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Effect of Bradyrhizobium Inoculation on Yield and Yield's Components of Soybean (*Glycine max* (L.)) Grown in Sudan

[1,5]Khalid A. Ibrahim, [2]Elsiddig A.E.Elsheikh, [3]Ahmed M. El Naim and [4]Elbasri A.Mohamed

[1]Department of soil &water sciences, Faculty of natural Resources & Environmental Studies, University of Kordofan, Elobeid,P.O.BOX,160
[2]Department of soil sciences & Environment, Faculty of Agriculture, University of Khartoum, Shambat, Sudan
[3]Department of Crop Sciences, Faculty of Natural Resources and Environmental Studies, University of Kordofan, Elobied, P.O.BOX,160,Sudan
[4]Department of Agricultural Engineering, Faculty of agricultural Technology and Fish Sciences, Al-Neelain University, Khartoum, P.O.Box (12702), Sudan.
[5]Department of Plant Production, College of Food Science and Agriculture, King Saud University, Riyadh, Kingdom of Saudi Arabic.

Abstract: Field experiments were carried out at Shambat, Sudan (Latitude 15º 40´N and Longitude 32º 32´E) in three consecutive seasons (2000/03) to investigate the effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on growth, nodulation and yield of soybean. The results showed that inoculation, chicken manure, sulphur and their interaction significantly (P=0.05) improved the dry weight of shoots and roots, nodulation, yield and yield components. Measured parameters increased by with increasing the level of chicken manure or sulphur and the highest value of each parameter above were observed with either 10 t/fed chicken manure or 100 kg/fed sulphur with or without Bradyrhizobium inoculation. The residual effect of chicken manure or sulphur significantly (P=0.05) improved all the measured parameters at 10 t/fed chicken manure or 100 kg/fed sulphur with or without Bradyrhizobium inoculation.

Key words: Bradyrhizobium, inoculation, chicken manure, sulphur, soybean, yield.

INTRODUCTION

The importance of legumes as food lied primarily in their high protein content that averages 20 – 25%. Soybean (*Glycine max* (L.)) is a unique crop, containing 32 – 45% proteins extracted substances. Seeds are used as a raw material for the production of top-quality dry oil, varnishes, soaps, plastics, candies, shampoos, pesticides, paints, disinfectants, strong glues and adhesives. The soybean meals and cakes contributed about 70% of the international trade in meals and cakes (ITC, 1990). Soybean is the world's oldest cultured plant. It originated in East Asia (China), where people began growing it some five thousand years ago. In America soybean becomes the most important grain legume crop (IITA, 2002). According to FAO (1999), soybean estimates over 160 million tonnes of soybean was produced worldwide in 2000. The leading producer is the USA which accounted for 49%, Latin America and Caribbean produced 34%, Asia 14% and Africa less than 1%. The average yield in 2000 was 2210 kg/ha, ranging from about 3520 kg/ha in Western Europe and 2650 kg/ha in the USA, to 990 kg/ha in Africa (IITA, 2002). Soybean research was discontinued in the late 1940's in Sudan. Recently, the interest in soybean has been increased and research work on soybean has been reactivated (Khalifa, 1987; Mukhtar and Abu-Naib, 1987; Mahdi and Abdel Aziz, 1992). This was due to increasing demand for soybean as a cash crop. Sudanese commercial production started the cultivation of soybean in 1981 at the Damazin Sudanese-Egyptian Integration Agricultural Project and at Agadi, by the Arab Organization for Development and Agricultural Investment started a pilot commercial field at Agadi. Grain legumes such as cowpea and soybean are good nitrogen fixers, and they usually meet all of their nitrogen needs other than that absorbed from the soil (Lindet *et al.*, 1997). Grain legumes fix about 15 – 210 kg/ha/y (Dakora and Keya, 1997). Several workers have demonstrated that traditional seed inoculation with *Bradyrhizobium* strains favours the formation of nodules on primary root near the inoculation point (Milic *et al.*, 1992; Soliman *et al.*, 1996). Bradyrhizobia in soil appear relatively immobile (Zahran, 1999). Thus the objective of this study to investigate the effect of Bradyrhizobium strains, S and CHM on nodulation, growth and yield of soybean in shambat soil of Sudan.

Corresponding Author: Khalid A. Ibrahim, Department of soil &water sciences, Faculty of natural Resources & Environmental Studies, University of Kordofan, Elobeid, P.O.BOX,160,
E-mail: Sudan, zeeteer@hotmail.com

MATERIALS AND METHODS

Materials:

soybean (Glycine max L.) cultivar Jupitar used in this study was supplied kindly by Arab Corporation for Agricultural Investment and Development, Khartoum , Sudan. Bradyrhizobium (TAL 109) was obtained from the Biofertilization Department, Environment and Natural Resources Institute, National Centre for Research, Khartoum, Sudan. The Yeast Extract Manitol (YEM) medium was prepared according to Cleyet-Marel method.Chicken manure was obtained from The Top Farm of Faculty of Agriculture, University of Khartoum, Shambat, Sudan. Elemental sulphur was obtained from El Geneed Sugar Industry, Sudan. Strains of Bradyrhizobium were preserved by streaking on YEM agar ,mixed with 3.0 g of calcium carbonate per litre, in slants in screw –caps test tubes and kept in the refrigerator at 4º C. Seeds were inoculated by mixing with a thick suspension of charcoal based Bradyrhizobium inoculums ,with average count of 1×10^9 c fu/g .Arabic gum solution(40%) was added for good adhesion .Seed inoculation was carried out in the soil and irrigated .Unless otherwise stated all chemicals and reagents used in this are of reagent grade.

Field Experiments:

Three field experiments were conducted during three successive seasons (2000/01, 2001/02 and 2002/03) at the Demonstration Farm of the Faculty of Agriculture, Shambat, University Khartoum, Sudan (Latitude 15º 40 N and Longitude 32º 32 E) with the following soil composition: 23% sand, 32% silt, 45% clay, 0.05 total N,0.033% P,1.40% organic matter, 8.0 PH value, Ece 0.52(dS/m) and cations (meq/l) 0.12K,4.50 Na,1.90 Ca and 0.70 Mg.

Chicken Manure and Sulphur Application:

Two separate experiments were conducted, for one experiment chicken manure was applied at different levels (0, 2.5, 5.0, 7.5 and 10.0 t/fed), distributed along the ridges and mixed with the soil and then the soil divided into plots. Thereafter, the plots were irrigated twice for two weeks before sowing. For the other experiment elemental sulphur was applied at different levels (0, 25, 50, 75 and 100 kg/fed). The soil was divided into plots and the plots were irrigated twice before sowing for two weeks.

Treatments:

The treatments were replicated three times in a split-split plot design. The treatments used during the first and second seasons were divided into groups as follows:

Un inoculated:

Only chicken manure was applied at different levels (0, 2.5, 5.0, 7.5 and 10.0 t/fed).

Inoculated:

The seeds were inoculated and the soil was amended with manure at different levels (0, 2.5, 5.0, 7.5 and 10.0 t/fed).

Un inoculated:

Only elemental sulphur was applied at different levels (0, 25, 50, 75 and 100 kg/fed).

Inoculated:

Only elemental sulphur was applied at different levels (0, 25, 50, 75 and 100 kg/fed).

In the third season the residual effect of either chicken manure or elemental sulphur in the presence or absence of Bradyrhizobium inoculation was investigated.

Sampling:

Three samples from each plot were taken at 4 and 8 weeks after sowing to determined the number of nodules per plant and at 12 weeks to determine the dry weights of shoots and roots. At harvesting five plants were taken to determine plant height (cm), number of pods per plant, 100-seed weight (g), grain yield (g) per plant and total yield per unit area (kg/ha). Analysis of variance was performed to determine the effect of each treatment and interaction between treatments, on the measured parameters. The treatment means were separated by the Least Significant Difference Test (LSD, $P\leq 0.05$).

RESULTS AND DISCUSSION

Weight and Number of Shoots, Roots, Nodules and Pods:

Bradyrhizobium inoculation significantly ($P< 0.05$) increased dry weights of shoot, dry weight of root, number of nodules / plant and number of pods /plant of soybean compared to the uninoculated control in all seasons (Tables 1, 2, 3, 4 and 5). Chicken manure and sulphur treatments significantly increased the dry weights of shoots and roots in all seasons, the significance increased with the level of treatments. Interactions between treatments significantly increased the dry weights of shoots, roots, nodules and pods in all seasons. These results are in agreement with those reported by (Elsheikh, 1998), Okereke and Unaeglu (1992), Salih (2002), Penfold *et al.* (1995), Pankhurst *et al.* (1997) and Schawarz *et al.* (1999).

Table 1: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on shoot dry weight (g) after 12 weeks of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	11.60	12.50	12.05	8.77	11.01	9.89	12.54	13.12	12.82
2.5t/fed	18.70	20.90	19.80	16.53	19.17	17.85	16.14	18.76	17.45
5.0t/fed	22.10	30.70	26.40	19.10	33.30	26.20	20.57	22.09	21.33
7.5t/fed	29.60	46.00	37.80	27.20	50.06	38.63	23.81	30.89	27.35
10 t/fed	46.70	52.70	49.70	45.62	55.30	50.46	33.32	34.60	33.96
Means	25.74	32.56		23.44	33.76		22.47	22.69	
Overall			29.15			28.60			28.58
				Sulphur					
Control	10.70	11.80	11.25	8.22	10.16	9.19	11.56	13.32	12.44
25kg/fed	16.10	16.90	16.50	15.79	16.43	16.11	12.42	18.96	15.69
50kg/fed	17.70	18.40	18.05	18.68	34.88	26.78	20.04	22.26	21.15
75kg/fed	26.80	44.60	35.70	26.84	47.94	37.39	24.16	30.62	27.39
100kg/fed	41.80	50.20	46.00	43.56	49.78	46.67	35.12	36.44	35.78
Means	22.62	28.38		22.62	31.84		20.66	24.32	
Overall			25.50			27.23			22.49
Treatment (s)					LSD (5%)				
				1st season	2nd season	Residual(3rd season)			
Amendments				1.84	2.31	0.63			
Inoculated				0.90	0.74	0.48			
Amendments × Inoculated				2.80	3.01	0.90			
Amendments × Level				4.43	4.76	1.42			
Amendments × Inoculated × Level				6.12	6.73	2.00			

Table 2: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on root dry weight (g) after 12 weeks of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	0.82	0.86	0.84	0.48	0.58	0.53	1.86	1.86	1.86
2.5t/fed	1.38	1.24	1.31	0.90	1.40	1.15	2.10	2.20	2.15
5.0t/fed	1.76	1.94	1.85	1.30	1.44	1.37	2.36	2.78	2.57
7.5t/fed	1.99	2.07	2.03	1.34	1.52	1.43	2.94	2.86	2.90
10 t/fed	2.12	2.26	2.19	1.47	1.61	1.54	3.46	3.30	3.39
Means	1.61	1.67		1.11	1.31		2.55	2.61	
Overall			1.64			1.21			2.58
Control	1.22	1.40	1.31	0.88	0.86	0.87	1.80	1.84	1.82
25kg/fed	1.38	1.44	1.41	0.81	1.13	0.97	2.06	2.18	2.12
50kg/fed	1.46	1.50	1.48	0.96	1.09	1.02	2.24	2.80	2.52
75kg/fed	1.49	1.71	1.60	1.04	0.98	1.01	2.82	2.84	2.83
100kg/fed	1.63	1.85	1.74	1.00	1.73	1.37	3.40	3.22	3.31
Means	1.44	1.58		0.94	1.16		2.46	2.58	
Overall			1.51			1.05			2.52
Treatment (s)					LSD (5%)				
				1st season	2nd season	Residual(3rd season)			
Amendments				0.06	0.09	0.10			
Inoculated				0.03	0.05	0.03			
Amendments × Inoculated				0.11	0.16	0.12			
Amendments × Level				0.25	0.31	0.12			
Amendments × Inoculated × Level				0.37	0.48	0.26			

Table 3: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on number of nodules per plant after 4 weeks of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	2.00	3.18	2.59	1.33	2.17	1.75	4.25	6.91	5.58
2.5t/fed	3.32	4.02	3.67	2.51	3.09	2.80	7.17	10.25	8.71
5.0t/fed	4.17	5.83	5.00	3.96	5.10	4.53	10.24	15.30	12.77
7.5t/fed	4.83	6.17	5.50	4.54	6.04	5.29	15.31	18.55	16.93
10 t/fed	2.18	3.66	2.92	1.93	2.91	2.42	5.20	7.32	6.26
Means	3.31	4.57		2.86	3.86		8.43	11.67	
Overall			3.94			3.36			10.05
					Sulphur				
Control	1.50	1.68	1.59	1.83	2.07	1.95	5.00	8.24	6.62
25kg/fed	2.87	3.37	3.12	2.68	3.42	3.05	7.35	12.41	9.88
50kg/fed	4.80	6.32	5.56	4.60	6.48	5.54	11.72	18.20	14.96
75kg/fed	6.24	9.12	7.68	6.04	9.70	7.87	17.09	23.45	20.27
100kg/fed	9.00	11.88	10.44	8.96	10.08	10.52	19.21	29.23	24.22
Means	4.88	6.48		6.23	6.35		12.07	18.31	
Overall			5.68			6.29			15.19

Treatment (s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.42	0.39	0.60
Inoculated	0.27	0.21	0.42
Amendments × Inoculated	0.64	0.58	0.84
Amendments × Level	1.10	1.00	1.33
Amendments × Inoculated × Level	1.60	1.42	1.88

Table 4: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on number of nodules per plant after 8 weeks of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	3.00	5.16	4.08	2.50	5.50	4.00	9.09	11.25	10.17
2.5t/fed	4.50	7.82	6.16	5.14	7.92	6.53	14.20	17.64	15.92
5.0t/fed	8.00	11.36	9.68	8.86	12.24	10.55	18.78	24.14	18.21
7.5t/fed	11.04	13.66	12.35	12.18	14.76	13.46	27.19	37.03	39.03
10 t/fed	3.83	5.17	4.50	2.04	5.62	3.83	10.24	13.20	11.72
Means	6.07	8.63		6.14	9.20		15.91	20.65	
Overall			7.35			7.67			18.28
					Sulphur				
Control	2.67	4.27	3.47	3.04	4.78	3.91	8.80	10.88	9.84
25kg/fed	4.13	6.87	5.50	4.52	7.40	5.96	13.94	17.42	15.68
50kg/fed	7.34	9.14	8.24	7.11	9.63	8.37	16.99	25.67	21.33
75kg/fed	9.90	10.82	10.36	10.02	11.98	11.00	28.00	36.80	32.40
100kg/fed	12.00	13.56	12.78	13.04	14.12	13.58	30.61	41.37	35.99
Means	7.21	8.93		7.54	9.58		19.66	26.42	
Overall			8.07			8.56			23.04

Treatment (s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	0.60	0.51	1.00
Inoculated	0.41	0.30	0.75
Amendments × Inoculated	0.86	0.73	1.37
Amendments × Level	1.40	1.16	2.08
Amendments × Inoculated × Level	2.04	1.64	3.00

Table 5: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on number of pods per plant of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	52.83	53.25	53.04	55.83	58.13	56.98	36.08	44.84	40.46
2.5t/fed	59.83	60.25	60.04	66.56	58.22	6.239	39.67	43.59	41.63
5.0t/fed	61.75	78.83	70.29	70.83	79.89	75.36	48.83	58.75	53.79
7.5t/fed	66.75	82.43	74.58	80.85	87.87	84.36	54.17	67.75	60.96
10 t/fed	71.75	88.33	80.04	87.70	96.44	92.07	61.83	78.51	70.17
Means	62.58	72.62		72.36	76.11		48.11	58.69	
Overall			67.60			74.23			53.40

Table 5: Continue

				Sulphur				
Control	46.00	47.76	46.88	47.68	49.53	48.63	36.58	41.26	38.92
25kg/fed	47.75	53.59	50.67	53.83	54.25	54.04	37.67	43.17	40.42
50kg/fed	47.97	55.51	51.74	58.58	59.76	59.17	47.75	56.75	52.25
75kg/fed	50.08	60.34	55.21	60.00	62.08	61.04	51.50	63.78	57.64
100kg/fed	60.92	64.42	62.67	63.58	66.82	65.20	60.42	74.32	67.37
Means	50.54	56.32		56.73	58.49		46.78	55.86	
Overall			53.43			57.61			51.32
Treatment (s)					LSD (5%)				
				1st season	2nd season	Residual(3rd season)			
Amendments				2.26	2.59		2.26		
Inoculated				1.39	1.60		1.39		
Amendments × Inoculated				2.69	3.66		2.69		
Amendments × Level				4.48	5.78		4.48		
Amendments × Inoculated × Level				6.14	8.18		6.14		

Table 6: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on 100-seed weight (g) of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	11.15	12.15	11.65	10.27	10.79	10.53	20.52	20.94	20.73
2.5t/fed	15.43	14.13	14.78	14.32	14.56	14.44	20.88	20.98	20.93
5.0t/fed	15.56	16.04	15.80	14.70	14.82	14.76	21.68	21.98	21.83
7.5t/fed	15.89	16.61	16.25	15.09	14.97	15.03	22.20	22.12	22.16
10 t/fed	15.96	16.88	16.42	15.15	15.35	15.25	22.97	23.03	23.00
Means	14.78	15.16		13.90	14.10		21.65	21.83	
Overall			14.97			14.00			21.74
					Sulphur				
Control	10.81	11.27	11.04	8.75	10.43	9.59	19.19	20.61	19.90
25kg/fed	14.34	15.40	14.87	13.21	13.77	13.49	19.44	20.80-	20.12
50kg/fed	14.96	15.40	1518	14.28	14.76	14.52	20.46	20.94	20.70
75kg/fed	15.16	15.92	15.54	14.67	15.45	15.06	20.74	21.22	20.98
100kg/fed	15.80	16.12	15.96	15.34	15.68	15.51	21.04	22.18	21.61
Means	14.22	14.82		13.25	14.01		20.17	21.15	
Overall			22.49			13.63			20.66
Treatment (s)					LSD (5%)				
				1st season	2nd season	Residual(3rd season)			
Amendments				0.56	0.49		0.56		
Inoculated				0.39	0.28		0.39		
Amendments × Inoculated				0.86	0.69		0.86		
Amendments × Level				1.20	1.10		1.20		
Amendments × Inoculated × Level				2.09	1.56		2.09		

Table 7: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on grain yield per plant (g) of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	30.78	31.02	30.90	27.42	29.12	28.27	52.24	58.48	55.36
2.5t/fed	33.39	36.44	34.92	29.69	32.13	30.91	57.04	69.00	63.02
5.0t/fed	37.71	42.13	39.92	31.90	35.66	33.78	64.43	76.59	70.51
7.5t/fed	38.67	45.9	42.18	35.49	39.17	37.33	73.00	85.92	79.46
10 t/fed	39.02	55.27	47.14	45.14	51.94	48.54	88.53	94.23	91.38
Means	35.91	42.11		33.92	37.60		67.04	76.84	
Overall			39.01			35.76			71.94
					Sulphur				
Control	29.53	30.01	29.77	25.42	28.14	26.78	48.18	54.66	51.42
25kg/fed	30.93	33.81	32.37	29.87	32.01	30.94	52.21	64.47	58.34
50kg/fed	37.89	39.91	38.90	31.25	36.09	33.67	59.89	71.95	65.92
75kg/fed	39.27	41.19	36.23	34.15	39.41	36.78	70.38	84.16	77.27
100kg/fed	45.20	48.82	47.01	45.95	52.19	49.07	86.63	98.35	92.49
Means	36.56	38.74		33.32	37.56		63.45	74.71	
Overall			37.65			35.44			69.08
Treatment (s)					LSD (5%)				
				1st season	2nd season	Residual(3rd season)			

Table 7: Continue

Amendments	1.26	2.04	1.39
Inoculated	0.72	1.01	0.87
Amendments × Inoculated	1.78	3.30	1.92
Amendments × Level	2.82	5.81	3.05
Amendments × Inoculated × Level	3.98	8.63	4.12

Table 8: Effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on yield (kg/ha) of soybean grown for three consecutive seasons.

Seasons	1st season			2nd season			Residual(3rd season)		
Treatment	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means	Uninoculated	Inoculated	Means
Chicken Manure									
Control	764.6	738.7	751.6	694.4	916.2	805.8	732.13	807.23	769.68
2.5t/fed	794.9	867.7	831.3	1072.4	1001.8	1037.1	937.20	713.00	825.10
5.0t/fed	897.9	1097.7	997.8	1198.6	1428.7	1313.7	836.87	896.79	866.83
7.5t/fed	801.3	1087.7	944.5	1411.0	1545.7	1478.3	905.53	848.03	876.78
10 t/fed	929.0	1316.3	1122.7	1155.3	1708.7	1432.0	846.90	866.90	856.90
Means	837.5	1021.6		1106.6	1320.2		851.72	826.39	
Overall			929.6			1213.4			839.06
					Sulphur				
Control	512.5	666.9	589.7	729.4	980.3	854.9	376.07	701.79	538.93
25kg/fed	498.4	709.8	604.1	713.8	1036.1	875.0	605.13	696.75	650.95
50kg/fed	708.3	712.1	710.2	821.7	1156.7	989.2	467.50	646.30	556.90
75kg/fed	577.9	671.2	624.6	875.1	1177.3	1026.2	666.20	811.56	738.88
100kg/fed	576.2	683.9	630.1	1018.5	1058.8	1038.7	689.43	776.69	733.06
Means	574.7	688.8		831.7	1081.8		560.86	726.26	
Overall			631.7			956.8			741.40

Treatment (s)	LSD (5%)		
	1st season	2nd season	Residual(3rd season)
Amendments	19.6	26.31	22.35
Inoculated	15.4	23.54	18.51
Amendments × Inoculated	22.3	33.25	26.42
Amendments × Level	17.5	25.09	20.16
Amendments × Inoculated × Level	31.2	43.14	36.48

Yield and Yield Components:

The effect of Bradyrhizobium inoculation and chicken manure or sulphur fertilization on grain yield (g/plant) and yield (kg/ha) of soybean are representing in Table 7 and 8. Inoculation with Bradyrhizobium, chicken manure and sulphur significantly ($P< 0.05$) increased the grain yield (g/plant) and yield (kg/ha) compared to the uninoculated untreated control in all seasons. However, grain yield (g/plant) increased gradually with the increase in the level of chicken manure and sulphur in all seasons. While the 100-seed weight un affected with inoculation in all seasons compared to the uninoculated control (Table 6). It was found to be positively correlated with grain yield of legume crops. These results agree with the findings of Abdelmula *et al.* (1993) in faba bean, Rajput *et al.*, (1991), Ishag (1994) and Abdel-Hafeez, (2001) in hyacinth bean, Abdelgani *et al.* (2003) in fenugreek and Salih (2002) in soybean.

Conclusion:

Bradyrhizobium inoculation, chicken manure and sulfur significantly affected the growth and yield of soybean crop .while the highest results of yield and yield components were associated with the additional of 10 t of manure/fed or 100kg S/fed .

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