fix the nitrogen in the soil (37, 41, 42, 57). This effects of Bradyrhizobium bacteria belong to the availability of the chromosomes that have a symbiosis islands which carry nod and nif genes that responsible for the nodulation and nitrogen fixation (44). Moreover, the effects of *B. japonicum* inoculation in combination with phosphate solubilizing bacteria has increases the dry weight on bean (43). This results agreed with another study which showed that the use of the. *B japonicum* species led to increase the number of pods, number of seeds, seed weight, grain protein, total protein content and development of plant leaves when tested in two soybean cultivars (58, 62). Furthermore, soybean seeds inoculated by rhizobial bacteria provides plants with an increased in the number of pods, number of seeds, weight of thousand grain and the yield of soybean in general (35). The ability of rhizobia bacteria to fix nitrogen is due to the nodules found in the roots of most legumes, which work to convert nitrogen from atmosphere into ammonia that is easily absorbed by the plant (39). Furthermore, Phosphate solubilizing bacteria which made inorganic soil phosphates such as Fe, Ca and Al soluble by production several organic acid, siderophores and hydroxyl and carboxyl grouped and make them available for plant utilization (53). In the same context, the inoculation by *Bacillus pumilus* resulted in a significantly increase of plant height, leaf number, leaf area, grain protein and nodulation of soybean plant (56). Moreover, the combined inoculation of soybean by phosphate solubilizing bacteria and symbiotic bacteria increased the dry weight of soybean (50). On the other hand, biofertilizer with *B. subtilis* also increased the cotton plant growth, number of bolls and improved yield of cotton fiber (59). However, mycorrhizal fungi are well known as a biofertilizer that allows plants to exploit the mineral elements in the soil due to their root-like structure and possess a network of mycelium external to the tree roots that extends into the soil. This mycelium absorbs nutrients and translocate them back to the host plant, as a result, there is an increase in the absorption surface area of the roots. In the study conducted by (51) indicated that many species of mycorrhizal fungi inoculants such as *Glomusetunicatum* species are reported to enhance the vegetative growth, chlorophyll, and nutrient level in maize. In addition, species of *Vignaradiata* were also reported to have a positive impact on the nitrogen, potassium, phosphorus, and protein content of the green grain (12). Moreover, the use of the species, *Glomus mosseae* was improved wheat and corn yield even under stress conditions (4, 7, 17, 26, 40). Furthermore, the combined inoculation of *Acaulospora lacunosa* and *Glomus constrictum* improved foliar nutrient status of onions *Allium porrum* (23). Other previous studies indicated that cyanobacteria play a crucial role in the preservation and build-up of soil fertility and maintaining optimum yield production, as a natural biofertilizer by development of sustainable agricultural practices depending on the utilization of beneficial outcomes of cyanobacterial growth (55). These findings agreed with the observations by (3) who noted that the application of algae extracts leads to an increase in chlorophyll concentration. This, in turn, enhances the absorption of light energy, resulting in enhanced sugar and biomass production. Furthermore, it facilitates the provision of vital energy for protein synthesis within the plant. The results also indicated a significant increase in the percentage of oil, carbohydrates, protein, and phenolic compounds, whether treated with combination of biofertilizer or *bradyrhizobium* isolation, compared to the control treatment. We also previously pointed out the efficiency of biofertilizers in increasing production, as it was reflected positively on the amount of oil, carbohydrates, protein, and phenolic compounds for example the phenolic compounds increased in *Coriandrum sativum* L. after treatments of *Bacillus halotolerans* biofertilizer (33).This finding agreed with aved, and Panwar (32) were proved carbohydrate also increased in response to biofertilizers treatments on *Glycine max* and *Vigna mungo*. In the same context, oil and proten content also increased after application of biofertilizers as agreed with (61).Moreover, the densities of some economically important pests (*P. japonica*, *S. littoralis* and *A. ipsilon*), were decreased after treatment with biofertilizers. Biofertilizers can be used as promoting of plant's resistance to insects and pathogens, as they work to increase the level of secondary metabolic compounds that may act as repellent or killing insects. Biofertilizers may also increase some of the properties related to plant growth, which may hinder the development and growth of the insect (11, 48). Also, the response of insects may vary depending on the stages of growth and development of the plant and climatic factors, and this may reflect the development of insects in certain stages of the development and growth of the plant. Therefore, the density of larvae of the pests under experiment differed between the first week and the thirteenth week of the stages of soybean plant development.

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