



Utilization of BioFertilizers in Field Crop Production 17- Effect of organic manuring, mineral and bio fertilizers on forage yield and nutritive value of Egyptian clover (berseem) grown in new reclaimed sandy soil.

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Abstract: Two field experiments were conducted during two successive winter seasons of 2013/2014 and 2014/2015 in Research and Production Station, National Research Centre, (Al Emam Malek Village).Al-Nubaria District,AlBehaira Governorate, Egypt.

Experiments aimed to study the effect of organic manuring, chemical and bio fertilizers on forage yield and its nutritive value of Egyptian clover berseem (*Trifolium alexandrinum*) grown under new reclaimed sandy soil.

Four treatments were used. 1-Control, 2-bio+organic fertilization, Bio fertilization (phosphorine and nitrobine +20m³chiken manure/fad.*).3-Mineral fertilization as 20kg N /fad.,and 4-Bio+Organic + Mineral fertilization.

Fresh and dry forage yield/fed. were determined for the three cuts as ton / fad. (first cut 60 days after sowing, second 50 days from the first while third cut 40 days later.) during the two seasons. Chemical composition and nutritional evaluation for dry forage yield was conducted.

The obtained results showed that treatment of bio+organic +mineral fertilization was the best treatment in fresh and dry berseem yields as well as chemical components and evaluation i.e. crude protein, crude fiber, ether extract, nitrogen free extract, ash, digestible crude protein and total digestible nutrient yield in both seasons (as combined analysis of two seasons.)

Key words: Egyptian clover- berseem- bio +organic fertilization.

fad*.=Faddan=4200m²

Introduction

Egyptian clover (*Trifolium alexandrinum*, L.) is the first forage crop in Egypt; its cultivated area was about 1 million hectare/ year in 2007 (FAO STAT 2012).

Sandy soil in dry land areas are marginal for crop production. They are coarse-textured and inherently low fertility, with very poor organic matter content and water holding capacity. In Egypt about 94% of the total area sandy soils, with organic matter less than 1%.

Organic manures contain higher level of relatively easily available nutrient elements which are essentially required for plant growth. No doubt chemical farming exerts diverse environmental consequences, irrespective of the achieved increases in crop harvest. Hence biofertilized ecological farming system is considered, now days, as a potent solution for the deteriorated status of agriculture^{1,2,3,4,5,6}. The present work aims at evaluating the use of

chicken manure associated with biofertilizers singly or in combination with mineral fertilization on fresh and dry cuttings yield as well as nutritive value of Egyptian clover (berseem) grown in new reclaimed sandy soil.

Materials and Methods

Two field experiments were conducted during two successive winter seasons of 2013/2014 and 2014/2015 in Research and Production Station, National Research Centre, Al-Nubaria District, Al Behaira Governorate, Egypt.

The aim of this study to evaluate the effect of adding chicken manure, mineral and biofertilizers on forage fresh and dry yield and its nutritive value of Egyptian clover (*Trifolium alexandrinum* L. var. Meskawy).

The experimental soil was analyzed according to Jackson, (1960)⁷.

Soil texture was sandy and its characteristics are shown in Table (1)

Table (1): Mechanical and chemical analyses of the experimental soil (average of the two seasons 2013/2014 and 2014/2015)

Sand%	Silt%	Clay%	Ca Co ₃ %	Organic matter%	E.C. mmhos/cm ³	pH	Soluble N ppm	Available P, ppm	Exchan. K, ppm
91.2	3.7	5.1	1.4	0.3	0.3	7.3	8.1	3.2	20.0

Four fertilization experimental treatments can be described as follows:

1. Control
2. Bio-Organic fertilization 20m³ chicken manure/fad. + nitroline and phosphorine
3. Mineral fertilization (N) 20kg N /fad.
4. Bio-organic + mineral fertilization.

Organic manure at the source of chicken manure (Table 2) was added at the recommended dose of 20m³/fad. mixed with the soil surface layer three days before sowing.

Table (2): Chemical composition of the chicken manure used. (average of the two seasons 2013/2014 and 2014/2015)

Organic matter%	Organic carbon%	C/N ratio	pH	E.C. mmhos/ cm ³	N%	P (ppm)	K (ppm)
50.35	29.20	14.4	7.6	2.2	2.08	118	108

Nitroline and phosphorine are commercial products of biofertilizers produced by General Organization of Agriculture Equalization Fund (GOAEF) under the supervision of the Ministry of Agriculture, Egypt used in biofertilization treatments (treatments 2,4).

Nitrogen fertilizer was added at a rate 100 kg/fad.of ammonium sulfate (20.6 % N) for the mineral fertilization treatments (treatments 3 and 4) divided into three portions. The first was added before seeding and the second after the first cut while the third portion was added after the second cut. Split plot design was used with three replicates. Experimental field area were well prepared through two ploughing and leveling then divided into experimental plot 3x3.5 m= 10.5 m²(1/400 fad.).

Experimental area divided to four equal parts for fertilization treatments. All berseem seeds were inoculated with the specific *Rhizobium* strain and were sown at the rate of 20 kg seeds /fad. On 26October 2013 and 1November 2014 for the first and second seasons, respectively. The preceding crop was sunflower in the two seasons.

Three cuts were taken from each of the two seasons. First cut was obtained 60 days post seeding date, the second cut was obtained after 50 days from the first one, while the third one was taken after 40 days from the second cut.

Total nitrogen percentage was determined according to Chapman and Pratt(1978)⁸. Crude protein content was estimated by multiplying the analyzed total nitrogen percent by 6.25% for E. clover. Crude fiber % was determined according to A.O.A.C. (2000)⁹

The investigated nutritive evaluation of obtained forage dry matter for the fertilization different treatments included crude protein (CP), Crude fiber(CF), Ash, Nitrogen free extract (NFE), Ether extract (EE), Digestible crude protein (DCP), Total digestible nutrient (TDN) and Total digestible nutrient yield (TDNY) were determined according to N.R.C. (1977)¹⁰.

Data were statistically analysed according to Snedecor and Cochran (1990)¹¹. The combined analysis was conducted for the data of the two seasons since the results of two growing seasons followed a similar trend. The least significant differences (L.S.D) at the level of 5% significance was used to compare the treatments means.

Results and Discussion

A. Growth characteristics

1-Plant height

Table (3) show the high response of berseem clover growth as indicated in plant height (cm) to adding either bio-organic or mineral fertilizers all over the three cuts. The response showed high significant differences between control treatment and other fertilization treatments for the three cuts. The higher taller berseem plants was found in the bio-organic + mineral fertilizer treatment especially for the third cut. Data also indicated that plant height increased for all fertilization treatments. The same results trend were found by Soleymani *et al.*, (2011)⁵ and Saad (2012)¹².

2-Leaf area cm³/m²

Data presented in Table(4) clear significant differences for berseem leaf area cm³/m² between fertilization treatment and control in the three cuts.

Data indicated that treatment of bio-organic + mineral fertilizers recorded the best leaf area in the three cuts. Leaf area cm³/m² increased from the first cut to the third cut, respectively. Results may be due to the integrate effect between fast effect of mineral fertilizers with slow release of bio-organic fertilizers to nutrient release in soil which reflect on leaf area as cm³/m². Results are in accordance with those obtained by¹³ working on lettuce plants.

Table (3): Plant height (cm) of berseem clover as affected by fertilization (combined analysis of 2013/2014 and 2014/2015)

Fertilization treatments	First cut	Second cut	Third cut
Control	34.8	43.5	54.6
Bio-organic	40.9	49.1	60.2
Mineral	42.5	52.0	63.9
Bio-organic+mineral	47.0	57.5	72.2
L.S.D.(5%)	2.1	2.9	3.2

Table (4): Leaf are cm³/m² of berseem clover as affected by fertilization(combined analysis of 2013/2014 and 2014/2015)

Fertilization treatments	First cut	Second cut	Third cut
Control	125.4	264.1	317.2
Bio-organic	168.6	296.8	336.3
Mineral	181.6	315.9	365.9
Bio-organic+mineral	203.5	344.5	401.8
L.S.D. (5%)	8.2	10.0	11.3

B. Forage yield

1- Fresh forage yield (Ton/ fad.):

Data presented in Table (5) clear that there were significant differences between control and other fertilization treatments for weight as (ton/fad.). The highest fresh forage yield recorded by 2nd cut followed by 1st cut and 3rd cut was the third. The highest berseem fresh forage yield 11.793 ton/fad. produced by bio-organic + mineral fertilizers treatment in the 1st cut followed by the same fertilization treatment in the 2nd cut (11.501ton/fad.). The third order was for mineral fertilization treatment in the 2nd cut which produced 10.440 ton/fad. Superiority of bio-organic+ mineral fertilization (32.48 ton/fad. for total three cuts) may be due to integrated slow effect of organic and bio fertilizers beside the fast effect of mineral fertilizers ^{2,3}. The same results were obtained by ^{14,15,16,5}. El – Kramany *et al* (2012)¹⁷ found similar results on berseem clover grown alone or in mixture with triticale.

2- Dry forage yield(Ton/fad.).

Results presented in Table(5) showed that treatment of bio-organic + mineral fertilizers significantly surpassed other fertilization treatments. Dry forage yield of bio-organic+ mineral fertilizers treatment increased from 1st to 2nd to 3rd cuts(1.143- 2.026 and 3.093 ton/fad.). The second order of treatments recorded by the mineral fertilization treatments which produced 0.934,1.838 and 2.746 ton/fad. for the successive three cuts. ^{1,16,5} stated similar results on Egyptian berseem clover.

Table (5): Fresh and dry forage yield (ton/fad.) of berseem clover as affected by fertilization treatments.(combined analysis of 2013/2014 and 2014/2015 seasons)

Fertilization treatments	1 st cut		2 nd cut		3 rd cut		Total	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Control	6.707	0.627	7.471	1.391	7.155	2.500	21.333	4.518
Bio-organic	8.493	0.819	9.495	1.678	7.351	2.539	25.359	5.036
Mineral	9.656	0.934	10.440	1.838	8.367	2.746	28.463	5.518
Bio-organic +Mineral	11.793	1.143	11.501	2.026	9.186	3.093	32.480	6.262
L.S.D at 5 %	0.382	0.068	0.461	0.092	0.365	0.137	1.028	0.258

C. Chemical Constituents and Nutritive Value:

The effect of different sources of fertilizers on the nutritive value for berseem clover forage over the three cuts was conducted. The following constituents under study were:

1-Crude Protein (CP):

Nitrogen fertilizer sole or in combination with bio-organic fertilizers clearly affected CP percentage in tissues of berseem clover forage plants in all cuts. Tables (6,7,8). CP percentage decreased gradually from 1st cut to 3rd cut for all fertilization treatments. On the other hand, crude protein content as kg/fad. Increased progressively from the earlier cut to the latter one due to the high dry mass of leaves and stems of berseem forage plants in the latter cut than in the earlier one. In general, the high CP content was reported by bio-organic+ mineral fertilization treatment (250.3 kg/fad.) in the 1st cut, 431.1 kg/fad. in the 2nd cut and 536.6 kg/fad. for the 3rd cut. Such increase of CP percentage and content under mineral fertilization sole or in combination with bio-organic fertilizers may be attributed to the increase in the available nitrogen in root medium as reported by¹⁸. Similar findings were obtained by^{14,5,17}

2-Crude Fiber (CF):

Data presented in Tables (6,7,8) show that crude fiber increased gradually from 1st cut to 3rd cut. The effect of different nitrogen fertilizers on berseem clover crude fiber concentration was clearly identified under different sources of fertilizers. The highest CF% obtained by bio-organic+mineral treatment.

Similar results were obtained for berseem total fiber content as kg/fad. The highest CF content reported by bio-organic + mineral treatment for the three cuts. Such increase of CF content by N fertilization is expected since N fertilization increased constitutively dry forage yield. These results are confirmed with^{14,1,19}.

3-Ash:

Data presented in Tables (6,7,8) reveal that ash% for berseem clover decreased gradually from 1st cut to 3rd cut irrespective to fertilization treatments. However, the highest ash% was recorded by bio-organic+mineral treatment overall the three cuts. Regarding ash content kg/fad. clearly increased by different sources of nitrogen fertilizers. Such effect was clear in all cuts and gradually increased from the 1st cut to 3rd one. The highest ash content was reported by bio-organic +mineral fertilization treatment. Similar results were obtained by^{5,19}.

4-Nitrogen free extracts (NFE):

The effect of different sources of fertilizers on the NFE%, as well as NFE content as kg/fad. for berseem clover are shown in Tables (6,7,8) which indicate that NFE% was decreased with fertilization. In addition, data presented in the same Tables clarified consistent increase in NFE% from the 1st cut to the 3rd one. However, the highest content of NFE obtained by bio-organic+mineral fertilization treatment due to large dry forage accumulated in this treatment oversight all three cuts.¹ came to similar findings.

5-Ether Extract (EE):

EE% of berseem clover forage relatively decreased from the 1st cut to the third one (Tables 6,7,8). Data in the same Tables showed that different sources of fertilizers greatly affect EE% in the tissues of berseem forage in all cuts. Bio-organic+mineral fertilizer treatment recorded the highest EE%. Regarding EE content as kg/fad. for berseem clover forage increased gradually from the 1st cut to the 3rd one. These results were to be expected since the forage dry matter increased from the 1st cut to the 3rd cut. The obtained data results (Tables 6,7,8) showed superiority of bio-organic + mineral fertilization treatment than other treatments. These results are in harmony with those obtained by^{1,17}.

6- Digestible Crude Protein (DCP):

Data of digestible crude protein percentage and content are presented in Tables (6,7,8). The obtained results indicate that DCP % decreased consistently from the first cut till the third one. The highest percentage of DCP% was recorded for berseem forage plants fertilized with bio-organic +mineral fertilizers. The absolute amount of DCP (kg/fad.) for berseem clover forage as affected by different sources and combination of fertilizers

was similar to that obtained on DCP% as a result of increasing dry matter accumulation from the early cut to the latter one. It may be concluded that bio-organic + mineral fertilizers treatment caused an increase in DCP%. Here it should be noticed that the absolute amount of DCP was higher in the latter cut than earlier one. This was true as a result of increasing dry matter accumulation from the early cut to the fir stone. It may be concluded that bio-organic+ mineral fertilizers treatment caused an increase in DCP% as well as DCP content in all cuts. This is may be to the increase. of available nitrogen concentration in the plant root medium.¹⁸. Similar findings were obtained by¹

7- Total Digestible Nutrient (TDN):

The effect of the different fertilizers sources on total digestible nutrient % of berseem clover forage was present in Tables (6,7,8) which indicated that there were no changes over sight the three cuts. However, the TDN% increased consistently in the bio-organic +mineral fertilizer treatment in all cuts. Regarding TDNY content as kg/fad. For berseem clover forage (Tables 6,7,8) revealed that it is increased clearly in the latter cuts than the earlier one. The data also show that there was an increase for TDNY content by fertilization. The bio-organic +mineral fertilizer treatment surpassed the other fertilization treatments over all the three cuts. These results are in agreement with those showed by ^{14,1,5}.

Table (6): Chemical constituents and total digestible nutrients of berseem clover as affected by fertilization for percentage and content (kg/fad.)First cut

(combined analysis of 2013/2014 and 2014/2015 seasons)

Fertilization treatments	CP		CF		Ash		NFE		EE		DCP		TDN	
	%	yield kg/fad.	%	yield kg/fad.	%	yield kg/fad.	%	Yield kg/fad	%	yield kg/fad.	%	yield kg/fad.	%	yield kg/fad.
Control	19.63	123.0	14.55	91.3	16.97	106.4	46.58	292.0	2.06	14.4	14.04	88.0	62.1	389.3
Bio-organic	20.41	167.1	16.98	139.0	18.13	148.5	41.88	342.9	2.53	23.0	14.96	132.5	64.9	531.5
Mineral	21.35	199.4	17.25	161.1	18.31	171.0	40.55	378.7	2.70	25.3	15.59	145.6	66.9	624.8
Bio-organic+ Mineral	21.90	250.3	18.63	212.9	19.59	223.9	37.05	423.4	3.03	34.6	16.05	183.4	68.6	784.0
L.S.D at 5 %		9.2		10.4		8.4		26.4		2.4		8.0		41.6

Table (7): Chemical constituents and total digestible nutrients of berseem clover as affected by fertilization for percentage and content (kg/fad.)second cut

(combined analysis of 2013/2014 and 2014/2015 seasons)

Fertilization Treatments	CP		CF		Ash		NFE		EE		DCP		TDN	
	%	yield kg/fad.	%	yield kg/fad.	%	yield kg/fad.	%	Yield kg/fad	%	yield kg/fad.	%	yield kg/fad.	%	yield kg/fad.
Control	18.93	263.3	16.60	230.9	15.17	211.0	47.01	653.9	1.96	27.3	13.40	186.3	61.0	848.5
Bio-organic	19.45	335.7	18.83	315.9	15.99	268.3	43.31	726.7	2.38	39.9	14.15	237.4	63.1	1058.8
Mineral	20.08	369.0	19.17	352.3	16.43	301.9	41.88	769.7	2.51	46.1	14.67	269.6	65.3	1200.2
Bio-organic+Mineral	20.85	431.1	21.09	437.3	18.45	373.7	37.44	758.5	2.70	54.7	15.08	305.5	67.1	1359.4
L.S.D at 5 %		11.5		16.2		13.2		39.1		4.6		9.6		50.8

Table (8): Chemical constituents and total digestible nutrients of berseem clover as affected by fertilization for percentage and content (kg/fad.). Third cut(combined analysis of 2013/2014 and 2014/2015 seasons)

Fertilization treatments	CP		CF		Ash		NFE		EE		DCP		TDN	
	%	yield kg/fad.	%	yield kg/fad.	%	yield kg/fad	%	Yield kg/fad	%	yield kg/fad	%	yield kg/fad	%	yield kg/fad.
Control	13.31	332.7	18.13	453.2	12.37	309.3	53.38	1334.5	1.45	36.2	10.43	260.7	60.4	1510.0
Bio-organic	16.12	409.5	20.40	517.9	14.06	356.9	47.54	1207.0	1.76	44.6	11.26	285.8	61.9	1571.6
Mineral	16.89	463.7	20.81	571.4	14.53	398.9	46.04	1264.4	1.85	50.8	11.90	326.7	62.9	1727.2
Bio-organic+ Mineral	17.35	536.6	22.83	706.1	16.04	496.1	43.14	1334.3	1.99	61.5	12.29	373.4	64.8	2004.2
L.S.D at 5 %		16.3		21.5		16.5		53.6		6.8		11.0		83.7

Conclusion

Generally It can be concluded that the treatment of bio-organic +mineral fertilization for berseem clover fresh and dry forage surpass other fertilizers treatments. It was the first and produce highest crude protein and other nutritive values than other fertilization treatments in this study under sandy soil conditions.

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