

Evaluation of Four Levels Bradyrhizobium japonicum inoculants with cobalt on yield components of two soybean cultivars in Gypsiferous soil.

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Abstract

Conducted a factorial field experiment following a Randomized Complete Block. The factors that are placed in main plot and sub plot and at the Soil and Water Resources Research Station, College of Agriculture, Tikrit University, Salah al-Din Province, during the summer season on June 10, 2024. Governorate to evaluate the efficiency of the local inoculum prepared from the bacterial isolate *Bradyrhizobium japonicum* and cobalt on the growth and yield of two soybean cultivars in gypsum soil. The results that instead of in the number of pods, weight of pods, total yield of seeds, nitrogen, content, content of protein in seeds, as the percentage increase in these traits 7.41%, 3.69%, 6.97%, 3.82%, and 3.71%, respectively. compared to the Yema soya variety. The results showed that the inoculation with both had a significant impact on all yield traits of soybean plants. According to the LSD test. The inoculated treatments outperformed the non-inoculated treatment significantly in the number of pods, weight of pods, total yield of seeds, nitrogen, content, content of protein in seeds, as the percentage increase in these traits. The percentage increase was 51.76%, 36.22%, 41.17%, 41.57%, 23.82% and 23.91%, respectively, compared to the non-inoculated treatment. The results showed that cobalt fertilization had a significant effect, as the treatment fertilized with cobalt at the level of 1 kg Co ha⁻¹ significantly outperformed the unfertilized treatment, with a percentage increase (16.37%, 16.74%, 20.89%, 26.13%, and 26.38%) in the number of pods, weight of pods, total yield of seeds, nitrogen, content, content of protein in seeds, as the percentage increase in these traits. The percentage increase was compared to the non-fertilized treatment respectively. The results showed that the interaction effect between varieties and bacteria were significant, as the treatment (variety Shaima and bacteria A3) was significantly superior in the number and weight of pods, grain yield, nitrogen concentration, and protein percentage in the seeds. The percentage increase was 63.55%, 42.99%, 55.25%, 30.05%, 29.97%) compared to the treatment (Yema soya variety and no bacteria added), respectively. The interaction between the varieties and cobalt was significant, as the treatment (Shaima variety and cobalt at a concentration of 1 kg ha⁻¹) was significantly outperformed in all the studied traits in the number of pods, weight of pods, total yield of seeds, nitrogen, content, content of protein in seeds, as the percentage increase in these traits (26.20%, 21.91%, 30.57%, 30.60%, 32.5%, 32.46%) compared to the treatment (Yema soya variety and no cobalt addition) respectively. The interaction between bacteria and cobalt was significant, as the treatment (A3 bacteria and cobalt at a concentration of 1 kg ha⁻¹) was significantly outperformed in all the studied traits in the number of pods, weight of pods, total yield of seeds, nitrogen, content, content of protein in seeds, as the percentage increase in these traits (79.46%, 59.42%, 72.72%, 63.07%, 63.43%) compared to the

treatment (not adding bacteria and not adding cobalt) respectively. The effects of the three way interactions among the study factors was significant in all of the studied traits.

Keywords: soybean, cobalt, sterilization, bio fertilizer, gypsum soil.

The research is extracted from the doctoral thesis of the first researcher.

Introduction

Biofertilizers are an important part of the nutritional supplement system that must receive attention because chemical fertilizers and organic fertilizers cannot meet the nutritional needs of crops under the intensive agricultural system and the low level of soil fertility. Therefore, a support and assistance process is necessary to raise the productive capacity of the soil by using microscopic organisms that have the ability to fix atmospheric nitrogen or that work to increase the solubility and availability of phosphorus present in the soil (34). The use of biofertilizers as an auxiliary factor in fertilization has led to a reduction in the use of mineral nitrogen fertilizers by an amount that may reach half the amount of fertilizers recommended (33).

Soybean cultivation in arid and semi-arid regions, including Iraq, faces some problems, the most important of which are: high gypsum content and excessive use of chemical fertilizers, especially nitrogenous ones, as a result of the low productivity of gypsum soils, due to their low content of nutrients. Excessive use of chemical fertilizers is not a sustainable strategy because of its negative effects and harmful aspects to the environment, as soil health is affected as a result of reliance on chemical fertilizers and pesticides, in addition to continuous monoculture (30).

The symbiosis between Rhizobia bacteria and legumes has a significant impact on the

success of legume crops, which makes the culture of adding biofertilizers an important practice in improving the growth and production of crops, especially in dry and semi-dry areas. This will encourage their purchase and use by farmers compared to manufactured chemical nitrogen fertilizers. Rhizobium bacteria convert approximately 20 million tons of atmospheric nitrogen N₂ into ammonia, i.e. 50-70% of the biologically fixed nitrogen in the world. High nitrogen fixation can determine the extent of the success of the symbiotic relationship between them and legumes and vice versa (31). The soybean plant obtains 50-60% of its nitrogen requirements through biological nitrogen fixation (20).

Cobalt is an essential element for improving the symbiotic relationship of rhizobia with legumes and increasing the efficiency of atmospheric nitrogen fixation (17) as well as increasing the formation of effective root nodules, which ultimately leads to an increase in yield (2,4). Cobalt is one of the essential elements for the process of fixing atmospheric nitrogen by legumes and stimulates the growth of rhizobia bacteria in the soil adjacent to the root system (5). Due to the scarcity or absence of studies, especially in gypsum soils, that focus on studying the combined effect of inoculation with *Bradyrhizobium japonicum* bacteria and cobalt fertilization, this study was conducted with the aim of evaluating the effect of local and commercial inoculum prepared from the bacterial isolate

Bradyrhizobium japonicum and cobalt levels on the growth and yield of two soybean varieties in gypsum soil.

Materials and methods work

Experiment site and soil characteristics

A field experiment was carried out during the summer season at the research station of the Department of Soil Sciences and Water

Resources / College of Agriculture / Tikrit University in Salah al-Din Governorate for the year 2024. Soybean crop, samples of soil before planting at a depth of (0-30) cm were mixed and air-dried, then ground, and some chemical, physical and biological properties were estimated as shown in Table (1).

Table 1. Physical, Chemical, and Biological Properties of Soil Before the cultivation

Property	Measurement Unit	Value
the pH of the soil		8
Electrical conductivity (EC)	d.s.m^{-1}	8
Exchange capacity (CEC) - Cation	mol.Kg^{-1} soil	4.22
Organic matter (OM)	kg^{-1}	7
Gypsum (CaSO_4)	kg^{-1}	65
Available Nitrogen (N)	g.kg^{-1}	3.20
Available Phosphorus (P)	g.kg^{-1}	1.4
Available Potassium (K)	g.kg^{-1}	34.78
Cobalt (cobalt Available)	ppm	63
Bulk density	cm^{-3}	31
Sand	kg^{-1}	45
Silt	kg^{-1}	20
Clay	kg^{-1}	35
Texture		Sandy Clay loam
Total bacterial count	F.U	$10^6 \times 4.7$
Total fungal count	F.U	$10^3 \times 2.8$

Third: Two types of soybean plants: (Their superiority is due to their inherent genetic and physiological characteristics. The Shaima variety is an upright variety with an indeterminate growth potential, while the Yema soya variety is a spreading variety with a limited growth potential.).

Statistical design: An experiment was conducted using split plot design with three. The field experiment data were statistically analyzed using the split-plot analysis of variance. The means were compared using the least significant difference (LSD) at a probability level of (5%).

Agricultural operations: The field soil was prepared by plowing, smoothing and levelling

process. Then, the land was divided into three equal sectors, leaving suitable distances as dividers. Each sector had two varieties along the length of the land. Each replicate was divided into experimental units with dimensions of 0.4×0.3 m. Each experimental unit included five lines, the distance between each line and the next was 0.7m. The seeds were planted along the line, and then the plants were thinned out until the distance between one plant and another became 0.15 m. The seeds of soybean plants (Shaima and Yema soya varieties) were planted on 7/10/2024. Nitrogen was added at a rate of 40 kg N ha^{-1} , using 46% N urea fertilizer as a nitrogen source before planting. Phosphorus

was added at a rate of 80 kg h⁻¹ using 20% P triple superphosphate fertilizer, and potassium was added at a rate of 120 kg h⁻¹ using 43% K potassium sulfate fertilizer in one batch before planting (8). The fertilizer quantities were mixed and then added to the experiment using the bundle method and adjacent to the planting lines.

The Studied experiment

Five plants were randomly selected from the middle lines to carry out the following measurements on them, such as the number of pods per plant, the weight of the pods, and the total yield. The yield of one plant was multiplied by the plant density (95238) (Plant density was calculated as the area occupied by one plant (the distance between one plant and another * the distance between one line and another), and the nitrogen concentration in the seeds and the percentage of total protein in the seeds were estimated according to the following equation: Protein percentage (%) = Nitrogen percentage (%) × 6.25. According to (1.)

Statistical analysis

The data of the field experiment were statistically analyzed using the analysis of variance method according to the split-plot arrangement, applying the randomized complete block design and the results of the experiment were statistically analyzed using the ANOVA table based on the statistical program (Genstat12thed), then the averages were compared according to the least significant difference (LSD) test at the significance level of 0.05 (9.)

Results and Discussion

The results of Table (2) show that there are significant According to the LSD test between the studied factors influencing the number of pods of soybean plants (pod per plant-1). The cultivar Shaima was significantly superior and

gave the highest average number of pods, reaching 79.22 pods per plant-1, compared to the cultivar Yema soya, which gave the lowest average, reaching 73.75 pods per plant-1, with a percentage increase of 7.41%. The reason for this is attributed to the variation in the genetic composition of the cultivars, which led to a difference in their flowering period, especially the early ones, which leads to an increase in their ability to form more pods on the plant. The reason for this may be attributed to the overlapping effect between the genetic composition and the environmental conditions in which the cultivar grows, which may affect a specific cultivar more than another in the number of emerging seeds. The reason for this may be attributed to the overlapping effect between the cultivars and the environmental conditions in which the cultivar grows (26). As for the bacterial factor, level A3 was superior and gave the highest average of 93.67 pods/plant-1, followed by level W and level T, which gave averages of 79.89 pods/plant-1 and 70.67 pods/plant-1, respectively, while the control treatment gave the lowest average of 61.72 pods/plant-1, with a percentage increase of 14.50%, 29.43%, and 51.76%, respectively. This may be attributed to the fact that inoculation with this bacteria improves the growth of leguminous plants by increasing the dry green and root weight, and the plant height, which was positively reflected on nitrogen and other elements, and then the characteristics of the crop improve, especially the number of pods and their weight. These results are consistent with what was indicated by (23). As for the cobalt factor, the concentration of 1 kg CO ha⁻¹ gave the highest average of 82.58 pods plant-1, followed by the level of 0.5 kg CO ha⁻¹, which gave 75.92 pods plant-1. Finally, the treatment without adding cobalt gave the

lowest average of 70.96 pods plant⁻¹, with a percentage increase of 16.37% and 6.98%. The reason may be indirectly due to the increase in vegetative characteristics, including dry weight and number of branches, which may lead to an increase in the number of flower-bearing branches and thus lead to an increase in fruits. The results obtained were consistent with what was obtained by (11) on broad beans and peas. As for the two-way interaction between varieties and bacteria, the treatment (variety Shaima and bacteria A3) was superior and gave the highest average of 97.22 pods per plant⁻¹, while the treatment (variety Yema soya and no bacteria added) gave the lowest average of 59.44 pods per plant⁻¹, with a percentage increase of 63.55%. As for the interaction coefficients between varieties and cobalt, the treatment (variety Shaima and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 85.92 pods plant⁻¹, while the treatment (variety Yema soya and no cobalt addition) gave the lowest average of 68.08 pods plant⁻¹, with a percentage increase of 26.20%. As for the interaction between bacteria and cobalt, the treatment (bacteria A3

and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 100.50 pods plant⁻¹, while the comparison treatment (no addition of bacteria and no addition of cobalt) gave the lowest average of 56.00 pods plant⁻¹, with a percentage increase of 79.46%. The interaction between cobalt fertilization and inoculation with microorganisms resulted in the treatments inoculated with rhizobia and supplied with cobalt being distinguished in the overall characteristics of the yield over all other treatments. Both (4) and (21) obtained similar results when they studied the effect of cobalt and inoculation on the growth and yield of bean seeds. As for the triple interaction between varieties, bacteria and cobalt, the treatment (variety Shaima, bacteria A3 and cobalt 1 kg CO ha⁻¹) was significantly superior and gave the highest average number of pods, reaching 104.00 pods plant⁻¹, while the comparison treatment (variety Yema soya, no addition of bacteria and no addition of cobalt) gave the lowest average, reaching 50.67 pods plant⁻¹, with a percentage increase of 105.24%.

Table (2): The effect of levels with *Bradyrhizobium japonicum* bacteria and three levels of cobalt fertilization and their interaction on the number of pod (pod plant⁻¹) for two varieties of soybean.

Variety	Levels	cobalt kg ha ⁻¹			Mean
		0	0.5	1	
Shaima	0	61.33	62.67	68.00	64.00
	A3	90.67	97.00	104.00	97.22
	W	76.33	80.00	94.00	83.44
	T	67.00	72.00	77.67	72.22
Yema soya	0	50.67	61.67	66.00	59.44
	A3	84.33	89.00	97.00	90.11
	W	72.00	76.00	81.00	76.33
	T	65.33	69.00	73.00	69.11
LSD 0.05		2.840			2.092
variety * cobalt					
Shaima		73.83	77.92	85.92	79.22
Yema soya		68.08	73.92	79.25	73.75
LSD 0.05		1.420			1.347
bacteria* cobalt					
0		56.00	62.17	67.00	61.72
A3		87.50	93.00	100.50	93.67
W		74.17	78.00	87.50	79.89
T		66.17	70.50	75.33	70.67
LSD 0.05		2.008			1.479
Mean		70.96	75.92	82.58	
LSD 0.05		1.004			

average of 35.23 g pod⁻¹, with a percentage increase of 3.69%. The reason for this is attributed to the variation in the cultivars' genetic composition, which led to a difference in their flowering period, especially the early ones, which leads to an increase in their ability to form more pods in the plant. The reason for this may be attributed to the overlapping effect between the genetic composition and the environmental conditions in which the cultivar grows, which may affect a specific cultivar without another in the number of emerging seeds. The reason for this may be attributed to the overlapping effect between the cultivars and the environmental conditions in which the cultivar grows (26). As for the bacterial factor, level A3 was superior and gave the highest

average of 41.33 g pod⁻¹, followed by level W and level T, which gave averages of 37.81 g pod⁻¹ and 34.05 g pod⁻¹, respectively, while the comparison treatment gave the lowest average of 30.34 g pod⁻¹, with a percentage increase of 36.22%, 8.04%, and 24.62%, respectively. This may be attributed to the fact that inoculation with this bacteria improves the growth of leguminous plants by increasing the dry green and root weight, and the plant height, which was positively reflected on nitrogen and other elements, and then the characteristics of the crop improve, especially the number of pods and their weight. These results are consistent with what was indicated (3). As for the cobalt factor, the concentration of 1 kg CO ha⁻¹ gave the highest average of

38.77 g pods-1, followed by the level of 0.5 kg CO ha-1, which gave 35.67 g pods-1. Finally, the treatment without adding cobalt gave the lowest average of 33.21 g pods-1, with a percentage increase of 16.74% and 7.40%, respectively. This is attributed to the effect of cobalt in increasing the growth of reproductive organs and thus increasing the speed of flowering, which may affect the increase in the pod area. These results are consistent with what was indicated by (15). As for the two-way interaction between varieties and bacteria, the treatment (variety Shaima and bacteria A3) was superior and gave the highest average of 42.14 g pod-1, while the treatment (variety Yema soya and no bacteria added) gave the lowest average of 29.47 g pod-1, with a percentage increase of 42.99%. As for the interaction coefficients between varieties and cobalt, the treatment (variety Shaima and cobalt at a concentration of 1 kg CO ha-1) was significantly superior and gave the highest average of 40.00 g pod-1, while the treatment (variety Yema soya and no cobalt addition) gave the lowest average of 32.81 g pod-1, with a percentage increase of 21.91%. As for the interaction between bacteria and cobalt, the treatment (bacteria A3 and cobalt at a

concentration of 1 kg CO ha-1) was significantly superior and gave the highest average of 46.92 g pod-1, while the control treatment (no addition of bacteria and no addition of cobalt) gave the lowest average of 29.43 g pod-1, with a percentage increase of 59.42%. The reason is that the cobalt element is one of the necessary elements to improve the symbiotic relationship of rhizobia with leguminous plants and to increase the efficiency of atmospheric nitrogen fixation (17). These results are consistent with what was indicated by (7) that the interaction between cobalt fertilization and inoculation with microorganisms resulted in the treatments inoculated with rhizobia and supplied with cobalt being distinguished in the overall characteristics of the product over all other treatments. As for the triple interaction between varieties, bacteria and cobalt, the treatment (variety Shaima, bacteria A3 and cobalt 1 kg co ha-1) was significantly superior and gave the highest average pod weight of 49.11 g pod-1, while the comparison treatment (variety Yema soya, no addition of bacteria and no addition of cobalt) gave the lowest average of 28.58 g pod-1, with a percentage increase of 71.83%.

Table (3): The effect of levels with *Bradyrhizobium japonicum* bacteria and three levels of cobalt fertilization and their interaction on the pod weight (g pod⁻¹) of two soybean varieties.

Variety	Levels	cobalt kg ha ⁻¹			Mean
		0	0.5	1	
Shaima	0	30.27	31.00	32.36	31.21
	A3	36.91	40.42	49.11	42.14
	W	35.06	38.86	41.02	38.31
	T	32.18	33.63	37.50	34.44
Yema soya	0	28.58	29.90	29.92	29.47
	A3	37.29	39.53	44.72	40.51
	W	33.11	38.78	40.01	37.30
	T	32.26	33.21	35.49	33.65
LSD 0.05		1.382			0.480
variety * cobalt					
Shaima		33.60	35.98	40.00	36.53
Yema soya		32.81	35.36	37.54	35.23
LSD 0.05		0.691			0.417
bacteria* cobalt					
0		29.43	30.45	31.14	30.34
A3		37.10	39.98	46.92	41.33
W		34.09	38.82	40.52	37.81
T		32.22	33.42	36.49	34.05
LSD 0.05		0.978			0.339
Mean.		33.21	35.67	38.77	
LSD 0.05		0.489			

of 3.11 tons/ha-1, with a percentage increase of 7.71%. This may be attributed to the prior superiority in most of the yield components, including the number of pods per plant-1, or the reason may be attributed to genetic differences that affect the chemical composition and enzyme activity of the seeds (14). These results are consistent with the findings of (3) to study the effect of six soybean genetic compositions on growth traits, yield, and seed quality (Lee 74, Al-Tanaqi, Laura, D, 146, G6). As for the bacterial factor, level A3 was superior and gave the highest average of 3.78 tons ha-1, followed by level W and level T, which gave averages of 3.41 tons ha-1 and 3.03 tons ha-1, respectively, while the comparison treatment

gave the lowest average of 2.67 tons ha-1, with a percentage increase of 41.57%, 27.71%, and 13.51%, respectively. This may be attributed to the role of potassium-dissolving bacteria in increasing seed weight by producing a good amount of cytokines, which has a significant effect on increasing growth and grain filling (18). These results are consistent with what (14) found in his study on soybean plants, as the treatments inoculated with the TAL-379, UK Isolate, and Local strains outperformed. Brady rhizobium sp significantly over the inoculated treatments. The total biological yield rates of the inoculated treatments were 7489.7, 8060.7 and 7541.2 kg ha-1, respectively, compared with the inoculated treatments, which gave 5473.3

kg ha⁻¹. As for the grain yield, the treatments inoculated with the three strains gave significant differences, and the average grain yield reached 1881.51, 2766.40 and 2398.25 kg ha⁻¹, respectively, compared with the inoculated treatments, which gave 1520.72 kg ha⁻¹. As for the cobalt factor, the concentration of 1 kg CO ha⁻¹ gave the highest average of 3.53 tons ha⁻¹, followed by the level of 0.5 kg CO ha⁻¹, which gave 3.22 tons ha⁻¹, and finally, the treatment without adding cobalt gave the lowest average of 2.92 tons ha⁻¹, with a percentage increase of 20.89% and 10.27%. This is attributed to the effect of cobalt in increasing the growth of reproductive organs and thus increasing the speed of flowering, which may affect the increase in the yield, as confirmed by (15); the reason may also be indirectly due to the increase in vegetative characteristics, including dry weight and number of branches, which may lead to an increase in the number of flower-bearing branches and thus lead to an increase in fruits and yield. Cobalt has a positive effect on nitrogen fixation, which may lead to an increase in yield. These results are similar to what was found by (12) that adding cobalt led to a significant increase in the yield of beans and chickpeas, reaching 24 and 28 kg/hectare, respectively, compared to the treatment not supplied with cobalt. As for the bilateral interaction between varieties and bacteria, the treatment (variety Shaima and bacteria A3) was superior and gave the highest average of 3.99 tons ha⁻¹, while the treatment (variety Yema soya and no addition of

bacteria) gave the lowest average of 57.2 tons ha⁻¹, with a percentage increase of 55.25%. As for the interaction coefficients between varieties and cobalt, the treatment (Shaima variety and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 3.67 tons ha⁻¹, while the treatment (Yema soya variety and no cobalt addition) gave the lowest average of 2.81 tons ha⁻¹, with a percentage increase of 30.60%. As for the interaction between bacteria and cobalt, the treatment (A3 bacteria and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 4.37 tons ha⁻¹, while the control treatment (no addition of bacteria and no addition of cobalt) gave the lowest average of 2.53 tons ha⁻¹, with a percentage increase of 72.72%. The reason is that cobalt is one of the necessary elements to improve the symbiotic relationship of rhizobia with leguminous plants and to increase the efficiency of atmospheric nitrogen fixation, in addition to increasing the formation of effective root nodules, which ultimately leads to an increase in the yield (4 and 2). As for the triple interaction between varieties, bacteria and cobalt, the treatment (variety Shaima, bacteria A3 and cobalt 1 kg co ha⁻¹) was significantly superior and gave the highest average total seed yield of 4.70 tons ha⁻¹, while the comparison treatment (variety Yema soya, no addition of bacteria and no addition of cobalt) gave the lowest average of 2.39 tons ha⁻¹, with a percentage increase of 96.65%.

Table (4): The effect of Levels with Bradyrhizobium japonicum bacteria and three levels of cobalt fertilization and the interaction between them on the total seed yield (tons per hectare-1) of two varieties of soybean.

Variety	Levels	cobalt kg ha ⁻¹			Mean
		0	0.5	1	
Shaima	0	2.67	2.76	2.87	2.77
	A3	3.37	3.90	4.70	3.99
	W	3.15	3.54	3.73	3.48
	T	2.89	2.96	3.38	3.08
Yema soya	0	2.39	2.61	2.70	2.57
	A3	3.11	3.55	4.05	3.57
	W	2.94	3.47	3.61	3.34
	T	2.80	2.94	3.20	2.98
LSD 0.05		0.107			0.110
variety * cobalt					
Shaima		3.02	3.29	3.67	3.33
Yema soya		2.81	3.14	3.39	3.11
LSD 0.05		0.053			0.163
bacteria* cobalt					
0		2.53	2.69	2.79	2.67
A3		3.24	3.73	4.37	3.78
W		3.05	3.51	3.67	3.41
T		2.85	2.95	3.29	3.03
LSD 0.05		0.076			0.078
Mean.		2.92	3.22	3.53	
LSD 0.05		0.038			

%3.80 compared to the Yema soya variety, which gave the lowest average, reaching 3.66%, with a percentage increase of 3.82%. This may be attributed to the difference in the response of soybean varieties to different growth factors on the genetic susceptibility of any variety. Also, the plant's phenotypic characteristics are directly related to the plant's ability to absorb nutrients and its efficiency in carrying out the process of carbon assimilation (10). This is consistent with what was indicated by (29) and ((6), who obtained significant differences between varieties. As for the bacteria factor, level A3 was superior and gave the highest average of 4.21%, followed by level W and level T,

which gave averages of 3.72% and 3.59% respectively, while the comparison treatment gave the lowest average of 3.40%, with a percentage increase of 5.58%, 9.41%, and 23.82%. As for the cobalt factor, the concentration of 1 kg CO ha⁻¹ gave the highest average of 4.15%, followed by the level of 0.5 kg CO ha⁻¹, which gave 3.74%, and finally, the treatment without adding cobalt gave the lowest average of 3.29%, with a percentage increase of 26.13% and 13.67%, respectively. The reason may be attributed to the role of these added bacteria as inoculum in increasing the formation of root nodules and secreting some growth regulators that help improve and grow the plant and increase the root system, thus increasing the root nodules,

and the role of elements in forming and activating these nodules, thus increasing the amount of nitrogen fixed and absorbed by the plant. This result is consistent with (22), and this result is in line with what was found (25). As for the two-way interaction between the varieties and bacteria, the treatment (variety Shaima and bacteria A3) was superior and gave the highest average of 4.37%, while the treatment (variety Yema soya and no addition of bacteria) gave the lowest average of 3.36%, with a percentage increase of 30.05%. As for the interaction coefficients between varieties and cobalt, the treatment (variety Shaima and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 4.24%, while the treatment (variety Yema soya and no cobalt addition) gave the lowest average of 3.20%, with a percentage increase of 32.5%. As for the interaction

between bacteria and cobalt, the treatment (bacteria A3 and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 4.99%, while the comparison treatment (no addition of bacteria and no addition of cobalt) gave the lowest average of 3.06 and a percentage increase of 63.07%. This result is consistent with (27) and (28) and also this result is consistent with (31), (24) and (19). As for the triple interaction between varieties, bacteria and cobalt, the treatment (variety Shaima, bacteria A3 and cobalt 1 kg CO ha⁻¹) was significantly superior and gave the highest average nitrogen content in the seeds, reaching 5.29%, while the comparison treatment (variety Yema soya, no addition of bacteria and no addition of cobalt) gave the lowest average, reaching 3.03%, with a percentage increase of 74.58%.

Table (5): The effect of Levels with *Bradyrhizobium japonicum* bacteria and three levels of cobalt fertilization and their interaction on the nitrogen content in the seeds (%) of two varieties of soybean.

Variety	Levels	cobalt kg ha ⁻¹			Mean
		0	0.5	1	
Shaima	0	3.08	3.47	3.76	3.43
	A3	3.73	4.10	5.29	4.37
	W	3.50	3.81	4.01	3.77
	T	3.20	3.72	3.92	3.61
Yema soya	0	3.03	3.31	3.75	3.36
	A3	3.40	4.05	4.70	4.05
	W	3.26	3.80	3.94	3.66
	T	3.12	3.69	3.87	3.56
LSD 0.05		0.102			0.088
variety * cobalt					
Shaima		3.38	3.77	4.24	3.80
Yema soya		3.20	3.71	4.06	3.66
LSD 0.05		0.051			0.133
bacteria* cobalt					
0		3.06	3.39	3.75	3.40
A3		3.57	4.08	4.99	4.21
W		3.38	3.80	3.98	3.72
T		3.16	3.71	3.90	3.59

LSD 0.05	0.072			0.062
Mean.	3.29	3.74	4.15	
LSD 0.05	0.036			

reaching 22.87%, with a percentage increase of 3.71%. The reason may be attributed to genetic differences that affect the chemical composition and enzyme activity of the seeds (14). The plant's phenotypic characteristics are also directly related to the plant's ability to absorb nutrients and its efficiency in carrying out the process of carbon assimilation (10). This is consistent with what was indicated by (29). As for the bacterial factor, level A3 was superior and gave the highest average of 26.32%, followed by level W and level T, which gave averages of 23.24% and 22.38%, respectively, while the control treatment gave the lowest average of 21.24%, with a percentage increase of 9.41%, 23.91% and 5.36%, respectively. The significant superiority in protein content in seeds as a result of inoculation with *Bradyrhizobium japonicum* bacteria may be due to the ability of these bacteria cells to compete in causing root infection in the nodules, and their activity in providing plant nutrients, especially nitrogen, which was reflected in the root system and dry green weight, which achieved the best number of pods, which led to the best production of the crop, which increased the protein content. This result is consistent with what was found (25). As for the cobalt factor, the concentration of 1 kg CO ha⁻¹ gave the highest average of 25.96%, followed by the level of 0.5 kg CO ha⁻¹, which gave 23.39%. Finally, the treatment without adding cobalt gave the lowest average of 20.54%, with a percentage increase of 26.38% and 13.87%, respectively. The reason is attributed to the role of the element in the formation and activity of root nodules and increasing their

numbers, thus increasing the amount of nitrogen fixed symbiotically and making it available to the plant, which led to an increase in the percentage of nitrogen and protein in the seeds. This result is consistent with (16). As for the two-way interaction between the varieties and bacteria, the treatment (variety Shaima and bacteria A3) was superior and gave the highest average of 27.32%, while the treatment (variety Yema soya and no addition of bacteria) gave the lowest average of 21.02%, with a percentage increase of 29.97%. As for the interaction coefficients between varieties and cobalt, the treatment (Shaima variety and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 26.52%, while the treatment (Yema soya variety and no cobalt addition) gave the lowest average of 20.02%, with a percentage increase of 32.46%. As for the interaction between bacteria and cobalt, the treatment (bacteria A3 and cobalt at a concentration of 1 kg CO ha⁻¹) was significantly superior and gave the highest average of 31.20%, while the comparison treatment (no addition of bacteria and no addition of cobalt) gave the lowest average of 19.09%, with a percentage increase of 63.43%. As for the triple interaction between varieties, bacteria and cobalt, the treatment (variety Shaima, bacteria A3 and cobalt 1 kg co ha⁻¹) was significantly superior and gave the highest average protein percentage in the seeds, reaching 33.04%, while the comparison treatment (variety Yema soya, no addition of bacteria and no addition of cobalt) gave the lowest average, reaching 18.96%, with a percentage increase of 74.26%.

Table (6): The effect of Levels with Bradyrhizobium japonicum bacteria and three levels of cobalt fertilization and their interaction on the percentage of protein in the seeds (%) of two varieties of soybean.

Variety	Levels	cobalt kg ha ⁻¹			Mean
		0	0.5	1	
Shaima	0	19.23	21.68	23.48	21.46
	A3	23.31	25.62	33.04	27.32
	W	21.89	23.79	25.06	23.58
	T	19.79	23.25	24.52	22.52
Yema soya	0	18.96	20.66	23.44	21.02
	A3	21.27	25.31	29.35	25.31
	W	20.35	23.73	24.62	22.90
	T	19.49	23.08	24.18	22.25
LSD 0.05		0.681			0.561
variety * cobalt					
Shaima		21.05	23.58	26.52	23.72
Yema soya		20.02	23.20	25.40	22.87
LSD 0.05		0.341			0.760
bacteria * cobalt					
0		19.09	21.17	23.46	21.24
A3		22.29	25.47	31.20	26.32
W		21.12	23.76	24.84	23.24
T		19.64	23.16	24.35	22.38
LSD 0.05		0.482			0.397
Mean		20.54	23.39	25.96	

LSD 0.05	0.241		-5Alexander, Martin. 1982. Introduction to Soil Microbiology. Second ed. John Wiley & Sons. New York.
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