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ROLE OF BIOFERTILIZERS IN IMPROVING COWPEA PRODUCTIVITY CULTIVATED IN SANDY SOIL

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ABSTRACT

Cowpea is one of the fast-growing legume crops during the summer and is well suited to the summer environmental conditions in Egypt. In addition, it is a high-quality crop for feeding sheep and cattle, also very important fodder for dairy cows. In this study, an experiment was designed to evaluate the effect of biofertilizers on cowpea productivity cultivated under sandy soil conditions at Ismailia Governorate. To reach such aim, an identified *Streptomyces* strain was used with a mixture of biochar, and organic fertilizer in several different treatments compared to bradyrhizobia. The experiment was carried out at the Ismailia Research Station, ARC. Results showed that the number of nodules at the 2nd cutting was always higher than that of the 1st cutting. No results were recorded indicating the presence of root nodules in cowpea plants to which bradyrhizobia was not added. Results showed that the fresh weight of cowpea plants under investigation as shown in the 16 treatments was inconsistent and reflects the importance of biofertilizers. Percentages of nitrogen and crude protein among two cutting of cowpea plants cultivated under showed that the 2nd cut was always higher in the percentage of crude protein than the 1st cut. Treatment T16 containing bradrhizobia and actinomycetes as biofertilizers, organic fertilizer, low concentration of nitrogen fertilizer and biochar appeared the highest fresh weight per Feddan compared to all treatments. Results of the yield (Ton/Fed) of dry weight of the cowpea plants has become in the same trend as the results of fresh weight. As a conclusion, treatment coded T16 which containing *Bradyrhizobium*sp. (*Vigna*)and actinomycetes as biofertilizers, organic fertilizer, low concentration of nitrogen fertilizer and biochar appeared the highest levels of no. of nodules, nodules dry weight, fresh and dry weights of cowpea shoots, percentage of crude protein and yield of fresh and dry weight (Ton/Feddan).

Key words: *Cowpea, Organic fertilizer, Biochar, Biofertilizers, Bradyrhizobium* sp. *(Vigna), actinomycetes, yield,* nodules dry weight*, Crude protein.*

INTRODUCTION

Cowpea (*Vignaunguiculata* L. Walp.) is a quick growing and high yielding crop feed to livestock and also makes a valuable contribution towards human food in tropical and subtropical parts of the world (Kumar et al.,2014). Cowpea is suitable for Egypt summer environmental conditions. Cowpea is the fastest growing annual summer forage legume. It is an excellent quality crop for fattening both sheep and cattle and is also regarded as good feed for milking cows. Biofertilizers are microorganisms that enrich the nutrient quality of soil. The main sources of biofertilizers are bacteria, fungi, and cynobacteria. The most striking relationship that these have with plants is symbiosis, in which the partners derive benefits from each other (Al Abboud et al.,2014). Biofertilizers are products applied on the surface of a plant or in soil and contain live microorganisms that promote plant growth and development. These products may include bacterial species such as *Rhizobium*, *Azotobacter*, and *Azospirilium* as well as blue green algae (BGA) (Kumar et al.,2017 and Noufal et al., 2018). The need for N fertilizers could be reduced by biological nitrogen fixation (Nicolás et al., 2006) in other mean bio-fertizers (Ewees and Abdel Hafeez, 2010). Inoculation of cowpea seeds with *Rhizobium japonicum* was effective. An essential role in crop establishment and yield, was reported as a result of using biological nitrogen fixation, where N fertilizer was not apply, and it save the most needed nitrogen of plants (Chen, 2006). Oad et al. (2004) reported that an increase in germination of seeds appears as a direct result to improving soil productivity by adding plant growth-promoting rhizobacteria (PGPR) which considered as a group of free-living bacteria that colonize the rhizosphere and benefit the root growth. Nitrogen fixation and plant growth promotion by rhizobacteria are important criteria for an effective biofertilizer. Rhizobiaare legume root nodule bacteria. Arhizobium is a legume root nodule bacterium, and fix N_2 (diazotroph) after becoming established inside root nodules of legumes (Fabaceae). Rhizobacteria, through nitrogen fixation, are able to convert gaseous nitrogen (N_2) to ammonia (NH_3) making it an available nutrient to the host plant which can support and enhance plant growth. The host plant provides the bacteria with amino acids so they do not need to assimilate ammonia. Several microorganisms are commonly used as biofertilizers including nitrogen-fixing soil bacteria (*Azotobacter*, *Rhizobium*), nitrogen-fixing cyanobacteria (*Anabaena*), phosphate-solubilizing bacteria (*Pseudomonas* sp.), and AM fungi (Kumari et al., 2019). Biofertilizers trap atmospheric nitrogen to the soil and convert them into plant usable forms. They also convert the insoluble phosphate forms into plant available forms. They stimulate root growth by producing some hormones and antimetabolites. Effects of PGPR can occur via local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens throughout the entire plant. Of these bacteria *Bacillus*, *Pseudomonas* spp., *Rhizobium japonicum* and *B. elkanii* strains. PGPR improve plant growth directly by producing plant growth regulators such as auxins, gibberellins and cytokinins; by eliciting root metabolic activities and/or by supplying biologically fixed nitrogen. Consequently, germination, root development, nutrient and water uptake are improved (Kumar et al., 2017). Biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirilium* and blue green algae (BGA) have been in use a long time. Rhizobium inoculant is used for leguminous crops. *Azotobacter* can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. Ștefuanescu and Palanciuc (2000) found greater seed yield of cowpea crop due to *Rhizobium japonicum* inoculation. Tran et al. (2001) found that the nutrient contents of cowpea plants, mainly N, P and K as well as soil available P and K were significantly improved by the application of bio-fertilizer (*Rhizobium fredii* and *Bradyhrizobium* sp.). *Rhizobium* seed inoculation alone significantly increased soil nitrogen content and soil available phosphorus compared to the control in both seasons (Hatim, 2013). The experiment was designed to evaluate the effect of biofertilizers on cowpea productivity cultivated under the sandy soil conditions in Ismailia Governorate. To reach such aim, an identified *Streptomyces* strain associated with rhizobia was used with a mixture of biochar, organic fertilizer among several different treatments compared to unfertilized. The experiment was carried out among two summer seasons at the Ismailia Research Station, Soil, Water and Environmental Research Institute.

MATERIALS AND METHODS

Location and season: During the two summer seasons of 2020 and 2021 at Section 9, Ismailia Agricultural Research Station, Ismailia Governorate, Egypt a field experiment was conducted. Soil properties: Physical and chemical properties and type of the soil of the experiments among the two seasons were determined according to method of Page et al. (1982) and Cottenie et al. (1982), as recording in Table (1). Properties of used fertilizers: Properties of organic fertilizer (farmyard manure) used in the two cultivated seasons as fertilizers were determined and recorded in Table (2). Regarding the biochar, it was found to be characterized by: Total N 0.144%, N-NH_4 770 ppm, N-NO_3 100 ppm, P 0.17 ppm, K 0.51 ppm, Fe 3.95 ppm, Mn 0.38 ppm, Zn 0.37 ppm, and Cu 0.49 ppm.

Table -1: Physical and chemical analyses of cultivated soil among the two growing seasons.

Parameters		1st Season	2nd Season
Sand	(%)	41.7	40.9
Find sand	(%)	42.0	41.4
Silt	(%)	10.8	11.8
Clay	(%)	05.5	05.9
Textures		Sandy	Sandy
pH	(1:2.5)	8.14	8.19
E.C.	(dSm^{-1} at 25ºC)	0.56	0.56
SP	(%)	27.1	28.2
Soluble cations	(mmol/L)		
Ca^{2+}		1.48	1.41
Mg^{2+}		0.49	0.49
Na^{+}		0.23	0.22
K^{+}		2.92	2.89
Soluble anions	(mmol/L)		
CO_3^{2-}		00.0	00.0
HCO_3^{-}		0.48	0.45
Cl^{-}		3.47	3.34
SO_4^{2-}		1.16	1.17

Nitrogen	(%)	0.017	0.018
Total soluble-N	(ppm)	97.10	97.40
Available-P	(ppm)	08.37	08.93
Available-K	(ppm)	94.70	94.20
DTPA-extract			
Fe	(ppm)	1.20	1.50
Mn	(ppm)	0.37	0.39
Zn	(ppm)	0.11	0.12
Cu	(ppm)	0.06	0.08

DTPA: Di-ethylene tri-amine penta acetic acid.

Table -2: Properties of organic fertilizer (farmyard manure) used in the two seasons.

Properties	First season	Second Season
pH	7.35	7.28
E.C. (dS/m at 25°C)	4.26	4.48
Organic-C (%)	15.25	16.84
Total N (%)	1.34	1.27
C/N ratio	11.83	13.26
Total-P (%)	0.64	0.95
Total-K (%)	1.35	1.30
Total soluble-N (ppm)	92.5	84.6
Available-P (ppm)	18.3	21.5
Available-K (ppm)	645.0	682.5
DTPA extractable (ppm):		
Fe	135.5	142.8
Mn	32.9	30.80
Zn	21.8	28.40
Cu	2.35	2.95
Total count of bacteria	$6.0X10^7$	$9.2X10^6$
Total count of fungi	$5.4X10^6$	$2.6X10^6$
Total count of actinomycetes	$3.8X10^6$	$2.7X10^6$
Dehydrogenase activity (µg TPF/g)	108.4	113.8

Source of cowpea seeds and cultivation distance: Cowpea seed was kindly obtained from Seed management, Agricultural Research Center (ARC), Giza, Egypt. Seeds were sown at 25 cm distance between plants and 50 cm between rows.

Source of Bradyrhizobia inoculum: *Bradyrhizobium* spp. (*Vigna)* strain 604 brought from Biofertilizer Unit Agric. Res. Microbial, Soils, Water and Environment, Res. Inst. Agric. Center Giza, Egypt. Rhizobia seed coating was applied for all treatments expect for the control treatment that received full dose of N, P and K. Each bacterial strain was applied either separately or all in combination as a liquid culture at the rate of 20 L fed^{-1} mixed with 400 L water/fed for cowpea plants as a foliar spray.

Source of *Streptomyces luteogriseus* strain: An identified halotolerant actinomycete strain named as *Streptomyces luteogriseus*-08 was obtained from Department of Agricultural Microbiology, ARC, Giza, Egypt. This isolate was previously isolated from soil of Taif KSA and completely identified by Mohamed et al. (2013).

Preparation of *Streptomyces* inoculums: Inoculum of the applied *Streptomyces* strain was prepared by scraping the heavy spores from the surface of the growth of starch nitrate slant in the presence of 5 mL sterilized d.H_2O as described by Osman et al. (2007). An aliquot of 2 mL standard inoculum was transferred aseptically to 50 mL of a broth medium (data not shown) modified from starch nitrate broth medium in a 250 mL conical flask. Inoculated flasks were incubated at 28±2°C for 6 days on a rotary shaker (160 rpm/min) (Figure 1). Thereafter, growth was centrifuged at 10000 rpm at 4°C for 5 minutes. The supernatants and pellets were then distributed in 50 mL Fisher tubes and kept at 4°C until used.

Microbial total count: The total counts of microbes in the soil sample that cultivated in it was determined by estimating the numbers of bacteria, fungi and actinomycetes before and after cultivation as described by the method Clark (1965).

Field Experiment Design: A number of sixteen treatments were designed as shown in Table (3) below. In these treatments cowpea seeds were inoculated with a combination of different biofertilizers (*Bradrhizobium* spp. (*Vigna*) and *Streptomyces luteogriseus*-08) were cultivated in soil fertilized with low concentration of mineral fertilizer in the presence and absence of organic fertilizer and/or biochar.

Table-3: Field Experiment Design.

Treatments			
Code	**Soil application**	**Mineral nitrogen**	**Biofertilizer**
T01	Without fertilization	40 kg/fed*	Uninoc.
T02		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*).
T03		20 kg/fed	*Streptomyces luteogriseus*-08
T04		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*)+*Streptomyces luteogriseus*-08
T05	Organic fertilizer	40 kg/fed*	Uninoc.
T06		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*).
T07		20 kg/fed	*Streptomyces luteogriseus*-08
T08		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*)+*Streptomyces luteogriseus*-08
T09	Biochar	40 kg/fed*	Uninoc.
T10		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*).
T11		20 kg/fed	*Streptomyces luteogriseus*-08
T12		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*)+*Streptomyces luteogriseus*-08
T13	Organic fertilizer + Biochar	40 kg/fed*	Uninoc.
T14		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*).
T15		20 kg/fed	*Streptomyces luteogriseus*-08
T16		20 kg/fed	*Bradyrhizobium* spp. (*Vigna*)+*Streptomyces luteogriseus*-08

*: It was used due to the recommendation of Ministry of Agriculture.

Dehydrogenase activity in soil: Determination of dehydrogenase enzyme in the soil sample before and after planting was conducted based on the method of Stevenson (1959).

Nodules number: Number of nodules per cowpea plant and dry weight of nodules/plant were determined post planting among the two cuttings.

Post-harvest measurements: It is worth to mention that for each treatment of the seven treatments three replicates were applied. From each replicates a number of cowpea plants were collected to determine the previous measurements. In each cut from the following parameters (Fresh weight (Kg/m^2), dry weight (Kg/m^2), yield of fresh weight (Ton/Feddan) and yield of dry weight (Ton/Feddan)) were determined on a random sample of ten guarded plants from each plot as reported by Pepe and Heiner (1975) and Helmy et al. (2014).

NPK content: NPK in soil sample according to the method Attanandana et al. (1999) was carried out before and after cultivation. NPK (mg/plant) and protein (N % X 6.25) contents in plant or seeds was also determined. From each ample 0.5 g was digested using mixture of sulfuric (H_2SO_4) and perchloric ($HClO_4$) acids (1:3) as described by Cottenie et al. (1982). Nitrogen was determined by micro Keldahl, according to Jackson (2005). Phosphorus was determined Spectrophotometrcally using ammonium molybdate/stannus chloride method according to Chapman and Pratt (1961). Potassium was determined by a flame photometer, according to Page et al., (1982). Variation for each season was determined as according to Gomez and Gomez (1984).

Percentage of crude protein: Estimating the percentage of protein in cowpea seeds was estimated according to the method of Hames et al. (2008).

RESULTS AND DISCUSSION

The results of soil chemical and mechanical analyses showed that the sample of soil cultivated in the experiment in Ismailia station, section 9 was of the sandy type.

It was able to establish a symbiotic association with its symbiotic bradyrhizobium which acquire most of its essential N. With respect to the importance of cowpea production worldwide, providing the suitable conditions for the optimum yield production is of great significance.

The *Streptomyces* strain under investigation was characterized by gray aerial mycelium (gray colour series) and the reverse side of substrate mycelium was dark gray. Spore chains belonged to section RF or spiral with hairy surface. This isolate was also found to produce melanoid, did not produce soluble pigments and had a good growth on Cazpek's medium. Concerning the utilization of carbon sources, the isolate was able to give a good growth in the presence of all sugars as sole carbon source. The isolate also showed antimicrobial activities and not inhibited with streptomycin (4µg mL^{-1}) and grew on NaCl concentrations up to 21% (Mohamed et al., 2013).

Determination of NPK elements in the soil applied in this study was poor in NPK elements before planting. The soil was poor in NPK elements as available N (49&52 ppm), available P (7.9&8.2 ppm) and available K (35&40 ppm) were recorded in uncultivated (inoculated-unfertilized) soil (T01) among the two seasons. On planting and treating, the NPK was determined in soil samples collected from the 15 fertilizer treatments as well as blank soil. Results in Table-4 showed that the NPK was raised up as available soil-nitrogen was ranged from 72 to 165 ppm (1st season) & 84 to 187 ppm (2nd season) ppm, available P ranged from 8.2to 9.6 ppm (1st season) & 8.4 to 10.2 (2nd season), and available K ranged from 38 to 72 ppm (1st season) & 42 to 96 (2nd season) compared to blank soil sample (T01).

Table -4: Average of NPK in soil cultivated with cowpea subjected to different fertilizers treatments among two seasons.

Treatments		Available elements (ppm)					
		1st Season			2nd Season		
Soil application	**Codes**	**N**	**P**	**K**	**N**	**P**	**K**
Without application	**T01**	049	7.9	35	52	8.2	40
	T02	072	8.2	38	84	8.4	42
	T03	081	8.3	42	98	8.3	46
	T04	112	9.2	58	124	8.5	48
Organic matter	**T05**	081	8.6	62	95	9.5	64
	T06	121	8.8	68	142	9.8	75
	T07	096	8.8	66	112	9.9	74
	T08	141	9.5	68	158	10.2	78
Biochar	**T09**	045	7.6	46	50	8.0	60
	T10	106	7.8	49	145	8.2	66
	T11	088	7.6	48	56	8.3	62
	T12	126	8.2	52	168	8.6	68
Organic matter + Biochar	**T13**	096	9.2	58	124	9.6	72
	T14	138	9.5	67	158	9.8	87
	T15	110	9.1	68	143	9.8	82
	T16	165	9.6	72	187	10.2	96

The average numbers of microbial counts were 14.6 X10^5,0.5 X10^4and2.6 X10^4 of bacteria, fungi and actinomycetes, respectively. In other mean, the numbers were few in untreated soil (T01) compared to soil cultivated with cowpea subjected to different fertilizer as well as bio-fertilizers treatments (T02-T16). On planting and treating, the microbial total counts was developed in the soil samples collected from the 15 fertilizer treatments compared to the blank-soil sample (T01) (Table 5). This was obvious from the numbers of bacteria, fungi and actinomycetes, while the bacterial count was the highest followed by total counts of actinomycetes and fungi. Data also, mention that inoculated treatment recorded higher counts of bacteria, fungi and actinomycetes compared with uninoculated treatments. Moreover, applied organic matter after cultivated clover recorded higher number of bacteria, fungi and actinomycetes than applied biochar after cultivated.

The activity of soil representing in the rate of dehydrogenase enzyme before planting was reached 2.59µg/g of soil/24 hours. The average rate of dehydrogenase enzyme among the two seasons reached 2.62 µg TPF/g soil/24 hr was fewer in soil of T01 treatment (unfertilized+uninooculated) than soils after 15 treatments containing cowpea seeds inoculated with different biofertilizers and cultivated in soil fertilized by different combinations of fertilizers (Organic fertilizer, biochar and mineral nitrogen). This was approved by the rate of DHA which ranged from 2.90 to 3.45 µg TPF/g soil/24 hr in the first season, and from 3.2 to 6.08 45 µg TPF/g soil/24 hr in the second season (Table 5).

Table-5: Averages of each of number of microbial total counts and dehydrogenase activity in soil cultivated with cowpea and subjected to different fertilizers treatments among two season.

Treatments		Microbial total counts						DHA (µg TPF/g soil/24 hr))	
		Bacteria (X10^5)		Fungi (X10^4)		Actinomycetes (X10^4)			
		Seasons							
Soil applications	**Codes**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**	**1st**	**2nd**
Without application	**T01**	14.0	15.2	0.45	0.55	2.00	3.20	2.56	2.68
	T02	17.5	18.5	0.50	0.62	2.50	3.50	2.90	3.20
	T03	20.0	16.4	0.45	0.50	5.00	6.20	2.90	3.27
	T04	21.5	22.5	0.55	0.65	5.50	6.50	3.00	4.20
Organic matter	**T05**	25.0	28.0	0.50	0.65	2.50	3.80	3.00	4.60
	T06	27.0	32.0	0.65	0.78	3.00	3.90	3.22	5.20
	T07	28.5	30.0	0.55	0.60	5.50	6.50	3.20	5.26
	T08	31.0	34.0	0.70	0.82	6.00	6.80	3.30	5.90
Biochar	**T09**	15.0	15.4	0.50	0.50	2.00	2.40	2.80	2.59
	T10	18.5	19.5	0.55	0.65	2.50	2.60	3.00	3.22
	T11	20.5	18.5	0.55	0.60	5.50	5.80	3.05	3.53
	T12	23.0	24.8	0.65	0.70	6.00	6.20	3.10	4.49
Organic matter + Biochar	**T13**	26.5	28.5	0.55	0.75	2.60	2.70	3.20	4.50
	T14	28.5	32.8	0.70	0.86	3.10	3.20	3.40	5.22
	T15	31.5	31.5	0.58	0.76	5.60	5.60	3.30	5.37
	T16	33.5	34.8	0.75	0.86	6.50	7.20	3.45	6.08

Data in Table (6) showed the number of nodules among the two cutting of cowpea plants cultivated in the open field and subjected to 15 different fertilizer treatments compared to the control

(Uninoculated+40kgN/fed, T01). As overall view the number of nodules at the 2nd cut was always higher than that of the 1st cut. No results were recorded indicating the presence of root nodules in cowpea plants to which Rhizobium was not added. The highest average number of root nodules was in cowpea plants that were subjected to T16 containing organic fertilizer and low concentration of mineral nitrogen fertilizer, rhizobia, biochar and actinomycetes, followed by treatments no. T14, T10, T06 and T04 that contain cowpea seeds inoculated with Rhizobium only. It was also noted that the addition of organic fertilizer (T06, T08) to the soil increased the number of root nodules to a higher degree than the addition of biochar (T10 and T12). The effect of soil amended with organic matter and/or biochar, results show that the maximum mean value was recorded in the treatment which cultivated in the soil amended with Organic matter and biochar.

The means values of nodule number in the first season were recorded 50.2 and 57.6nodule/plant in the first and second cutting, respectively. In the second season, the maximum values of nodule number recorded 56.5 and 66.0 nodule/plant in the first and second cutting in the same order. The same trend for the maximum values of nodule dry weight was recorded in the cowpea seed cultivated in the soil amended with the two compounds.

Table-6: Nodule number per cowpea plant of two cutting cultivated under different treatments among two seasons.

Codes	Soil applications	Mineral nitrogen Kg/Fed	Bio-fertilizers	Nodules number (#/plant)			
				1st Season		2nd Season	
				1st cutting	2nd cutting	1st cutting	2nd cutting
T01	Without	40	Uninoc.	00.0	00.00	0.00	0.00
T02		20	*Brady.*	35.3	43.33	42.6	51.0
T03		20	*Strepto.*	00.0	00.00	0.00	0.00
T04		20	*Brady.+Strept.*	41.0	47.67	52.7	56.7
T05	Organic	40	Uninoc.	00.0	00.00	0.00	0.00
T06		20	*Brady.*	53.7	60.33	60.7	67.0
T07		20	*Strepto.*	00.0	00.00	0.00	0.00
T08		20	*Brady.+Strept.*	57.0	66.67	64.0	74.0
T09	Biochar	40	Uninoc.	00.0	00.00	0.00	0.00
T10		20	*Brady.*	39.7	47.67	44.3	50.7
T11		20	*Strepto.*	00.0	00.00	0.00	0.00
T12		20	*Brady.+Strept.*	43.0	48.67	45.0	56.4
T13	Organic + Biochar	40	Uninoc.	00.0	00.00	0.00	0.00
T14		20	*Brady.*	59.0	67.00	63.0	76.7
T15		20	*Strepto.*	00.0	00.00	0.00	0.00
T16		20	*Brady.+Strept.*	59.7	67.33	64.3	77.0
LSD 0.05				**3.4**	**4.6**	**4.6**	**5.2**
Effect of soil application							
Without				0.0	0.0	0.0	0.0
Organic				46.9	54.6	52.7	61.4
Biochar				0.0	0.0	0.0	0.0
Organic+Biochar				50.2	57.6	56.5	66.0
LSD 0.05				**1.7**	**2.3**	**2.3**	**2.6**
Effect of Biofertilizers							
Uninoc., 40kgN/fed				19.08	22.75	23.83	26.93
Brady., 20kgN/fed				27.68	31.75	31.18	35.25
Strepto.,20kgN/fed				20.68	24.09	22.33	26.78
Brady.+Strept., 20kgN/fed				29.68	33.58	31.83	38.43
LSD 0.05				**1.70**	**2.30**	**2.30**	**2.60**

Regardless of soil amended with organic matter and/or biochar, data in Table (7) indicate the nodules number and dry weight population in response of rhizobia and actino inoculation combined with activation dose of nitrogen. Results recorded the highest significant difference compared with rhizobia inoculation alone or rhizobia inoculation combined with actinomyces. In the first season, the inoculated rhizobia plus actino recorded maximum values of nodule number which recorded 29.68 and 33.58 nodule/plant in the first and second cutting, respectively. The same trend in the second season, where the maximum values of nodule number recorded 31.83 and 38.43 nodule/plant in the first and second cutting in the same order. Regarding to the nodule dry weight of cowpea plants result in Table-6showed that the root nodules dry weight of the cowpea plants under investigation has become in the same direction as the results of the number of root nodules. The highest values of thesis parameter were recorded in the cowpea seed inoculated with rhizobia and actino combined with activation dose of nitrogen in the cutting and seasons.

Table-7: Nodule dry weight per cowpea plant of two cutting cultivated under different treatments among two seasons.

Codes	Soil applications	Mineral nitrogen Kg/Fed	Bio-fertilizers	Nodules dry weight (mg/plant)			
				1st Season		2nd Season	
				1stcutting	2ndcutting	1stcutting	2ndcutting
T01	Without	40	Uninoc.	0.00	0.00	0.00	0.00
T02		20	*Brady.*	0.51	0.82	0.56	0.87
T03		20	*Strepto.*	0.00	0.00	0.00	0.00
T04		20	*Brady.+Strept.*	0.57	0.92	0.62	1.05
T05	Organic	40	Uninoc.	0.0	0.00	0.00	0.00
T06		20	*Brady.*	0.74	1.01	0.79	1.14
T07		20	*Strepto.*	0.00	0.00	0.00	0.00
T08		20	*Brady.+Strept.*	0.85	1.05	0.91	1.18
T09	Biochar	40	Uninoc.	0.00	0.00	0.00	0.00
T10		20	*Brady.*	0.61	0.77	0.66	0.83
T11		20	*Strepto.*	0.00	0.00	0.00	0.00
T12		20	*Brady.+Strept.*	0.64	0.88	0.66	0.92
T13	Organic +	40	Uninoc.	0.00	0.00	0.00	0.00
T14	Biochar	20	*Brady.*	0.80	1.03	0.84	1.16
T15		20	*Strepto.*	0.00	0.00	0.00	0.00
T16		20	*Brady.+Strept.*	0.82	1.07	0.85	1.20
LSD 0.05				**0.04**	**0.06**	**0.04**	**0.08**
Effect of soil application							
Without				0.0	0.0	0.0	0.0
Organic				0.7	0.9	0.7	1.0
Biochar				0.0	0.0	0.0	0.0
Organic + Biochar				0.7	1.0	0.8	1.1
LSD 0.05				**0.02**	**0.03**	**0.02**	**0.04**
Effect of Bio-fertilizers							
Uninoc., 40kgN/fed				0.27	0.44	0.30	0.48
Brady., 20kgN/fed				0.40	0.52	0.43	0.58
Strepto. ,20kgN/fed				0.31	0.41	0.33	0.44
Brady.+Strept. , 20kgN/fed				0.41	0.53	0.42	0.59
LSD 0.05				**0.02**	**0.03**	**0.02**	**0.04**

Fresh weight (kg/m^2) among two cutting of cowpea shoots cultivated under different fertilizer treatments was recorded in Table (8). Results showed that the fresh weight of cowpea plants under investigation is shown in the 16 treatments was inconsistent and reflect the importance of biofertilizers. In the sense that the absence of rhizobium led to a decrease in the fresh weight of plants. Also, the presence of organic fertilizer increased the fresh weight of the plants compared to the use of biochar. Whereas, the highest fresh weight was obtained when all were added in a combination as shown in T16.Results of the dry weight of the cowpea plants under investigation has become in the same direction as the results of fresh weight (**Table 8**). Regardless biofertilizer inoculation combined with activation dose of nitrogen, results in Table (8) showed significant differences among amended soil with organic matter and/or biochar. The highest values of shoot dry weight in the first season (23.23 and 25.12kg/m^2 in the same order in the first and second cutting). The corresponding values in the second season were 28.34 and 29.29 kg/m^2, respectively. On the contrary plant cultivated in the soil without organic matter or biochar scored the lowest values of shoot frish and dry weight.Data show also, the inoculation with rhizobia and actino combined with activation dose of nitrogen was recorded the highest significant difference compared with uninoculated plant in the two seasons.

Table-8: Fresh and dry weights (kg/m^2) of cowpea shoots during two cutting cultivated under different treatments among two seasons.

Codes	Soil applications	Mineral nitrogen Kg/Fed	Biofertilizers	Fresh weights (kg/m^2)				Dry weights (kg/m^2)			
				1st Season		2nd Season		1st Season		2nd Season	
				1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd Cutting
T01	Without	40	Uninoc.	18.47	21.23	22.07	23.53	12.77	14.53	16.75	14.59
T02		20	*Brady.*	21.17	23.50	25.07	26.70	14.97	16.77	18.88	16.55
T03		20	*Strepto.*	19.77	21.27	24.13	25.13	13.47	14.73	17.29	15.58
T04		20	*Brady.+Strept.*	22.33	23.80	26.50	27.13	15.47	17.50	13.29	16.82
T05	Organic	40	Uninoc.	19.57	21.30	24.83	25.70	13.60	15.27	17.78	15.93
T06		20	*Brady.*	22.60	24.60	30.17	28.43	16.20	18.10	20.70	17.63
T07		20	*Strepto.*	21.10	22.07	29.80	26.20	14.90	15.90	19.93	16.24

T08		20	*Brady.+Strept.*	23.70	25.47	31.87	30.17	17.13	18.97	22.37	18.70
T09	Biochar	40	Uninoc.	19.23	21.13	22.67	24.47	13.37	15.13	17.03	15.17
T10		20	*Brady.*	21.57	23.37	26.13	27.70	15.40	16.93	19.44	17.17
T11		20	*Strepto.*	20.00	21.33	23.50	24.00	13.90	15.30	18.05	14.88
T12		20	*Brady.+Strept.*	22.57	24.03	26.77	27.37	16.30	17.63	19.90	16.97
T13	Organic + Biochar	40	Uninoc.	20.97	24.03	24.43	25.63	15.17	17.63	18.41	15.89
T14		20	*Brady.*	23.27	26.43	27.13	31.93	16.70	19.87	20.31	19.80
T15		20	*Strepto.*	21.80	23.87	25.13	30.07	15.60	17.27	19.33	18.64
T16		20	*Brady.+Strept.*	24.30	27.17	28.23	32.47	17.27	20.33	21.18	20.13
LSD 0.05				2.20	2.25	2.13	3.12	1.23	2.13	1.78	1.85
Effect of soil application											
Without				19.56	21.92	23.50	24.83	13.73	15.64	17.49	15.40
Organic				22.15	24.48	27.13	28.69	15.82	17.92	19.83	17.79
Biochar				20.67	22.14	25.64	26.35	14.47	15.80	18.65	16.34
Organic + Biochar				23.23	25.12	28.34	29.29	16.54	18.61	19.19	18.16
LSD 0.05				1.1	1.15	1.06	1.66	0.62	1.70	0.88	0.63
Effect of Bio-fertilizers											
Uninoc., 40kgN/fed				20.44	22.45	24.44	25.62	14.17	15.88	16.55	15.89
Brady., 20kgN/fed				21.74	23.36	29.17	27.63	15.46	17.06	20.20	17.13
Strepto. , 20kgN/fed				20.84	22.47	24.77	25.89	14.74	16.25	18.61	16.05
Brady.+Strept. , 20kgN/fed				22.59	25.38	26.23	30.03	16.19	18.78	19.81	18.62
LSD 0.05				1.10	1.15	1.06	1.66	0.62	1.70	0.88	0.63

Percentages of nitrogen and crude protein (Table 9) among two cutting of cowpea plants cultivated under showed that the 2^{nd} cut was always higher in the percentage of crude protein than the 1^{st} cut. Also, treatments containing rhizobium as a biofertilizer showed a high percentage of crude protein compared to rhizobium-free treatments. Also, the presence of organic fertilizer was effective in its effect compared to biochar. Finally, when rhizobium, organic fertilizer, biochar and actino mycetes were added in the presence of low nitrogen fertilizer, the highest crude protein content was obtained (T16).

Table-9: Percentages of nitrogen and crude protein of cowpea shoots cultivated under different treatments among two seasons.

Codes	**Soil applications**	**Mineral nitrogen Kg/Fed**	**Biofertilizers**	**Nitrogen (%)**				**Crude protein (%)**			
				1^{st} Season		**2^{nd} Season**		**1^{st} Season**		**2^{nd} Season**	
				1^{st} cutting	**2^{nd} cutting**	**1^{st} cutting**	**2^{nd} cutting**	**1^{st} cutting**	**2^{nd} cutting**	**1^{st} cutting**	**2^{nd} Cutting**
T01	Without	40	Uninoc.	22.8	2.31	2.26	2.32	13.01	14.38	14.13	14.50
T02		20	*Brady.*	2.25	2.36	2.35	2.50	14.06	14.58	14.69	15.63
T03		20	*Strepto.*	2.15	2.14	2.27	2.39	13.41	14.79	14.19	14.94
T04		20	*Brady.+Strept.*	2.33	2.40	2.39	2.60	14.57	15.10	14.94	16.25
T05	Organic	40	Uninoc.	2.23	2.29	2.30	2.28	13.91	14.44	14.38	14.25
T06		20	*Brady.*	2.40	2.45	2.36	2.43	14.96	15.10	14.75	15.19
T07		20	*Strepto.*	2.26	2.35	2.28	2.26	14.12	14.58	14.25	14.13
T08		20	*Brady.+Strept.*	2.41	2.54	2.37	2.41	15.08	15.21	14.81	15.06
T09	Biochar	40	Uninoc.	2.12	2.21	2.15	2.12	13.22	13.33	13.44	13.25
T10		20	*Brady.*	2.33	2.46	2.37	2.33	14.54	14.96	14.81	14.56
T11		20	*Strepto.*	2.21	2.32	2.28	2.22	13.84	14.27	14.25	13.88
T12		20	*Brady.+Strept.*	2.26	2.46	2.31	2.35	14.12	15.00	14.44	14.69
T13	Organic + Biochar	40	Uninoc.	2.30	2.35	2.29	2.30	14.36	14.48	14.31	14.38
T14		20	*Brady.*	2.39	2.47	2.42	2.40	14.96	15.17	15.13	15.00
T15		20	*Strepto.*	2.27	2.40	2.33	2.50	14.16	14.54	14.56	15.63
T16		20	*Brady.+Strept.*	2.48	2.58	2.48	2.52	15.50	15.58	15.50	15.63
LSD 0.05				2.30	2.35	2.29	2.30	14.36	14.36	14.36	14.36
Effect of soil application											
Without				2.18	2.29	2.25	2.26	13.63	14.16	14.07	14.10
Organic				2.34	2.44	2.38	2.42	14.63	14.95	14.85	15.10
Biochar				2.22	2.30	2.29	2.34	13.88	14.55	14.31	14.65
Organic + Biochar				2.37	2.50	2.39	2.47	14.82	15.22	14.92	15.41
LSD 0.05				2.18	2.29	2.25	2.26	13.63	14.16	14.07	14.10
Effect of Bio-fertilizers											
Uninoc., 40kgN/fed				2.20	2.30	2.32	2.45	13.76	14.71	14.49	15.33
Brady., 20kgN/fed				2.33	2.41	2.33	2.35	14.52	14.83	14.55	14.66
Strepto. ,20kgN/fed				2.23	2.36	2.28	2.26	13.93	14.39	14.24	14.10
Brady.+Strept, 20kgN/fed				2.36	2.45	2.38	2.43	14.75	14.94	14.88	15.16
LSD 0.05				2.20	2.30	2.32	2.45	13.76	14.71	14.49	15.33

Results in Table (10) showed that yield of fresh weight (Ton/Fed) was higher in the presence of rhizobium and organic fertilizer as biofertilizers compared to control (No rhizobium or organic fertilizer). Treatment T16 containing rhizobium and actinomycetes as biofertilizers, organic fertilizer, low concentration of nitrogen fertilizer and biochar appeared the highest fresh weight per Feddan compared to all treatments. Results of the yield (Ton/Fed) of dry weight of the cowpea plants under investigation has become in the same trend as the results of fresh weight (Table 10). Amended soil with organic matter and/or biochar, results in Table (10)showed the highest frish and dry weight yield of clover (Ton/fed) in the first season and second cutting. The same trend in the second season, On the contrary plant cultivated in the soil without organic matter or biochar scored the lowest values of thesis parameters. Data show also, the inoculation with rhizobia and actino combined with activation dose of nitrogen were recorded the highest significant difference compared with uninoculated plant in the two seasons.

Table-10: Yields of fresh and dry weights (Ton/Feddan) of cowpea shoots during two cutting cultivated under different fertilizer treatments among two seasons.

Codes	Soil applications	Mineral nitrogen Kg/Fed	Biofertilizers	Fresh weight yield (Ton/Feddan)				Dry weight yield (Ton/Feddan)			
				1st Season		2nd Season		1st Season		2nd Season	
				1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd cutting	1st cutting	2nd Cutting
T01	Without	40	Uninoc.	73.88	084.80	88.00	94.00	11.40	13.00	13.80	15.08
T02		20	*Brady.*	84.68	090.40	100.8	106.8	13.24	14.44	15.68	16.40
T03		20	*Strepto.*	79.08	085.20	96.40	100.4	12.36	13.08	15.04	15.56
T04		20	*Brady.+Strept.*	89.32	095.20	106.0	108.4	12.60	14.60	16.52	17.24
T05	Organic	40	Uninoc.	78.28	085.20	99.20	102.8	11.92	13.68	15.48	15.56
T06		20	*Brady.*	90.40	098.40	120.8	113.6	13. 76	15.08	18.80	17.76
T07		20	*Strepto.*	84.40	088.40	119.2	104.8	13. 64	13.80	18.56	15.88
T08		20	*Brady.+Strept.*	94.80	102.00	127.2	120.8	14.80	15.48	19.88	18.32
T09	Biochar	40	Uninoc.	76.92	084.40	90.80	96.00	12.08	12.96	14.12	14.80
T10		20	*Brady.*	86.28	093.60	104.4	110.8	13.48	14.60	16.28	16.24
T11		20	*Strepto.*	80.00	085.20	94.00	98.00	12.36	13.12	14.68	14.96
T12		20	*Brady.+Strept.*	90.28	096.00	106.8	109.6	14.04	13.92	16.68	16.96
T13	Organic + Biochar	40	Uninoc.	83.88	090.00	88.00	94.00	13.08	14.72	13.80	15.08
T14		20	*Brady.*	93.08	105.60	100.4	120.0	14.44	16.08	15.68	16.96
T15		20	*Strepto.*	87.20	095.60	97.60	102.4	14.36	14.72	15.28	16.20
T16		20	*Brady.+Strept.*	97.20	108.80	108.4	127.6	11.40	13.00	13.80	15.08
LSD 0.05				3.45	4.26	3.87	4.58	0.85	0.85	0.85	0.85
Effect of soil application											
Without				78.24	86.10	91.50	96.70	12.12	13.59	14.30	15.13
Organic				88.61	97.00	106.60	112.80	13.73	15.05	16.61	16.84
Biochar				82.67	88.60	101.80	101.40	13.18	13.68	15.89	15.65
Organic + Biochar				92.90	100.50	112.10	116.60	13.21	14.25	16.72	16.90
LSD 0.05				1.73	2.13	1.94	2.29	0.43	0.73	0.68	0.93
Effect of Bio-fertilizers											
Uninoc., 40kgN/fed				81.74	88.90	97.80	102.40	12.40	13.78	15.26	16.07
Brady., 20kgN/fed				86.97	93.50	116.60	110.50	13.53	14.51	18.18	16.88
Strepto. , 20kgN/fed				83.37	89.80	99.00	103.60	12.99	13.65	15.44	15.74
Brady.+Strept. , 20kgN/fed				90.34	100.00	98.60	111.00	13.32	14.63	14.64	15.83
LSD 0.05				1.73	2.13	1.94	2.29	0.43	0.73	0.68	0.93

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