Pak. J. Biotechnol. Vol. 20(1)10-20 http://doi.org/10.34016/pjbt.2023.20.01.583.

Research Article

OPEN ACCESS

Available on http://www.pjbt.org
Pakistan Journal of Biotechnology
(PJBT)
(P-ISSN: 1812-1837 and E-ISSN: 2312-7791)

BERSEEM CLOVER PRODUCTIVITY IN THE PRESENCE OF A COMBINATION OF BIOFERTILIZERS, ORGANIC FERTILIZER AND BIOCHAR UNDER SANDY SOIL CONDITIONS

Alaaeldin Abdel-Hamid Abo El-Soud[1], SayedAhmed El-Tohamy[2], Mohamed Mohsen El-Kholi[2], BakrAbdel-GalilAbdel-Wahab[2], Gamal Abdel-Fattah Ahmed Mekhemar[2], Amani Mostafa Ahmed[2], Gehan Mohamed Salem[1], Rasha Atef Shoman[2], Rabab Abdallah Abdel-Naser Amer[1], Heba Belal Abdel-Samie Kandil[1], Sonya Hamouda Mohamed[1*]

[1]Department of Agricultural Microbiology, Soils, Water and Environmental Research Institute, Agricultural Research Centre, P.O. Box, 12619, Giza, Egypt
[2]Department of Environment, Soils, Water and Environmental Research Institute, Agricultural Research Centre, P.O. Box, 12619, Giza, Egypt

*Corresponding author Email: drsonyasweri@gmail.com
Article Received 18-11-2022, Article Revised 11-01-2023, Article Accepted 27-02-2023

ABSTRACT

Several studies have shown that berseem clover is one of the most important winter leguminous crops as fodder for animals due to its high content of crude protein. In Egypt, its cultivation is abundant in soils whether ancient or reclaimed soils. This study was designed to evaluate the effect of biofertilizers on berseem clover productivity cultivated under sandy soil conditions in Ismailia Research Station, Ismailia Governorate. To reach such aim, an identified actinomycete strain (*Streptomyces luteogriseus*-08) was used with a mixture of biochar, and organic fertilizer in several different treatments compared to rhizobia. The experimental results revealed that the percentages of crude protein in the two cuts indicated that the 2nd cutting was always higher in the percentage of crude protein than the 1st cutting. On planting and treating, the microbial total counts, dehydrogenase activity and NPK contents were developed in the soil samples collected from the 15 fertilizer treatments compared to the control-soil sample. In addition, treatments containing rhizobium as a biofertilizer showed a high percentage of crude protein compared to rhizobium-free treatments. The presence of organic fertilizer was more suitable for improving the growth of berseem clover plants compared to biochar. Combination of *Rhizobium legumin Sarum* bv. *trifolii* , *Streptomyces luteogriseus*-08, organic fertilizer, biochar in the presence of low nitrogen fertilizer (T16 treatment) gave the highest numbers of nodules, dry weight of nodules, and fresh weight of berseem clover nodules. Dry and fresh weights of berseem clover shoots, crude protein (%) and expected yield (Ton/Feddan) were higher in T16 compared to the control and the other 14 treatments.

Key words: *Berseem clover, biofertilizers, Rhizobium legumino sarum*bv. *trifolii, Yield, Crude protein (%), Streptomyces luteogriseus*-08.

INTRODUCTION

Egyptian clover (*Trifolium alexandrinum* L.) is considered the main winter forage legume in old and new lands of Egypt. This is due to its high yield and quality especially crude protein content (Ghaffarzade, 1997). Ryegrass (*Lolium multiflorum* L.) is a native annual winter grass and adapted to a wide varieties of soils and produce quick cover after cutting, of high production and quality (Ramadan, 1997). Thus, the principal benefits of mixing ryegrass with Egyptian clover are the increase of total dry fertilizer production and forage quality (James et al.,1998). Organic and biofertilizers seem to be more appropriate agronomic practices as they are considered the important aspects in agronomic clean farming. Among these organic materials are crop residues, farmyard compost, green manure and bio-fertilizer as microbial fertilizers and rhizobium, blue green algae and azolla. These were used to improve soil health and increased the yield which plays an important role for minimizing the harmful effect of pesticides and herbicides (Ananata,2005).Egyptian clover is one of keeping the soil, prevents wind and increases soil organic fertilizer content, particularly in newly reclaimed lands and enhancing soil structure and chemical and physical feature, (Graves et al.,1996).Thus, there is a need to develop berseem clover cultivar and appropriate

fertilizer types for high forage production and good quality (El-Nahrawy,2005). In Egypt clover is considered long winter forage legume. Its cultivated area almost accumulates more than 1.25 million fed of the total area of around 7.5 million fed. Leguminous crop with high yield and nutritive value, berseem has become one of the basic entries of successive crops in irrigated soils or as a green manure in cash crop rotations (Kuneluis, 1997).Ismail and Hassanen (2019) investigated the effect of foliar application of compost tea humic acid, and biofertilizer on forage production, seeds, chemical analysis of plant and soil. In general, berseem clover treated with humic acid increased the plant height, fresh, dry yield, seeds yield, weight of 1000 seeds, crude protein, fiber fraction, total carbohydrate, digestible crude protein of forage yield as compared with control. Mixtures of forage crops (cereals and legumes) clearly have many advantages and are superior to their monocultures in providing greater yield and quality. In this respect grass-legume mixtures have high crude protein concentration and low fiber concentration than pure grass stand (Chen 2006 Damadola et al.,2009). Thalooth et al. (2015) conducted two field experiments during two successive winter seasons of 2008/2009 and 2009/2010 to investigate the potentialities of mixing Egyptian clover with ryegrass under bio, organic and mineral fertilization treatments and their combination to increase forage yield and quality grown under sandy soil conditions. The experiment included the combination of five mixing ratios (Egyptian clover alone, ryegrass alone, 75% Egyptian clover: 25% ryegrass, 50% Egyptian clover: 50% ryegrass and 25% Egyptian clover: 75% ryegrass) and eight fertilizer sources, which include control, organic fertilization, bio fertilization, chemical fertilizer, organic + biofertilizer, organic + chemical fertilizer, bio + chemical fertilizer and combination of organic and chemical and bio fertilizers. On the other hand, it reported the lowest dry weight of weeds g/m^2. 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-fertilizers (Ewees and Abdel Hafeez, 2010). 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 bio fertilizer. Rhizobia are legume root nodule bacteria. A *Rhizobium* is a legume root nodule bacterium, and fixes N_2 (diazotroph) after becoming established inside root nodules of legumes (Fabaceae). Rhizobacteria, through nitrogen fixation can able to convert gaseous nitrogen (N_2) to ammonia (NH_3) making it an available nutrient to the hostplant 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. PGPR improve plant grow the 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 berseem clover, maize, mustard, cotton, potato and other vegetable crops. *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 bio-fertilizers on berseem clover productivity cultivated under the sandy soil conditions in Ismailia Governorate. To reach such aim, an identified *Streptomyces* strain associated with rhizobium as biofertilizers was used with a mixture of biochar, organic fertilizer and ½recommended dose (RD) of mineral nitrogen among several different treatments compared to unfertilized.

MATERIALS AND METHODS

Location and season: Among two winter seasons of 2020 and 2021 at Section 9, Ismailia Agricultural

Research Station, Ismailia Governorate, Egypt, this field experiment was carried out.

Soil analyses: Among the two seasons, each of mechanical and chemical analyses and soil type were determined according to method of Page et al. (1982) and Cottenie et al. (1982), and results are recorded in Table (1).

Table-1: Mechanical and chemical analyses of cultivated soil among two seasons.

Parameters	Properties	1st Season	2nd Season
Mechanical analysis	Sand (%)	42.0	41.7
	Fine sand (%)	39.5	39.1
	Silt (%)	11.5	11.8
	Clay (%)	07.0	07.4
	Texture grade	Sandy	Sandy
	pH (1:2.5)	7.65	7.80
	E.C. (dSm^{-1} at 25ºC)	0.49	0.51
	SP (%)	26.0	26.3
Soluble cations (mmol/L)	Ca^{2+}	1.50	1.60
	Mg^{2+}	0.50	0.59
	Na^{+}	0.22	0.26
	K^{+}	2.45	2.50
Soluble anions (mmol/L)	CO_3^{2-}	0.00	0.00
	HCO_3^{-}	0.50	0.56
	Cl^{-}	3.50	3.63
	SO_4^{2-}	0.92	0.94
Macroelements	Total-N (%)	0.018	0.019
	Total soluble-N (ppm)	100.0	101.1
	Available-P (ppm)	08.24	8.840
	Available-K (ppm)	101.0	98.20
Microelements (DTPA-extract)	Iron(ppm)	0.930	0.810
	Manganese(ppm)	0.310	0.330
	Zinc(ppm)	0.120	0.130
	Copper(ppm)	0.050	0.055

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

Characters of used fertilizers: Properties of organic fertilizer (farmyard manure) (Table 2) used among the two successive seasons were determined. In other side, characteristics of biochar conditioner used in the two experiment seasons at Ismailia station was as follows: % Total N (0.144), $N\text{-}NH_4$ (770 ppm), $N\text{-}NO_3$ (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-2: Properties of organic fertilizer (farmyard manure) used in the two seasons.

Properties	1st season	2nd season
pH	7.85	7.68
E.C. (dS/m at 25ºC)	4.66	5.04
Organic-C (%)	16.15	17.24
Total N (%)	1.64	1.57
C/N ratio	12.80	13.96
Total-P (%)	0.74	1.05
Total-K (%)	1.55	1.40
Total soluble-N (ppm)	95.5	89.4
Available-P (ppm)	19.3	22.4
Available-K (ppm)	656.0	691.5
DTPA extractable (ppm):		
Fe	134.5	141.7
Mn	31.9	29.90
Zn	22.1	29.10
Cu	2.65	3.05
Microbial total counts:		
Bacteria	$6.2X10^7$	$9.4X10^6$
Fungi	$5.6X10^6$	$2.8X10^6$
Actinomycetes	$3.9X10^6$	$2.9X10^6$
Dehydrogenase activity (µg TPF/g)	107.4	112.7

Source of berseem clover seeds and cultivation distance: From the Seed Management, Agricultural Research Center (ARC), Giza, Egypt, Berseem clover seeds was kindly obtained. Seeds were sown at 25 cm distance between plants and 50 cm between rows.

***Rhizobium* inoculum source:** The strainARC-102of *Rhizobium leguminosarum* bv. *Trifolii* was obtained from Biofertilizer Unit; Agric. Res. Microbial; Soils, Water and Environment Res. Inst.; Agric. Res. Center; Giza; Egypt. Seeds were coated with rhizobium and applied for all treatments expect for the control treatment that received the recommended dose of mineral nitrogen. 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 berseem clover plants as a foliar spray.

Streptomycete source and preparation of its inoculum: An identified halotolerant streptomycete strain labelled as *Streptomyces luteogriseus*-08 was obtained from Department of Agricultural Microbiology, ARC, Giza, Egypt. This isolate was previously isolated from Taif soil, KSA and completely identified by Mohamed et al. (2013). 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±2ºC for 6 days on a rotary shaker (160 rpm/min) in Molecular Biology Laboratory, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. Thereafter, growth was centrifuged at 10000 rpm at 4ºC for 5 minutes. The supernatants and pellets were then distributed in 50 mL Fisher tubes and kept at 4ºC until used.

Microbial total counts: Before and after cultivation the total counts of microbes (bacteria, fungi and actinomycetes) in the soil sample as described by the method Clark (1965).

Design of field experiment: As shown in Table (3), number of 16 treatments were designed. In these treatments berseem clover seeds were inoculated with a combination of different biofertilizers (*Rhizobium leguminosarum* bv. *Trifolii* and *Streptomyces luteogriseus*-08) and cultivated in soil fertilized with low concentration of mineral fertilizer in the presence and absence of organic fertilizer and/or biochar.

Table-3: Design of field experiment.

Treatments	Description
T01	RD of mineral nitrogen (Control)
T02	*Rhizobium leguminosarum* bv. *trifolii* + ½RD of mineral nitrogen
T03	*Streptomyces luteogriseus* + ½RDof mineral nitrogen
T04	*Rhizobium leguminosarum* bv. *trifolii* + *Streptomyces luteogriseus*-08 + ½RD of mineral nitrogen
T05	Organic fertilizer+ RD of mineral nitrogen
T06	Organic fertilizer+ *Rhizobium leguminosarum* bv. *trifolii* + ½RD of mineral nitrogen
T07	Organic fertilizer+ *Streptomyces luteogriseus*-08 + ½RD of mineral nitrogen
T08	Organic fertilizer+ *Rhizobium leguminosarum* bv. *trifolii* + *Streptomyces luteogriseus*-08+ ½RD of mineral nitrogen
T09	Biochar + RD of mineral nitrogen
T10	Biochar + *Rhizobium leguminosarum* bv. *trifolii* + ½RD of mineral nitrogen
T11	Biochar + *Streptomyces luteogriseus*-08 + ½ RD of mineral nitrogen
T12	Biochar + *Rhizobium leguminosarum* bv. *trifolii* + *Streptomyces luteogriseus* + ½RD of mineral nitrogen
T13	Biochar + organic fertilizer+ RD of Mineral nitrogen
T14	Biochar + organic fertilizer +*hizobium leguminosarum* bv. *trifolii* + ½RD of mineral nitrogen
T15	Biochar + organic fertilizer+ *Streptomyces luteogriseus*-08+ ½RD of mineral nitrogen
T16	Biochar + organic fertilizer+ *Rhizobium leguminosarum* bv. *trifolii* + *Streptomyces luteogriseus*-08+ ½RD of mineral nitrogen

RD: 40 kg N/fed due to the Ministry of Agriculture.

Activities of dehydrogenase in soil: Based on the method of Stevenson (1959) activity of dehydrogenase enzyme was determined in the soil sample before and after planting.

Numbers of nodules: Nodule numbers per berseem clover plant and its dry weight were determined post planting among the two cuttings.

Measurements post-harvest: Four parameters, *i.e.*, 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 for all 16 treatments on a random sample of ten guarded plants from each plot as reported by Pepe and Heiner (1975) and Helmy et al. (2014).

Content of NPK: According to the method Atta Nandana et al. (1999) NPK content (mg /plant) in soil sample was measured before and after cultivation. Percentage of crude protein (N % X 6.25) in shoots or seeds was also determined according to the method of Hames et al. (2008).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).

RESULTS AND DISCUSSION

The results of soil chemical and mechanical analyses showed that the sample of soil cultivated in the experiment at Ismailia Research Station, Ismailia Governorate was sandy type. It was able to establish a symbiotic association with its symbiotic *Rhizobium* and acquire most of its essential nitrogen. With respect to the importance of berseem clover 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 color 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, was not inhibited with streptomycin (4 μgmL^{-1}) and grew on NaCl concentrations up to 21% (Mohamed et al., 2013).

The soil of this experiment was appeared to be poor in NPK elements (52-55 ppm N, 7.2-7.6 ppm P and 46-49 ppm K) in unfertilized soil and cultivated with control berseem clover seeds (Biofertilizers-free seeds). On planting NKP amounts were raised with ranges of 89-199 ppm, 8.6-10.7 and 48-91 of N, P and K respectively, among the two successive seasons in the 15 fertilizer treatments compared control (T01) (Table4).

Table-4: Means of NPK elements in soil cultivated with berseem clover subjected to different fertilizers treatments.

Treatments	N (ppm)		Available P (ppm)		Available K (ppm)	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
T01	0.52	0.55	7.2	7.6	46	49
T02	0.89	0.95	8.6	8.9	48	51
T03	0.75	0.89	7.9	8.1	44	47
T04	120	126	8.9	8.6	52	49
T05	106	110	7.8	8.2	63	66
T06	141	145	9.0	9.3	68	72
T07	130	135	8.8	9.1	64	69
T08	150	160	9.6	10.1	69	73
T09	074	079	7.4	7.9	54	57
T10	112	125	8.8	9.1	60	63
T11	105	095	8.2	8.6	58	61
T12	132	150	9.2	9.6	68	72
T13	118	128	8.5	8.9	68	65
T14	158	173	9.2	9.5	78	83
T15	140	163	8.9	9.4	72	76
T16	186	199	10.2	10.7	84	91

The averages of total microbial numbers (Bacteria (18-19.2$X10^5$), Fungi (0.6-0.65$X10^5$) and Actinomycetes (3.5-3.9$X10^4$) were few in control-soil sample (Uninoc. +40 kg N/fed, T01) compared to soil cultivated with berseem clover seeds subjected to different treatments among the two seasons. Total counts of bacteria, fungi and Actinomycetes were increased in the soil samples after cultivated with clover treated with different treatments of 15 treatments compared to the control-soil sample (Uninoc.+40 kgN/fed, T01) (Table 5). This was obvious from the numbers of bacteria (21-39.1 $X10^5$), fungi (0.7-0.99 $X10^5$) and actinomycetes (4.0-6.9 $X10^4$), while the bacterial count was the highest followed by total counts of fungi and actinomycetes. 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 rate of dehydrogenase enzyme which reached up to 4.10 and 4.5 µg TPF/g soil/24 hr among the two seasons was fewer in control-soil sample (Uninoc.+40 kgN/fed, T01) than soil cultivated with berseem clover subjected to different fertilizer

treatments. This was approved by the rate of DHA in the 15 treatments which ranged from 4.85 to 6.48 µg TPF/g soil/24 hr (Table5).

Table-5: Average number of microbial total counts and dehydrogenase activities in soil after cultivated with berseem clover and subjected to different fertilizers treatments.

Treatments	Soil amended	Biofertilizers	Mineral nitrogen Kg/Fed	Microbial total counts (cfu/gm soil)						DHA (µg TPF/g soil/24 hr)	
				Bacteria ($X10^5$)		Fungi ($X10^5$)		Actinomycetes ($X10^4$)			
				Seasons							
				1st	2nd	1st	2nd	1st	2nd	1st	2nd
T01	Without	Uninoc.	40	18.0	19.2	0.60	0.65	3.5	3.9	4.10	4.50
T02		*Rhizobium*	20	21.0	22.2	0.70	0.74	4.0	4.4	4.85	5.15
T03		*Strept.*	20	24.0	26.3	0.55	0.58	5.5	5.8	4.90	5.26
T04		*Rhizobium+Strept.*	20	26.0	28.4	0.70	0.75	6.0	6.4	5.40	5.80
T05	Organic	Uninoc.	40	28.5	31.1	0.70	0.74	3.5	3.8	5.30	5.60
T06		*Rhizobium*	20	30.5	33.4	0.82	0.84	4.5	4.8	5.50	5.85
T07		*Strept.*	20	30.0	32.4	0.67	0.69	4.5	4.8	5.35	5.65
T08		*Rhizobium+Strept.*	20	34.5	36.7	0.80	0.82	6.0	6.4	6.10	6.50
T09	Biochar	Uninoc.	40	22.5	25.5	0.65	0.68	3.5	3.8	4.30	4.80
T10		*Rhizobium*	20	24.0	27.3	0.80	0.83	4.0	4.3	4.65	4.90
T11		*Strept.*	20	23.5	25.4	0.70	0.73	5.0	5.6	4.80	5.01
T12		*Rhizobium+Strept.*	20	26.5	29.5	0.85	0.88	6.0	6.5	5.25	5.50
T13	Organic + Biochar	Uninoc.	40	30.5	32.1	0.80	0.84	4.0	4.4	5.45	5.70
T14		*Rhizobium*	20	32.5	34.2	0.85	0.89	5.0	5.4	5.65	5.80
T15		*Strept.*	20	32.0	33.1	0.70	0.74	5.5	5.9	5.70	5.95
T16		*Rhizobium+Strept.*	20	35.0	39.1	0.95	0.99	6.5	6.9	6.23	6.48

The nodules number of berseem clover plants among the two cutting, which cultivated in the open field through a number of 15 different fertilized treatments compared to the control-soil sample (Uninoc.+40 kg N/fed, T01) are shown in Table (6). Nodule number at the 1st cutting of berseem clover were lower than that of the 2nd cutting. Low nodule numbers on berseem clover roots were recorded in the eight treatments (T01, T03, T05, T07, T09, T11, T13 and T15) out of the 16 treatments which were rhizobium-free. Treatment No.16 (Seed inoulated with *Rhizobium leguminosarum bv. trifolii* + *Streptomyces luteogriseus*-08+ 20 kg N/fed cultivated in soil amended with organic matter and biochar) showed the highest number of root nodules followed by treatment No. 15 which did not inoculated with actinomycetes cultivated in the same soil.

Results also indicated that when organic fertilizer was added to the soil in the two treatments of (T06 and T08), its activity was increased, and this was obvious from increasing the number of root nodules. While addition of biochar in the treatments of T09 and T11 was not effective compared to organic fertilizer in T06 and T08. Furthermore, the nodule dry weight of the berseem clover plants in this study has become in the same trend as the results of the root nodules number.

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 28.50 and 50.25 nodule/plant in the first and second cutting, respectively. In the second season, the maximum values of nodule number recorded 32.75 and 56.50 nodule/plant in the first and second cutting in the same order. The maximum values of nodule dry weight were recorded in the seed clover cultivated in the soil amended with the two compounds.

Regardless of soil amended with organic matter and/or biochar, data in **Table (6)** 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 34.50 and 58.50 nodule/plant in the first and second cutting, respectively.

Table-6: Averages of nodule numbers and dry weight per plant of clover among two cutting during two successive seasons cultivated under different fertilizer treatments.

Codes	Soil amended	Biofertilizers	Mineral nitrogen Kg/Fed	Nodule number(#/plant)				Nodule dry weight (mg/plant)			
				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	Uninoc.	40	8	21	11	16	21.2	26	29.2	32.1
T02		*Rhizobium*	20	25	46	29	34	54.6	66.5	70.6	73.4
T03		*Strept.*	20	12	21	15	18	32.8	40.4	44.3	47.2
T04		*Rhizobium+Strept.*	20	30	56	36	48	66.5	82.2	86.2	89.1
T05	Organic	Uninoc.	40	9	13	12	18	17.8	24.9	28.2	32.2
T06		*Rhizobium*	20	30	30	28	38	52.7	63.9	66.3	69.2
T07		*Strept.*	20	14	23	17	27	23.7	29.6	33.2	36.2
T08		*Rhizobium+Strept.*	20	35	44	36	52	43.7	54.7	58.3	61.2
T09	Biochar	Uninoc.	40	14	23	17	26	26.5	30.9	34.1	37.2
T10		*Rhizobium*	20	32	57	39	61	56.7	57.7	61.2	64.3
T11		*Strept.*	20	16	35	19	39	31.5	37.2	40.1	43.7
T12		*Rhizobium+Strept.*	20	36	65	45	70	62.8	74.6	78.1	81.2
T13	Organic + Biochar	Uninoc.	40	15	31	18	36	24	28.5	32.1	35.1
T14		*Rhizobium*	20	40	64	46	73	61	74.8	78.9	81.2
T15		*Strept.*	20	18	37	21	41	25.2	30.2	34.8	37.2
T16		*Rhizobium+Strept.*	20	41	69	46	76	65.9	80.4	85.3	88.5
LSD 0.05				**2.20**	**2.25**	**2.13**	**3.12**	**1.23**	**2.13**	**1.78**	**1.85**
Effect of soil amended											
Without				18.75	36.00	22.75	29.00	43.78	53.78	57.58	60.45
Organic				22.00	27.50	23.25	33.75	34.48	43.28	46.50	49.70
Biochar				24.50	45.00	30.00	49.00	44.38	50.10	53.38	56.60
Organic + Biochar				28.50	50.25	32.75	56.50	44.03	53.48	57.78	60.50
LSD 0.05				**1.10**	**1.13**	**1.07**	**1.56**	**0.62**	**1.07**	**0.89**	**0.93**
Effect of Bio-fertilizers											
Uninoc., 40kgN/fed				11.50	22.00	14.50	24.00	22.38	27.58	30.90	34.15
Rhizobium, 20kgN/fed				31.75	49.25	35.50	51.50	56.25	65.73	69.25	72.03
Strepto.,20kgN/fed				15.00	29.00	18.00	31.25	28.30	34.35	38.10	41.08
Rhizobium+Strept., 20kgN/fed				35.50	58.50	40.75	61.50	59.73	72.98	76.98	80.00
LSD 0.05				**1.10**	**1.13**	**1.07**	**1.56**	**0.62**	**1.07**	**0.89**	**0.93**

The same trend in the second season, where the maximum values of nodule number recorded 40.75 and 61.50 nodule/plant in the first and second cutting in the same order. Regarding to the nodule dry weight of clover plants result show that the values of thesis parameter were recorded in the seed clover inoculated with rhizobia and actinomycetes combined with activation dose of nitrogen. In the first season, the mean values were 59.73 and 72.98 mg nodules/plant for nodules dry weight in the first and second cutting, respectively. The corresponding values in the second season were 76.98 and 80.00 mg nodules/plant in the same order.

The importance of biofertilizers was inconsistent and reflected from the results of fresh weight of berseem clover plants in this study through the 16 treatments. Data in Table (7) show the fresh weight (kg/m^2) among the two cuts of berseem clover shoots cultivated under different fertilizer treatments. The experimental results paid an attention to the importance of rhizobia to legume crops. This was clear from the decreasing in the fresh weight of berseem clover shoots in the absences of rhizobium. Similar observation was noted in the case of organicfertilizerthat increased the fresh weight of the berseem clover shoots compared to the use of biochar. As overall, treatment No. 16 (T16) gave the highest fresh weight due to combination of Biochar + organic fertilizer+ *Rhizobium japonicum bv. trifolii* + *Streptomyces luteogriseus*-08 + ½RDof mineral nitrogen (20 kg N/fed).Results of the dry weight of the berseem cloverplantsin this study has become in the same direction as the results of fresh weight (Table 7).

Regardless biofertilizer inoculation combined with activation dose of nitrogen, results in Table (7)showed significant differences among amended soil with organic matter and/or biochar. The highest values of shoot dry weight in the first season (3.38and 4.08 kg/m^2 in the same order in the first and second cutting). The corresponding values in the second season were 3.68 and 4.48kg/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 actinomycetes combined with activation dose of nitrogen were recorded the highest significant difference compared with uninoculated plant in the two seasons.

Table-7: Fresh and dry weights (kg/m^2) of clover shoots among two cutting cultivated under different treatments during two seasons.

Codes	Soil amended	Biofertilizers	Mineral nitrogen Kg/Fed	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	Uninoc.	40	2.1	2.9	2.3	2.6	0.432	0.51	0.462	0.501
T02		*Rhizobium*	20	3.2	3.9	3.5	3.8	0.582	0.71	0.61	0.642
T03		*Strept.*	20	2.7	3.2	2.9	3.1	0.48	0.59	0.51	0.551
T04		*Rhizobium+Strept.*	20	3.4	4.1	3.7	4.0	0.606	0.74	0.636	0.674
T05	Organic	Uninoc.	40	2.9	3.5	3.1	3.4	0.534	0.63	0.562	0.591
T06		*Rhizobium*	20	3.3	4.0	3.6	3.9	0.588	0.72	0.61	0.642
T07		*Strept.*	20	3.0	3.7	3.2	3.5	0.57	0.67	0.6	0.632
T08		*Rhizobium+Strept.*	20	3.4	4.1	3.7	4.5	0.618	0.76	0.642	0.673
T09	Biochar	Uninoc.	40	2.7	3.3	3.0	3.6	0.48	0.59	0.51	0.542
T10		*Rhizobium*	20	3.2	3.9	3.5	4.2	0.582	0.71	0.612	0.634
T11		*Strept.*	20	2.9	3.6	3.2	3.9	0.528	0.65	0.558	0.61
T12		*Rhizobium+Strept.*	20	3.4	4.1	3.6	4.5	0.606	0.74	0.634	0.654
T13	Organic + Biochar	Uninoc.	40	3.1	3.6	3.3	3.9	0.558	0.65	0.588	0.601
T14		*Rhizobium*	20	3.5	4.3	3.8	4.7	0.624	0.77	0.652	0.692
T15		*Strept.*	20	3.2	4.0	3.5	4.4	0.576	0.7	0.601	0.634
T16		*Rhizobium+Strept.*	20	3.7	4.4	4.1	4.9	0.666	0.82	0.695	0.71
LSD 0.05				**0.2**	**0.4**	**0.2**	**0.4**	**0.12**	**0.18**	**0.14**	**0.16**
Effect of Soil amended											
Without				2.85	3.53	3.10	3.38	0.53	0.64	0.55	0.59
Organic				3.15	3.83	3.40	3.83	0.58	0.70	0.60	0.63
Biochar				3.05	3.73	3.33	4.05	0.55	0.67	0.58	0.61
Organic + Biochar				3.38	4.08	3.68	4.48	0.61	0.74	0.63	0.66
LSD 0.05				**0.10**	**0.20**	**0.10**	**0.20**	**0.06**	**0.09**	**0.07**	**0.08**
Effect of Bio-fertilizers											
Uninoc., 40 kg N/fed				2.70	3.33	2.93	3.38	0.50	0.60	0.53	0.56
Rhizobium, 20 kg N/fed				3.30	4.03	3.60	4.15	0.59	0.73	0.62	0.65
Strepto. ,20 kg N/fed				2.95	3.63	3.20	3.73	0.54	0.65	0.57	0.61
Rhizobium+Strept. ,20 kg N/fed				3.48	4.18	3.78	4.48	0.62	0.77	0.65	0.68
LSD 0.05				**0.10**	**0.20**	**0.10**	**0.20**	**0.06**	**0.09**	**0.07**	**0.08**

Data in Table (8)showed the percentages of nitrogen content and crude protein among two cuts of berseem clover shoots cultivated under different fertilizer treatments in the two successive seasons conductedin the open field.The experimental resultsillustrated that the percentages of crude protein in the two cuttings indicated that the 2nd cut was always higher in the percentages of each of nitrogen content and crude protein than the 1st cutting.

In addition, treatments containing rhizobium as a biofertilizer showed a high percentage of crude protein compared to rhizobium-free treatments. The presence of organic fertilizerwasmore suitable for improving the growth of berseem clover plants compared to biochar.

Table-8: Nitrogen and crude protein percentages among two cutting clover cultivated under different fertilizer treatments among two seasons.

Codes	Soil amended	Biofertilizers	Mineral nitrogen Kg/Fed	Nitrogen (%)				Crude protein (%)			
				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	Uninoc.	40	2.21	2.29	2.41	2.62	13.81	14.31	15.06	16.38
T02		*Rhizobium*	20	2.45	2.8	2.65	3.1	15.31	17.5	16.56	19.38
T03		*Strept.*	20	2.32	2.77	2.42	2.91	14.5	17.31	15.13	18.19
T04		*Rhizobium+Strept.*	20	2.54	3.01	3.1	3.25	15.88	18.81	19.38	20.31
T05	Organic	Uninoc.	40	2.34	2.39	2.53	2.67	14.63	14.94	15.81	16.69
T06		*Rhizobium*	20	2.42	2.91	2.54	3.32	15.13	18.19	15.88	20.75
T07		*Strept.*	20	2.45	2.7	2.64	3.12	15.31	16.88	16.5	19.5
T08		*Rhizobium+Strept.*	20	2.53	3.07	2.73	3.36	15.81	19.19	17.06	21
T09	Biochar	Uninoc.	40	2.11	2.48	2.34	3.2	13.19	15.5	14.62	20
T10		*Rhizobium*	20	2.32	2.96	2.54	3.32	14.5	18.5	15.88	20.75
T11		*Strept.*	20	2.21	2.77	2.41	2.96	13.81	17.31	15.06	18.5
T12		*Rhizobium+Strept.*	20	2.35	2.91	2.53	3.23	14.69	18.19	15.81	20.19

T13	Organic + Biochar	Uninoc.	40	2.38	2.58	2.57	2.97	14.88	16.13	16.06	18.56
T14		*Rhizobium*	20	2.48	2.96	3.01	3.21	15.5	18.5	18.81	20.06
T15		*Strept.*	20	2.54	2.83	2.73	3.31	15.88	17.69	17.06	20.69
T16		*Rhizobium+Strept.*	20	2.66	3.18	2.93	3.41	16.63	19.88	18.31	21.31
Effect of Soil amended											
Without				2.38	2.72	2.65	2.97	14.88	16.98	16.53	18.57
Organic				2.44	2.77	2.61	3.12	15.22	17.30	16.31	19.49
Biochar				2.25	2.78	2.46	3.18	14.05	17.38	15.34	19.86
Organic + Biochar				2.52	2.89	2.81	3.23	15.72	18.05	17.56	20.16
Effect of Bio-fertilizers											
Uninoc., 40kgN/fed				2.26	2.44	2.46	2.87	14.13	15.22	15.39	17.91
Rhizobium, 20kgN/fed				2.42	2.91	2.69	3.24	15.11	18.17	16.78	20.24
*Strepto.*20kgN/fed				2.38	2.77	2.55	3.08	14.88	17.30	15.94	19.22
*Rhizobium+Strept.*20kgN/fed				2.52	3.04	2.82	3.31	15.75	19.02	17.64	20.70

Combination of rhizobium, actinomycetes organic fertilizer, biochar in the presence of low nitrogen fertilizer gave the highest crude protein content as shown in (T16). The presence of organic amended and *Rhizobium* plus Streptomyces as biofertilizers gave higher yield of fresh weight (Ton/Fed)compared to control soil (Uninoc.+40 kg N/fed, T01) (Table 9). Treatment T16 containing *Rhizobium* and streptomycete isolate as biofertilizers, organic fertilizer, low concentration of nitrogen fertilizer and biochar appeared the highest fresh weight per Feddan compared to all treatments. Similar observations were recorded in case of berseem clover yield of dry weight (Ton/Fed) of this study among the two cuttings in the two seasons.

Table-9: Fresh and dry weights (Ton/Feddan) of clover among two cutting cultivated under different treatments during two seasons.

Codes	Soil amended	Biofertilizers	Mineral nitrogen Kg/Fed	Fresh weights (Ton/Feddan)				Dry weights (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	Uninoc.	40	8.4	11.6	9.2	10.4	1.728	2.04	1.848	2.004
T02		*Rhizobium*	20	12.8	15.6	14	15.2	2.328	2.84	2.44	2.568
T03		*Strept.*	20	10.8	12.8	11.6	12.4	1.92	2.36	2.04	2.204
T04		*Rhizobium+Strept.*	20	13.6	16.4	14.8	16	2.424	2.96	2.544	2.696
T05	Organic	Uninoc.	40	11.6	14	12.4	13.6	2.136	2.52	2.248	2.364
T06		*Rhizobium*	20	13.2	16	14.4	15.6	2.352	2.88	2.44	2.568
T07		*Strept.*	20	12	14.8	12.8	14	2.28	2.68	2.4	2.528
T08		*Rhizobium+Strept.*	20	13.6	16.4	14.8	18	2.472	3.04	2.568	2.692
T09	Biochar	Uninoc.	40	10.8	13.2	12	14.4	1.92	2.36	2.04	2.168
T10		*Rhizobium*	20	12.8	15.6	14	16.8	2.328	2.84	2.448	2.536
T11		*Strept.*	20	11.6	14.4	12.8	15.6	2.112	2.6	2.232	2.44
T12		*Rhizobium+Strept.*	20	13.6	16.4	14.4	18	2.424	2.96	2.536	2.616
T13	Organic + Biochar	Uninoc.	40	12.4	14.4	13.2	15.6	2.232	2.6	2.352	2.404
T14		*Rhizobium*	20	14	17.2	15.2	18.8	2.496	3.08	2.608	2.768
T15		*Strept.*	20	12.8	16	14	17.6	2.304	2.8	2.404	2.536
T16		*Rhizobium+Strept.*	20	14.8	17.6	16.4	19.6	2.664	3.28	2.78	2.84
LSD 0.05				1.3	1.6	1.5	1.8	0.012	0.014	0.012	0.013
Effect of Soil amended											
Without				11.40	14.10	12.40	13.50	2.10	2.55	2.22	2.37
Organic				12.60	15.30	13.60	15.30	2.31	2.78	2.41	2.54
Biochar				12.20	14.90	13.30	16.20	2.20	2.69	2.31	2.44
Organic + Biochar				13.50	16.30	14.70	17.90	2.42	2.94	2.54	2.64
LSD 0.05				0.40	0.80	0.40	0.80	0.24	0.36	0.28	0.32
Effect of Bio-fertilizers											
Uninoc., 40kgN/fed				10.80	13.30	11.70	13.50	2.00	2.38	2.12	2.24
Rhizobium, 20kgN/fed				13.20	16.10	14.40	16.60	2.38	2.91	2.48	2.61
Strepto. ,20kgN/fed				11.80	14.50	12.80	14.90	2.15	2.61	2.27	2.43
Rhizobium+Strept. ,20kgN/fed				13.90	16.70	15.10	17.90	2.50	3.06	2.61	2.71
LSD 0.05				0.40	0.80	0.40	0.80	0.24	0.36	0.28	0.32

Acknowledgment

The authors would like to thank Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation for funding this research experiment. The research team would like to

thank Dr. Badawy Othman, Emeritus Prof. of Agric. Microbiology, Department of Agric. Microbiol., Faculty of Agric., Ain Shams University for his sincere help to prepare the inoculum of streptomycete strain used in this study. Authors would like to thank Eng. AmlAlaaeldin Mohamed for her sincere help, before passing away, to accomplish this work.

REFERENCES

Al Abboud, M.A., Ghany, T.M.A. and Alawlaqi, M.M. (2014). Role of biofertilizers in agriculture: a brief review. Mycopath, **11**(2).

Ananata, G. (2002). A review on organic farming for sustainable. Agriculture B.Sc. (Ag.), Final Year Exam Roll No: 32.

Attanandana, T., Chairerk, S., Somchai, K. and Boonsan, T. (1999). Simple determination of NPK in the soils. Warasan Din LaePui.

Chapman, H.D., and Pratt, P.F. (1962). Methods of analysis for soils, plants and waters. Soil Science,**93**(1), 68.

Chen, J.H. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use,**16**(20), 1–11.

Clark, F.E. (1965). Agar-Plate Method for total microbial count. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties,**9**, 1460–66.

Cottenie, A., Verloo, M., Kikens, L., Velghe, G. and Camerlynck, R. (1982). Analytical problems and method in chemical plant and soil analysis. Hand Book Ed. A. Cottenie, Gent, Belgium, 190.

El-Nahrawy, M.A.Z. (2005).The vital role of Egyptian clover in agriculture. Proceedings of the 11th Conference of Agronomy, November 15-16, 2005, Assiut University, Assiut, Egypt.55-62.

Ewees, M.S.A. and Abdel Hafeez, A.A.A. (2010). Response of maize grain yield to a partial substitution of N-mineral by applying organic manure, bio-inoculation and elemental sulphur as an alternative strategy to avoid the possible chemical pollution. Egypt. J. Soil Sci.,**50**(1), 141–166.

Ghaffarzadeh, M. (1997). Economic and biological benefits of intercropping berseem clover with oat in corn-soybean-oat rotation. Journal of Production Agriculture, **10**, 314-319.

Gomez, K.A., and Gomez, A.A. (1984). Statistical procedures for agricultural research. John Wiley & Sons.

Graves, W.L, Williams, W.A., and Thomson, C.D. (1996). Berseem clover: A winter annual forage for California agriculture. University of California Division of Agriculture and Natural Resources, Publication No. 21536, pp. 12.

Hamdollah, E., Ahmad, G. and Abdollah, J. (2009). Intercropping of cereals and legumes for forage production. Notulae Scientia Biologicae, **1**, 7-13.

Hames, B., Scarlata, C. and Sluiter, A. (2008). Determination of protein content in biomass. National Renewable Energy Laboratory, 1-5.

Hatim, A.S. (2013). Effect of bio-organic fertilizers on soil fertility and yield of groundnut (*Arachishypogaea* L.) in Malakal, Area, Republic of South Sudan. J. Nat. Resour& Environ. Stu.,**19**(12), 14- 18.

Helmy, A.A., Abo El-Soud, A.A., Desoky, A.H. and Rajab, M.N. (2014). The negative impacts of mineral nitrogen fertilization on inoculated berseem clover with rhizobium. Egypt. J. Appl. Sci.,**29**(5), 317-335.

Ismail, F. S. and Hassanen S.A. (2019). Improvement of Egyptian clover yield and quality by using bio and organic fertilizers in newly cultivated saline soil. J. Soil Sci. and Agric. Eng., Mansoura Univ., **10**(3), 147 – 147-155.

Jackson, M.L. (2005). Soil chemical analysis: Advanced course. UW-Madison Libraries Parallel Press.

James, J., Beth, W. and Joan, Mc.K. (1998). Forage Production from Spring Cereals and Cereal-Pea Mixtures. Ministry of Agriculture Food & Rural Affairs C.F., Ontario

Kumar, R., Kumawat, N.and Sahu, Y. K. (2017). Role of biofertilizers in agriculture. Pop Kheti,**5**(4), 63–66.

Kumari, B., Mallick, M.A., Solanki, M.K., Solanki, A.C., Hora, A. and Guo, W. (2019). Plant growth promoting rhizobacteria (PGPR): modern prospects for sustainable agriculture. In Plant Health under Biotic Stress (pp. 109–127). Springer.

Kunelius, T. (1997). Growing Berseem Clover, Percian Clover and Hairy Vetch. Crops and Livestock Research Centre, Agriculture and Agri-Food Canada. Agri-Info, 97-05, Bulletin. 10 p.

Mohamed, S.H., Al-Saeedi, T.A., and Sadik, A.S. (2013). Halotolerant streptomycetes isolated from soil at Taif region, Kingdom of Saudi Arabia (KSA) I: Purification, salt tolerance range, biological and molecular identification. African Journal of Biotechnology,**12**(19), 2565-2574.

Nicolás, M.F., Hungria, M. and Arias, C.A.A. (2006). Identification of quantitative trait loci controlling nodulation and shoot mass in progenies from two

Brazilian soybean cultivars. Field Crops Research,**95**(2–3), 355–366.

Noufal, E.H.A., Ali, M.A.M. and Abd El-Aal, M.M.M. (2018). Effect of rhizobium inoculation and foliar spray with salicylic and ascorbic acids on growth, yield and seed quality of pea plant (*Pisumsativum* L.) grown on a salt affected soil, New Valley-Egypt. 4th International Conference on Biotechnology Applications in Agriculture Held During, 4–7.

Oad, F.C., Agha, S.K. and Buriro, U.A. (2004). Growth and yield characters of inoculated and un-inoculated soybean undernitrogen broadcast and fertigation practices. International Journal of Agriculture and Biology (Pakistan).

Osman, G., Mostafa, S. and Mohamed S.H. (2007). Antagonistic and insecticidal activities of some *Streptomyces* isolates. Pakistan Journal of Biotechnology,**4**(1-2): 65-71.

Page, A.L., Miller, R.H. and Keeney, D.R. (1982). Methods of soil analysis part 2: chemical and microbiological properties second edition. Agronomy 920 Am. Soc. Agron. Inc. Soil Sci. Soc. Am. Inc. Pub. Madison, Wisconsin, USA.

Pepe, J.F. and Robert E.H. (1975). Plant Height, Protein Percentage, and Yield Relationships in Spring Wheat 1.Crop.

Ramadan, A.W. (1997). Response of Berseem-Ryegrass Mixture to Fertilization. M.Sc. Thesis, Ain Shams University, Cairo.

Stevenson, I.L. (1959). Dehydrogenase Activity in Soils. Canadian Journal of Microbiology,**5**(2), 229–35.

Thalooth A.T., Sary G.A., El-Nagar H.M., El-Kramany M.F., Kabesh M.O. and Hanna G.S. (2015). Yield and Quality Response of Ryegrass, Egyptian clover and their mixtures to different sources of fertilizers. Agricultural Sciences, **6**, 137-145.