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Yield and Quality of Egyptian Clover and Ryegrass Mixtures under Nitrogen Levels and Bio-Fertilizer

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Abstract: A field experiment was conducted during the two successive winter seasons of 2017/2018 and 2018/2019 to investigate the potentialities of mixing Egyptian clover with Ryegrass under nitrogen levels and bio-fertilizer treatments and their combination to increase forage yield and quality at Sids Agricultural Research Station, (ARC), Egypt. The experimental design was a split block with three replicates, main plots included the combination of four mixing ratios (Egyptian clover alone, 75% Egyptian clover: 25% Ryegrass, 50% Egyptian clover: 50% Ryegrass and Ryegrass alone), whereas the five nitrogen levels and bio-fertilizer were arranged horizontally (20 Kg N fed^{-1} (control), 40 Kg N fed^{-1}, 60 Kg N fed^{-1}, 20 Kg N fed^{-1} with bio-fertilizer and 40 Kg N $fed^{-1}$with bio-fertilizer). Results showed that mixing system had significant effect on fresh and dry forage yield at the five cuts and at total fresh and dry forage yield. Maximum values of studied traits were obtained with mixing system (75% Egyptian clover: 25% Ryegrass). On the contrary, minimum values were recorded with Ryegrass alone. Nitrogen levels and bio-fertilizer indicated significant differences in fresh and dry forage yield at the five cuts and at total fresh and dry forage yield. The highest values were obtained by using (40 Kg N fed^{-1} + bio-fertilizer followed by 60 Kg N fed^{-1} and 40 Kg N fed^{-1}) as compared with the control (20 Kg N fed^{-1}). Regarding the effect of mixing ratios and nitrogen levels and bio-fertilizer interaction the obtained results indicated that best treatment was at 75% Egyptian clover: 25% Ryegrass mixture fertilized by 40 Kg N fed^{-1} with bio-fertilizer in fresh and dry forage production. Chemical analysis of forage plants showed that the mixture of 75% Egyptian clover: 25% Ryegrass surpassed that of other treatments except Egyptian clover 100% for crude protein, ash and organic matter. The results also revealed that the highest record of DCP, and crude fiber was obtained by forage mixture of 75% Egyptian clover: 25% Ryegrass fertilized with 40 Kg N fed^{-1} with bio-fertilizer. Such higher yield of these characters has secured a balanced ratio which is really needed for farm animals.

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1. Introduction

Egyptian clover (*Trifolium alexandrinum*, L.) is considered the main winter forage legume crop in Egypt. This is because of its high yield and quality especially crude protein content. Ryegrass (*Lolium multifloorum*, L.) is an annual winter grass and adapted to a wide types of soils and produce quick cover after cutting, of high production and quality. Thus, the main benefit of mixing Ryegrass with Egyptian clover to increase the productivity of total dry matter and forage quality **Ghaffarzadeh, (1997)**.

Mixtures of forage crops (grasses and legumes) have many usefulness and are superior to their monocultures in providing greater yield and quality. Also, grass-legume mixtures have high crude protein and low fiber concentration than pure stand of grass. **Hamdollah et al. (2009).**

Bio-fertilizer used to improve soil health and to increase the yield which plays an important role for minimizing the harmful effect of pesticides and herbicides **Ananata, (2002).**

Grasses and legumes are considered as important forage crops because of their nutritional value, especially protein content in legumes and crude fiber in grasses **Rakeih et al., (2008)**. However, monocultures of legumes or grasses do not provide satisfactory results for forage production and nutritive value **Osman and Nersoyan, (1986)**. Therefore, mixing grasses with legumes for forage production has been a common cropping system especially in the Mediterranean countries **Lithourgidis et al., (2006)**. In mixtures, companion grasses are expected to provide structural support for legumes' growth, improve light interception, and facilitate mechanical harvest, whereas legumes improve the quality of forage **Thompson et al., (1992)**. Also, mixtures are increasing total dry matter content **Holland and Brummer, (1999)**, and may increase crude protein percentage, protein yield, and length of optimum harvest period over pure grasses **Carr et al., (1998)**. Thus, mixing legume forages with grass forages can be an effective way to improve

forage quality and nutritive value of the end product **Ross et al., (2004)**. Literature also revealed that intake of fodder is low when fed as pure fodders, either of legumes or grasses, compared with their grass-legume mixtures **Ansar et al., (2010)**.

Despite the advantages associated with mixed cropping, its management is rather difficult than sole cropping due to differences in the agronomic practices of the component crops of the mixture. Differences in sowing time, fertilizer and water requirements, growth behavior, phenology and harvesting time of the associated crops pose many problems to the farmers in managing the mixtures. Hence, devising the suitable agronomic practices for mixtures under different ecological zones had been the aim of many researcher **(Tuna and Orak, 2007; Nadeem et al., 2010)**.

Amongst the most important cultural practices that need to be carefully adjusted to achieve maximum benefit from the forage grass-legume mixtures, are the nitrogen (N) fertilizer level and the appropriate mixing rate. Although, the grass-clover relationship is highly affected by N fertilization **(Caradus et al., 1993; Shareif et al., 1996)**, inconsistent results have been reported on the effects of N fertilization on crude protein (CP) and fiber concentrations of forages **Balabanli et al., (2010)**. While **Min et al., (2002)** reported that N application at high rates increased CP concentration compared with the control treatment.

The main aim of the current study was to determine the effect of mixing ratio and nitrogen levels and bio-fertilizer on forage yield of Egyptian clover- Ryegrass mixture. Also, assess the dry matter content and prominent quality measures of Egyptian clover- Ryegrass mixtures under the Egyptian agricultural system.

2. Materials and Methods

Experimental Design and Treatments:

A field experiment was conducted at Sids Agricultural Research Station, (ARC), Egypt during 2017/2018 and 2018/2019 winter seasons to study the effect of mixture rates of Egyptian clover var. Giza 6 (*Trifolium alexandrinum* L.) and Ryegrass (*Lolium multiflorum* L.), nitrogen levels, bio-fertilizer and their combinations on forage yield and quality.

Soil sample from the experimental site were taken at random from the upper 30 cm of the soil surface for physical and chemical analysis according to **Cottenie et al., (1982) (Table 1)**.

Table (1): Physical and chemical properties of the experimental sites.

Crosse sand (%)	Fin sand (%)	Silt (%)	Clay (%)	Texture		O.M (%)	SAR	$CaCO_3$ (%)
5.90	22.58	30.80	40.72	Clay		0.75	6.19	6.18
pH	EC (dS/m)	Cations (meq/l)				Anions (meq/l)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_3	Cl^{-}	SO^{--}_4
7.83	3.50	8.15	5.55	20.45	0.85	2.35	13.95	18.70
Macronutrients (mg/kg)			Micronutrients (mg/kg)					
N	P	K	Fe		Mn		Zn	
44.85	4.90	179	2.34		1.77		0.69	

Sowing dates were October, 4 and 7 in the two successive seasons 2017/2018 and 2018/2019, respectively. The experimental design was a split block design with three replications. The four mixture systems were assigned to the main plots as follows:

1- Egyptian clover 100% alone (20 kg fed^{-1}).
2- 75% Egyptian clover: 25% Ryegrass.
3- 50% Egyptian clover: 50% Ryegrass.
4- Ryegrass 100% alone (10 kg fed^{-1}).

The five nitrogen levels and bio-fertilizer were arranged horizontally as follows:

1- 20 Kg fed^{-1} mineral (N) fertilization (control treatment).
2- 40 Kg fed^{-1} mineral (N) fertilization.
3- 60 Kg fed^{-1} mineral (N) fertilization.
4- 20 Kg fed^{-1} mineral (N) fertilization + Bio-fertilizer
5- 40 Kg fed^{-1} mineral (N) fertilization + Bio-fertilizer

Bio-fertilizer was *Rhizobium* inoculation for Egyptian clover, and N_2-fixing bacteria for grasses *Azospirillum* and *Bacillus* for Ryegrass. Fertilized Nitrogen as Urea (46.5% N) added after 15 days from planting and after each cut. To avoid *Rhizobium* cross contamination, plots of un-inoculated seeds were sown first. The inoculated seeds were prepared by three grams of Arabic gum added to two table spoons full of water and mixed to form a solution, 1 kg of Egyptian clover seed was weighed and 2 table spoons full of Arabic gum solution were added and mixed well; 10 g of legume inoculants was added and mixed well so that all the solution are coated, the inoculated seeds were left under shade to dry and sown immediately. N-fixing bacteria for grasses

Azospirillum and *Bacillus* (1mL^{-1}) in fresh liquid culture at a rate of 1.5 L fed^{-1} for Ryegrass then seeds were inoculated immediately prior to sowing. Experimental field well prepared then divided into experimental plots 12 m^2 (3m x 4m).

Recommended rates of phosphorus (150 kg fed^{-1}) in form of super phosphate (15.5% P_2O_5) and potassium (50 kg fed^{-1}) in form of potassium sulphate (48% K_2O) were applied to all the experimental plots just after land preparation.

Five cuts of Egyptian clover and four cuts of Ryegrass were taken from each of the two seasons. First cut was at 60 days from seeding date, and then after 30 days.

Parameter Assessments:

Fresh and dry Forage Yield

Samples of Egyptian clover and Ryegrass and their mixtures were taken from each experimental plot from one m^2 just before each cut to determine fresh and dry forage yields (kg fed^{-1}), then transformed to ton fed^{-1}.

Chemical analysis:-

Plant samples were taken from each cut then oven dried at 70 ˚C until constant weight, followed by fine grinding to estimate the following:

1- Crude protein content: Total nitrogen was determined in the dry matter by using the modified micro-kjeldahl method as described in **A.O.A.C. (2000)**. The crude protein content was calculated by multiplying the total nitrogen percentage by the factor of 6.25. Digestible crude protein (DCP) was calculated according to **Bredon et al. (1963)** using the following equation:
DCP= 0.9596CP – 3.55.

2- Ash and crude fibers content: Ash and crude fibers contents were estimated according to the method described in the **A.O.A.C. (2000).** Organic matter (OM%) were estimated by using the following equation: OM% =100 – (Ash%).

3- Total digestible nutrients (TDN); was estimated according to **Adams *et al*., (1964)**, using the following equations:
TDN= 74.43 + 0.35 CP – 0.73 CF (legume).
TDN= 50.41 + 1.04 CP – 0.07 CF (grass).

4- Digestible energy (DE) = k cal / g dry matter; was calculated according to **Heaney and Pigden (1963)**. Whereas: DE = 0.546 + 0.055 TDN.

Statistical analysis

Data were statistically analyzed according to **Snedecor and Cochran (1990).** Bartlett's test was done to detect the homogeneity of error variances. The test was non significant for all traits, thus combined analysis across the two seasons was carried out for all studied traits, the least significant differences (LSD) at the level of 5% significance was used to compare the treatments mean **Steel et al., (1980).**

3. Results and Discussion

A. Fresh and dry forage yield (ton fed^{-1})

A. 1. Effect of mixing ratio on:

A. 1. 1. Fresh forage yield (ton fed^{-1})

Data in Table 2 revealed that mixing ratio had significant effect on fresh forage yield at the five cuts and at total fresh forage yield (ton fed^{-1}). The highest values were obtained from mixing ratio (75% Egyptian clover: 25% Ryegrass) which recorded 65.27 ton fed^{-1}. These results were similar to those obtained by **Thalooth et al., (2015).** The other mixing ratio (50% Egyptian clover: 50% Ryegrass) recorded lower values and amounted 58.59 ton fed^{-1}, compared to sole crops 100% Egyptian clover and/or 100% Ryegrass, which recorded 61.51and 27.68 ton fed.$^{-1}$, respectively.

A.1.2. Dry forage yield (ton fed^{-1})

Mixing ratio affected significantly dry forage yield at each cut and total dry forage yield (Table 2). The highest values were obtained with mixing ratio (75% Egyptian clover: 25% Ryegrass) and comprised 8.43 ton fed^{-1}. While with 50% Egyptian clover: 50% Ryegrass mixing ratio were recorded (8.05 ton fed^{-1}), as compared with sole crops (Egyptian clover 100% and Ryegrass 100%). which recorded 8.08and 4.47ton fed^{-1}., respectively.

These findings are in agreement with **James et al., (1998)** who found that mixing Ryegrass with Egyptian clover are increase total dry yield production and forage quality.

A.2. Effect of nitrogen levels and bio-fertilizer on:

A.2.1. Fresh forage yield (ton fed^{-1})

Results of fresh forage yield at each cut and total fresh forage yield (ton fed^{-1}) as affected by nitrogen levels and bio-fertilizer are presented in Table (3). Results indicated significant differences in the above mentioned trait. The highest values were obtained by using 40 Kg N fed^{-1} + bio-fertilizer followed by 60 Kg N fed^{-1} and 40 Kg N fed^{-1}, as compared to the control (20 Kg N fed^{-1}). The increases were 8.60%, 6.10% and 4.77%, respectively. While, the lowest value was obtained by using 20 Kg N fed^{-1} + bio-fertilizer which increased by 3.18%, as compared to control. These results may be due to the effect of *Rhizobium* bacteria on nodules of Egyptian clover roots and its effect in fixing nitrogen from the ambient air beside the integrated effect of Bio + N fertilizers. Also, Bio-fertilizer have a potential importance to improve growth and increase yield not only due to high N_2- fixation activity, but also due to plant growth promotion by production of auxins, cytokinins, gibberlins and

ethaylene and increased nutrient uptake **Kloepper, (2003)**.

Table (2): Effect of Egyptian clover, Ryegrass and their mixtures on fresh and dry forage yield (ton fed^{-1}) (combined over two seasons).

Mixing ratio	1st cut	2nd cut	3rd cut	4th cut	5th cut	Total yield (tonfed^{-1})
Fresh forage yield (ton fed^{-1})						
Egyptian clover 100%	8.98	14.31	15.09	15.65	7.48	61.51
75% Egyptian clover: 25% Ryegrass	9.99	16.03	16.25	16.00	7.00	65.27
50% Egyptian clover: 50% Ryegrass	8.77	12.86	15.06	15.70	6.20	58.59
Ryegrass 100%	7.68	5.48	8.87	5.65	---	27.68
L.S.D 0.05	0.172	1.046	0.581	0.294	0.437	2.212
Dry forage yield (ton fed^{-1})						
Egyptian clover 100%	1.14	1.78	1.99	2.19	0.98	8.08
75% Egyptian clover: 25% Ryegrass	1.30	1.72	1.98	2.26	1.17	8.43
50% Egyptian clover: 50% Ryegrass	1.26	1.65	1.95	2.23	0.96	8.05
Ryegrass 100%	1.14	0.89	1.40	1.04	---	4.47
L.S.D 0.05	0.113	0.057	0.022	0.020	0.129	0.314

A.2.2. Dry forage yield (ton fed^{-1})

Results in Table (3) revealed that dry forage yield (ton fed^{-1}) was significantly affected by nitrogen levels and bio-fertilizer. Application of 40 Kg N fed^{-1} + bio-fertilizer recorded the highest values of dry forage yield (ton fed^{-1}) followed by 60 Kg N fed^{-1}and 40 Kg N fed^{-1} as compared to the control (20 Kg N fed^{-1}). Relative percentages in dry yield as compared to control treatment were 3.62, 1.11 and 0.28 %, respectively. These results are in agreement with **Kennedy et al., (2004)** who proposed that bio-fertilizer can help to supply of nutrients contributing to optimized yield is maintained.

Table (3): Effect of nitrogen levels and bio-fertilizer on fresh and dry forage yield (ton fed^{-1}) (combined over two seasons).

Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Total yield (ton fed^{-1})
Fresh forage yield (ton fed^{-1})						
20 Kg N fed^{-1} (Control)	8.49	11.10	13.29	12.84	5.23	50.95
40 Kg N fed^{-1}	8.77	11.70	14.37	13.54	5.00	53.38
60 Kg N fed^{-1}	8.65	13.34	13.68	13.31	5.08	54.06
20 Kg N fed^{-1}+bio-fertilizer	9.09	11.87	13.51	12.97	5.13	52.57
40 Kg N fed^{-1}+bio-fertilizer	9.28	12.83	14.22	13.59	5.41	55.33
L.S.D 0.05	0.104	0.151	0.227	0.114	0.118	0.530
Dry forage yield (ton fed^{-1})						
20 Kg N fed^{-1} (Control)	1.22	1.49	1.73	1.87	0.87	7.18
40 Kg N fed^{-1}	1.19	1.55	1.82	1.93	0.71	7.20
60 Kg N fed^{-1}	1.15	1.61	1.87	1.93	0.70	7.26
20 Kg N fed^{-1}+bio-fertilizer	1.25	1.38	1.84	1.93	0.80	7.20
40 Kg N fed^{-1}+bio-fertilizer	1.24	1.52	1.90	1.98	0.80	7.44
L.S.D 0.05	0.042	0.061	0.024	0.011	0.027	0.019

A.3. Interaction effects on:

A.3.1. Fresh forage yield (ton fed^{-1})

Results of fresh forage yield (ton fed^{-1}) as affected by the interactions between mixing ratio and nitrogen levels and bio-fertilizer are presented in Table (4). Results indicated significant interaction effects on fresh forage yield (ton fed^{-1}). The highest values were obtained by the interaction between mixing ratio of 75% Egyptian clover: 25% Ryegrass and 40 Kg N fed^{-1} + bio-fertilizer followed by 60 Kg N fed^{-1}and 40 Kg N fed^{-1}, which recorded 66.32, 66.11 and 65.61 ton fed^{-1}, respectively. On the other hand, the lowest values were observed by the interaction between Ryegrass 100% and 20 Kg N fed^{-1} followed by Ryegrass 100% and 20 Kg N fed^{-1}+bio-fertilizer. These results are in agreement with **Mousa, (1995)** indicated that Egyptian clover-Ryegrass mixture inoculated with *Rhizobium* and mixture of N-fixing bacteria produced higher fresh yield.

A.3.2. Dry forage yield (ton fed^{-1})

Results in Table (4) revealed a significant interaction effect between mixing ratios and nitrogen levels and bio-fertilizer on dry forage yield. This significant effect means that the effect of mixing ratio

was not the same under the different nitrogen levels and bio-fertilizer. The effect of mixing ratios was more pronounced at 75% Egyptian clover: 25% Ryegrass mixture in combination with 40 Kg N fed^{-1} + bio-fertilizer and it gives the highest value.

These results were in agreement with those obtained by **Butler and Muir, (2012) and Thalooth et al., (2012).** These results may be due to the effect of *Rhizobium* bacteria on nodules of E. clover roots and its effect in fixing nitrogen from the ambient air beside the integrated effect of Bio + N fertilizers.

Table (4): Effect of Egyptian clover, Ryegrass, their mixtures, nitrogen levels and bio-fertilizer interaction on fresh and dry forage yield (ton fed^{-1}) (combined over two seasons).

Mixing ratio	Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Total yield (ton fed^{-1})
Fresh forage yield (ton fed^{-1})							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	8.99	14.18	14.26	14.67	8.49	60.59
	40 Kg N fed^{-1}	8.30	12.49	16.83	16.72	6.58	60.92
	60 Kg N fed^{-1}	8.12	16.33	14.58	15.97	6.83	61.83
	20 Kg N fed^{-1}+bio-fertilizer	9.41	13.33	15.45	15.09	7.42	60.70
	40 Kg N fed^{-1}+bio-fertilizer	10.09	15.25	14.32	15.82	8.08	63.56
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	9.53	16.40	15.83	15.75	6.49	64.00
	40 Kg N fed^{-1}	10.00	15.83	16.25	16.28	7.25	65.61
	60 Kg N fed^{-1}	10.49	15.68	16.43	16.00	7.51	66.11
	20 Kg N fed^{-1}+bio-fertilizer	9.43	16.23	16.00	15.90	6.76	64.32
	40 Kg N fed^{-1}+bio-fertilizer	10.49	16.00	16.75	16.08	7.00	66.32
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	8.51	11.18	15.34	15.37	5.92	56.32
	40 Kg N fed^{-1}	9.25	11.84	15.66	15.82	6.17	58.74
	60 Kg N fed^{-1}	8.13	14.92	14.79	15.45	5.99	59.28
	20 Kg N fed^{-1}+bio-fertilizer	9.01	13.26	14.24	15.23	6.34	58.08
	40 Kg N fed^{-1}+bio-fertilizer	8.96	13.09	15.25	16.64	6.58	60.52
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	6.92	2.67	7.75	5.58	---	22.92
	40 Kg N fed^{-1}	7.54	6.63	8.75	5.34	---	28.26
	60 Kg N fed^{-1}	7.88	6.42	8.92	5.84	---	29.06
	20 Kg N fed^{-1}+bio-fertilizer	8.50	4.67	8.34	5.67	---	27.18
	40 Kg N fed^{-1}+bio-fertilizer	7.58	7.00	10.57	5.84	---	30.99
L.S.D 0.05		0.491	1.04	0.576	0.390	1.224	0.203
Dry forage yield (ton fed^{-1})							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	1.12	1.90	1.88	2.10	0.96	7.96
	40 Kg N fed^{-1}	1.14	1.82	1.92	2.18	1.00	8.06
	60 Kg N fed^{-1}	1.13	1.92	1.99	2.22	0.89	8.15
	20 Kg N fed^{-1}+bio-fertilizer	1.12	1.52	2.14	2.24	1.04	8.06
	40 Kg N fed^{-1}+bio-fertilizer	1.21	1.75	2.03	2.20	1.01	8.20
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	1.31	1.73	1.85	2.10	1.21	8.20
	40 Kg N fed^{-1}	1.30	1.93	2.07	2.24	0.82	8.36
	60 Kg N fed^{-1}	1.24	1.76	2.02	2.20	1.15	8.37
	20 Kg N fed^{-1}+bio-fertilizer	1.35	1.71	1.96	2.29	1.30	8.61
	40 Kg N fed^{-1}+bio-fertilizer	1.31	1.48	2.00	2.46	1.35	8.60
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	1.45	1.86	1.97	2.25	1.33	8.86
	40 Kg N fed^{-1}	1.22	1.38	1.93	2.33	1.00	7.86
	60 Kg N fed^{-1}	1.11	1.76	2.00	2.23	0.77	7.87
	20 Kg N fed^{-1}+bio-fertilizer	1.22	1.54	1.99	2.18	0.88	7.81
	40 Kg N fed^{-1}+bio-fertilizer	1.31	1.71	1.88	2.14	0.84	7.88
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	1.00	0.48	1.22	1.03	---	3.73
	40 Kg N fed^{-1}	1.10	1.07	1.35	0.96	---	4.48
	60 Kg N fed^{-1}	1.13	1.00	1.47	1.07	---	4.67
	20 Kg N fed^{-1}+bio-fertilizer	1.33	0.77	1.27	1.02	---	4.39
	40 Kg N fed^{-1}+bio-fertilizer	1.13	1.14	1.70	1.10	---	5.07
L.S.D 0.05		0.203	0.161	0.104	0.087	0.189	0.172

B- Forage quality.

The forage quality measurements included crude protein, ash, crude fiber, organic matter and nutritive value such as digestible crude protein (DCP), total digestible nutrients (TDN) and digestible energy (DE) were evaluated as function parameters versus mixtures ratio of Egyptian clover with Ryegrass, nitrogen levels and bio-fertilizer.

B.1. Effect of mixing ratios on forage quality:

In respect to the effects of forage mixing ratios on forage chemical composition, results are in Table (5) which indicated that crude protein, digestible crude protein, crude fiber, ash and organic matter contents were significantly affected by studied forage treatments, (pure stands& mixtures).

Concerning crude protein (CP) and digestible crude protein (DCP) percentages results showed that the highest values were observed with 100 % Egyptian clover followed by 75 % Egyptian clover: 25 % Ryegrass, and the lowest values with 100 % Ryegrass in all cuts. The CP and DCP percentages in Ryegrass plants were significantly lower than that of Egyptian clover and Ryegrass mixture in the five cuts.

Table (5): Effect of Egyptian clover, Ryegrass and their mixtures on forage quality (combined over two seasons).

Mixing ratio	1st cut	2nd cut	3rd cut	4th cut	5th cut	Mean
CP%						
Egyptian clover 100%	20.80	19.86	16.37	13.02	11.96	16.40
75% Egyptian clover: 25% Ryegrass	20.23	17.40	13.89	9.89	8.91	14.06
50% Egyptian clover: 50% Ryegrass	18.34	15.47	12.38	8.44	7.45	12.42
Ryegrass 100%	14.95	13.06	11.08	8.13	---	11.81
L.S.D 0.05	1.054	1.116	1.083	1.421	1.325	1.724
DCP%						
Egyptian clover 100%	16.41	15.51	12.16	8.94	7.93	12.19
75% Egyptian clover: 25% Ryegrass	15.86	13.15	9.78	5.94	5.00	9.95
50% Egyptian clover: 50% Ryegrass	14.05	11.30	8.33	4.55	3.60	8.37
Ryegrass 100%	10.80	8.98	7.08	4.25	---	7.78
L.S.D 0.05	1.146	1.282	1.193	1.210	1.328	1.380
CF%						
Egyptian clover 100%	19.74	22.27	23.77	25.57	27.42	23.75
75% Egyptian clover: 25% Ryegrass	20.72	23.15	25.57	29.08	30.21	25.75
50% Egyptian clover: 50% Ryegrass	22.91	25.00	27.18	30.63	31.38	27.42
Ryegrass 100%	22.76	24.91	25.99	30.10	---	25.94
L.S.D 0.05	0.341	0.216	0.663	0.514	0.448	0.775
Ash%						
Egyptian clover 100%	16.94	15.68	14.08	12.89	11.93	14.30
75% Egyptian clover: 25% Ryegrass	18.15	16.76	15.40	13.84	12.55	15.34
50% Egyptian clover: 50% Ryegrass	15.70	13.60	12.23	11.35	10.65	12.71
Ryegrass 100%	15.85	14.16	12.96	12.52	---	13.87
L.S.D 0.05	0.544	0.329	0.633	0.350	0.521	0.438
OM%						
Egyptian clover 100%	83.06	84.32	85.92	87.11	88.07	85.70
75% Egyptian clover: 25% Ryegrass	81.85	83.24	84.60	86.16	87.45	84.66
50% Egyptian clover: 50% Ryegrass	84.30	86.40	87.77	88.65	89.35	87.29
Ryegrass 100%	84.15	85.84	87.04	87.48	---	86.13
L.S.D 0.05	0.571	0.625	0.430	0.331	0.348	0.271

Results of Egyptian clover and Ryegrass pure stands forage quality revealed that the highest values of CP and DCP percentages were observed in the first cut for all treatments. It is clear that CP and DCP percentages of Ryegrass and Egyptian clover mixing ratios were significantly increased in all cuts compared to Ryegrass pure stands. This trend may be explained by the fact that the grass component in the mixtures will give better quality in close proximity to the legume, these results are in agreement with those obtained by **Omar (1997) and Thalooth et al., (2015).**

Regarding the ash contents, results in Table (5) indicated that the highest ash percentages were found with the mixing ratio of 75% Egyptian clover: 25% Ryegrass as well as Egyptian clover and Ryegrass

pure stand. The same results were obtained by **Thalooth et al., (2015).** The lowest values were with 50% Egyptian clover: 50% Ryegrass treatments, especially in the 5th cut. Results also indicate that the highest values of ash percentage were obtained in the first cut for all forage treatments (pure stands and its mixtures).

In respect to the effect of forage treatments on organic matter (OM) contents, results showed a reverse trend to that of ash content. The highest values of organic matter percentage were obtained with 50% Egyptian clover: 50% Ryegrass, but the lowest values were recorded with 75% Egyptian clover: 25% Ryegrass. Meanwhile the organic matter percentages were increased from the 5th cut up to the 1st cut.

Concerning the effect of the forage treatments on crude fiber contents, results indicated that the lowest values of crude fiber were obtained with Egyptian clover pure stand and the highest values were with 50% Egyptian clover: 50% Ryegrass especially in the 5th cut. These results are in agreement with those obtained by **Thalooth et al., (2015)** who reported that CF yield (kg fed^{-1}) of the proposed binary forage mixtures was higher for the later cut than the earlier cuts

B.2. Effect of Nitrogen levels and bio-fertilizer on forage quality:

Data in Table (6) showed that crude protein, digestible crude protein, crude fiber, ash and organic matter percentages were significantly affected by nitrogen levels and bio-fertilizer treatments in the five taken cuts. Results indicated that the highest values of above mentioned traits were obtained with 40 Kg N fed^{-1}+ bio-fertilizer except organic matter which gave the highest value at 20 Kg N fed^{-1} . On the other hand, the lowest values were obtained at 20 Kg N fed^{-1} for all traits except organic matter which gave the lowest value at 40 Kg N fed^{-1} +bio-fertilizer for the five taken cuts. In other words, increasing nitrogen levels and bio-fertilizer led to significantly increasing crude protein, digestible crude protein, crude fiber and ash percentages and decreasing organic matter percentage.

The highest values of crude protein, digestible crude protein and ash percentages were recorded in the 1st cut under 40 Kg N fed^{-1} +bio-fertilizer, respectively. Meanwhile the highest values of crude fiber and organic matter percentages were obtained in the 5th cut with 40 Kg N fed^{-1} +bio-fertilizer and 20 Kg N fed^{-1}, respectively. This may be due to the increase in dry matter in 5th cut, consequence increase crude fiber. Many investigators confirmed the stimulating effect of inoculation in creating a favorable habitat for legume growth and biological nitrogen fixation which leads to increasing productivity and quality **Rizk et al., (2011) Badawi and El-Sayed, 2015.**

B.3. Effect of the interaction between mixing ratios and nitrogen levels and bio-fertilizer on forage quality:

Regarding the effect of mixing ratios and nitrogen levels and bio-fertilizer interaction on crude protein (CP%), digestible crude protein (DCP%), ash% organic matter (OM%) and crude fiber (CF%), are shown in Tables (7, 8and 9). Results indicated that the highest values of crude protein and digestible crude protein were obtained in the 1st cut at the interaction between 75% Egyptian clover: 25% Ryegrass and 40 Kg N fed^{-1} +bio-fertilizer it recorded 22.35% and 17.90% followed by 100% Egyptian clover and 40 Kg N fed^{-1} +bio-fertilizer (21.69% and 17.26%), respectively. In the 2nd cut the highest value was found at the interaction between Egyptian clover 100% and 40 Kg N fed^{-1} +bio-fertilizer and recorded 20.81% and 16.42% for CP% and DCP%, respectively. At the 3rd, 4th and 5th cuts the highest values were found at the interaction between Egyptian clover and 40 Kg N fed^{-1} +bio-fertilizer followed by 75% Egyptian clover: 25% Ryegrass and 40 Kg N fed^{-1} +bio-fertilizer interaction. In respect to the mean values of the five cuts, the highest values of CP% and DCP% were recorded (17.22% and 12.97%) at the interaction between 100% Egyptian clover and 40 Kg N fed^{-1} +bio-fertilizer followed by100% Egyptian clover and 60 Kg N fed^{-1} which recorded (16.85% and 12.62%) for CP% and DCP%, respectively. This is due to the increase in total dry matter content in mixtures **Holland and Brummer, (1999)**, and then increase crude protein % and protein yield **Carr et al., (1998)**. So that, mixing legume forages with grass forages can be an effective way to improve forage quality and nutritive value of the end product **Ross et al., (2004).** Also, **Min et al., (2002)** reported that N application at high rates increased CP concentration compared with the control treatment.

Ash percentage recorded the highest value at 1st cut at 75% Egyptian clover: 25% Ryegrass and 60 Kg N fed^{-1} recorded (19.14%), main while the highest value of ash% at the mean of the five cuts were obtained at the interaction between75% Egyptian clover: 25% Ryegrass and 40 Kg N fed^{-1} +bio-fertilizer recorded (16.02%). While, the lowest value of ash% recorded 11.87% at the interaction between 100% Ryegrass and 20 Kg N fed^{-1}. Results obtained by **Nyamagouda and Angadi, (2002)** and **Soleymani et al., (2011)**. are similar to those findings in this work Concerning crude fiber percentage, results showed that the highest values were observed

with 50% Egyptian clover: 50% Ryegrass and 40 Kg N fed^{-1} +bio-fertilizer interaction at 5th cut, and its lowest values were recorded in the 1st cut at the interaction between Egyptian clover 100% and 20 Kg N fed^{-1}. Results indicated that the highest values of organic matter were obtained with 50% Egyptian clover: 50% Ryegrass and 20 Kg N fed^{-1}. In this respect, **Mouriño et al., (2003)** and **Fulkerson et al., (2007)** reported that grasses have much higher hemicellulose. Similar results were recorded by **Rizk et al., (2007)** and **Soleymani et al., (2011)**. Such higher yield of CF of 75% E. clover: 25% Ryegrass mixture under the combined source of fertilizers is responsible for a number of benefits beside the higher production of yield and quality as securing balanced ratio concerning crude protein and energy which is really needed for ruminants ration.

Table (6): Effect of nitrogen levels and bio-fertilizer on forage quality (combined over two seasons).

Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Mean
CP%						
20 Kg N fed^{-1} (Control)	17.48	15.09	12.51	9.07	8.86	12.60
40 Kg N fed^{-1}	18.66	16.58	13.53	9.82	9.40	13.60
60 Kg N fed^{-1}	18.96	17.18	13.74	10.15	9.55	13.92
20 Kg N fed^{-1}+bio-fertilizer	18.04	16.14	13.09	9.54	9.22	13.21
40 Kg N fed^{-1}+bio-fertilizer	19.76	17.24	14.28	10.77	10.18	14.45
L.S.D 0.05	0.251	0.034	0.115	0.201	0.113	0.274
DCP%						
20 Kg N fed^{-1} (Control)	13.22	10.93	8.45	5.15	4.95	8.54
40 Kg N fed^{-1}	14.36	12.36	9.43	5.87	5.47	9.50
60 Kg N fed^{-1}	14.64	12.94	9.63	6.19	5.61	9.80
20 Kg N fed^{-1}+bio-fertilizer	13.76	11.94	9.01	5.60	5.30	9.12
40 Kg N fed^{-1}+bio-fertilizer	15.41	12.99	10.15	6.78	6.22	10.31
L.S.D 0.05	0.204	0.136	0.164	0.230	0.108	0.183
CF%						
20 Kg N fed^{-1} (Control)	22.21	23.85	27.80	21.54	24.35	23.95
40 Kg N fed^{-1}	23.94	25.60	28.82	22.35	25.60	25.26
60 Kg N fed^{-1}	24.79	26.63	28.51	22.09	26.34	25.67
20 Kg N fed^{-1}+bio-fertilizer	23.46	25.95	28.72	21.95	25.50	25.12
40 Kg N fed^{-1}+bio-fertilizer	24.76	26.10	30.37	23.33	26.64	26.24
L.S.D 0.05	0.154	0.131	0.086	0.115	0.172	0.283
Ash%						
20 Kg N fed^{-1} (Control)	15.73	14.17	12.55	11.54	8.36	12.47
40 Kg N fed^{-1}	16.32	15.13	13.57	12.56	11.59	13.83
60 Kg N fed^{-1}	17.07	15.18	13.86	12.79	12.01	14.18
20 Kg N fed^{-1}+bio-fertilizer	16.50	14.91	13.92	12.78	11.62	13.95
40 Kg N fed^{-1}+bio-fertilizer	17.67	15.87	14.44	13.59	12.20	14.75
L.S.D 0.05	0.114	0.030	0.137	0.104	0.090	0.101
OM%						
20 Kg N fed^{-1} (Control)	84.27	85.83	87.45	88.46	91.64	87.53
40 Kg N fed^{-1}	83.68	84.87	86.43	87.45	88.41	86.17
60 Kg N fed^{-1}	82.93	84.82	86.14	87.21	87.99	85.82
20 Kg N fed^{-1}+bio-fertilizer	83.50	85.09	86.08	87.22	88.38	86.05
40 Kg N fed^{-1}+bio-fertilizer	82.33	84.13	85.56	86.41	87.80	85.25
L.S.D 0.05	0.131	0.238	0.150	0.205	0.123	0.110

Table (7): Effect of Egyptian clover, Ryegrass, their mixtures, nitrogen levels and bio-fertilizer interaction on crude protein (CP%) and digestible crude protein (DCP%) (combined over two seasons).

Mixing ratio	Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Mean
CP%							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	19.52	18.58	15.32	12.29	11.36	15.41
	40 Kg N fed^{-1}	20.93	20.25	16.62	12.83	11.92	16.51
	60 Kg N fed^{-1}	21.33	20.31	16.86	13.53	12.23	16.85
	20 Kg N fed^{-1}+bio-fertilizer	20.55	19.35	15.88	12.63	11.74	16.03
	40 Kg N fed^{-1}+bio-fertilizer	21.69	20.81	17.16	13.85	12.57	17.22
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	19.14	15.37	13.19	9.23	8.32	13.05
	40 Kg N fed^{-1}	20.12	18.05	13.79	9.74	8.75	14.09
	60 Kg N fed^{-1}	20.37	18.18	14.07	10.11	9.05	14.36
	20 Kg N fed^{-1}+bio-fertilizer	19.21	16.16	13.59	9.64	8.70	13.46
	40 Kg N fed^{-1}+bio-fertilizer	22.35	19.26	14.80	10.74	9.74	15.38
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	18.08	14.08	11.85	7.87	6.90	11.76
	40 Kg N fed^{-1}	18.36	14.79	12.52	8.54	7.53	12.35
	60 Kg N fed^{-1}	18.51	16.86	12.40	8.34	7.38	12.70
	20 Kg N fed^{-1}+bio-fertilizer	17.67	16.29	12.20	8.24	7.22	12.32
	40 Kg N fed^{-1}+bio-fertilizer	19.07	15.31	12.95	9.20	8.23	12.95
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	13.20	12.32	9.69	6.88	---	10.52
	40 Kg N fed^{-1}	15.25	13.25	11.18	8.19	---	11.97
	60 Kg N fed^{-1}	15.65	13.37	11.64	8.63	---	12.32
	20 Kg N fed^{-1}+bio-fertilizer	14.73	12.77	10.68	7.64	---	11.46
	40 Kg N fed^{-1}+bio-fertilizer	15.94	13.58	12.22	9.29	---	12.76
L.S.D 0.05		0.951	1.223	1.180	0.816	1.282	1.168
DCP%							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	15.18	14.28	11.15	8.24	7.35	11.24
	40 Kg N fed^{-1}	16.53	15.88	12.40	8.76	7.89	12.29
	60 Kg N fed^{-1}	16.92	15.94	12.63	9.43	8.19	12.62
	20 Kg N fed^{-1}+bio-fertilizer	16.17	15.02	11.69	8.57	7.72	11.83
	40 Kg N fed^{-1}+bio-fertilizer	17.26	16.42	12.92	9.74	8.51	12.97
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	14.82	11.20	9.11	5.31	4.43	8.97
	40 Kg N fed^{-1}	15.76	13.77	9.68	5.80	4.85	9.97
	60 Kg N fed^{-1}	16.00	13.90	9.95	6.15	5.13	10.23
	20 Kg N fed^{-1}+bio-fertilizer	14.88	11.96	9.49	5.70	4.80	9.37
	40 Kg N fed^{-1}+bio-fertilizer	17.90	14.93	10.65	6.76	5.80	11.21
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	13.80	9.96	7.82	4.00	3.07	7.73
	40 Kg N fed^{-1}	14.07	10.64	8.46	4.64	3.68	8.30
	60 Kg N fed^{-1}	14.21	12.63	8.35	4.45	3.53	8.63
	20 Kg N fed^{-1}+bio-fertilizer	13.41	12.08	8.16	4.36	3.38	8.28
	40 Kg N fed^{-1}+bio-fertilizer	14.75	11.14	8.88	5.28	4.35	8.88
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	9.12	8.27	5.75	3.05	---	6.55
	40 Kg N fed^{-1}	11.08	9.16	7.18	4.31	---	7.93
	60 Kg N fed^{-1}	11.47	9.28	7.62	4.73	---	8.28
	20 Kg N fed^{-1}+bio-fertilizer	10.58	8.70	6.70	3.78	---	7.44
	40 Kg N fed^{-1}+bio-fertilizer	11.75	9.48	8.18	5.36	---	8.69
L.S.D 0.05		0.104	0.165	0.282	0.151	0.129	0.104

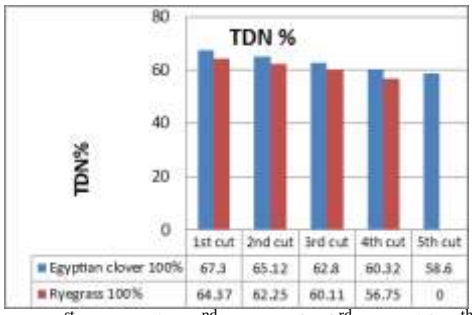
Table (8): Effect of Egyptian clover, Ryegrass, their mixtures, nitrogen levels and bio-fertilizer interaction on ash% and organic matter (OM%) (combined over two seasons).

Mixing ratio	Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Mean
Ash%							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	16.55	14.91	13.39	12.36	11.24	13.69
	40 Kg N fed^{-1}	16.16	16.58	14.57	12.53	11.84	14.34
	60 Kg N fed^{-1}	17.16	15.66	14.23	13.25	12.40	14.54
	20 Kg N fed^{-1}+bio-fertilizer	16.85	15.18	14.16	13.18	12.14	14.30
	40 Kg N fed^{-1}+bio-fertilizer	17.98	16.09	14.04	13.16	12.04	14.66
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	16.94	15.84	14.85	13.56	12.13	14.66
	40 Kg N fed^{-1}	17.54	16.36	15.31	14.33	12.77	15.26
	60 Kg N fed^{-1}	19.14	17.08	15.62	13.40	12.40	15.53
	20 Kg N fed^{-1}+bio-fertilizer	18.75	16.76	15.36	13.06	12.17	15.22
	40 Kg N fed^{-1}+bio-fertilizer	18.34	17.75	15.84	14.85	13.30	16.02
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	15.34	13.37	11.20	10.22	10.07	12.04
	40 Kg N fed^{-1}	16.05	13.66	11.95	11.02	10.17	12.57
	60 Kg N fed^{-1}	16.05	13.88	12.68	11.79	11.22	13.12
	20 Kg N fed^{-1}+bio-fertilizer	14.14	12.74	12.32	11.45	10.56	12.24
	40 Kg N fed^{-1}+bio-fertilizer	16.92	14.34	13.02	12.30	11.26	13.57
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	14.09	12.56	10.76	10.05	---	11.87
	40 Kg N fed^{-1}	15.54	13.92	12.44	12.35	---	13.56
	60 Kg N fed^{-1}	15.92	14.10	12.91	12.74	---	13.92
	20 Kg N fed^{-1}+bio-fertilizer	16.25	14.94	13.84	13.45	---	14.62
	40 Kg N fed^{-1}+bio-fertilizer	17.45	15.29	14.84	14.05	---	15.41
L.S.D 0.05		0.141	0.230	0.127	0.134	0.152	0.167
OM%							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	83.45	85.09	86.61	87.64	88.76	86.31
	40 Kg N fed^{-1}	83.84	83.42	85.43	87.48	88.16	85.67
	60 Kg N fed^{-1}	82.84	84.34	85.77	86.76	87.60	85.46
	20 Kg N fed^{-1}+bio-fertilizer	83.15	84.82	85.84	86.82	87.87	85.70
	40 Kg N fed^{-1}+bio-fertilizer	82.02	83.91	85.96	86.84	87.96	85.34
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	83.06	84.16	85.15	86.44	87.87	85.34
	40 Kg N fed^{-1}	82.46	83.64	84.69	85.67	87.24	84.74
	60 Kg N fed^{-1}	80.86	82.92	84.39	86.60	87.60	84.47
	20 Kg N fed^{-1}+bio-fertilizer	81.25	83.24	84.64	86.94	87.83	84.78
	40 Kg N fed^{-1}+bio-fertilizer	81.66	82.25	84.16	85.15	86.71	83.99
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	84.66	86.63	88.80	89.79	89.93	87.96
	40 Kg N fed^{-1}	83.95	86.34	88.05	88.98	89.84	87.43
	60 Kg N fed^{-1}	83.95	86.12	87.32	88.21	88.78	86.88
	20 Kg N fed^{-1}+bio-fertilizer	85.86	87.26	87.68	88.56	89.44	87.76
	40 Kg N fed^{-1}+bio-fertilizer	83.08	85.66	86.98	87.70	88.74	86.43
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	85.91	87.44	89.24	89.96	---	88.14
	40 Kg N fed^{-1}	84.46	86.08	87.56	87.66	---	86.44
	60 Kg N fed^{-1}	84.08	85.90	87.09	87.26	---	86.08
	20 Kg N fed^{-1}+bio-fertilizer	83.75	85.06	86.16	86.56	---	85.38
	40 Kg N fed^{-1}+bio-fertilizer	82.55	84.71	85.16	85.95	---	84.59
L.S.D 0.05		0.265	0.131	0.164	0.238	0.151	0.160

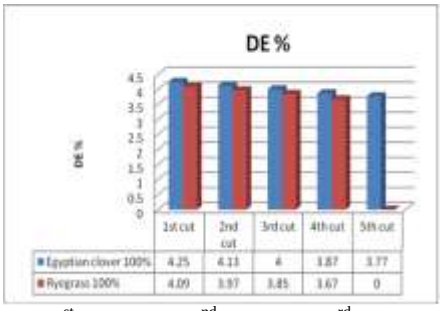
Table (9): Effect of Egyptian clover, Ryegrass, their mixtures, nitrogen levels and bio-fertilizer interaction on crude fiber (CF%) (combined over two seasons).

Mixing ratio	Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Mean
CF%							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	17.32	19.25	21.05	22.34	24.62	20.92
	40 Kg N fed^{-1}	19.27	22.57	23.98	24.94	27.33	23.62
	60 Kg N fed^{-1}	21.90	23.44	25.29	26.37	27.97	24.99
	20 Kg N fed^{-1}+bio-fertilizer	20.81	23.34	24.42	25.32	27.25	24.23
	40 Kg N fed^{-1}+bio-fertilizer	19.40	22.75	24.13	28.90	29.91	25.02
75% Egyptian clover: 25% Ryegrass	**20 Kg N fed^{-1} (Control)**	19.33	21.92	23.13	29.65	31.16	25.04
	40 Kg N fed^{-1}	21.30	23.44	25.87	29.04	30.12	25.95
	60 Kg N fed^{-1}	23.23	24.31	26.30	27.25	28.74	25.97
	20 Kg N fed^{-1}+bio-fertilizer	19.08	22.63	28.36	28.66	29.66	25.68
	40 Kg N fed^{-1}+bio-fertilizer	20.67	23.45	24.19	30.80	31.36	26.09
50% Egyptian clover: 50% Ryegrass	**20 Kg N fed^{-1} (Control)**	22.07	24.14	26.16	29.26	30.37	26.40
	40 Kg N fed^{-1}	22.60	24.32	26.40	30.91	31.95	27.24
	60 Kg N fed^{-1}	23.62	25.63	28.47	30.94	31.65	28.06
	20 Kg N fed^{-1}+bio-fertilizer	22.40	23.66	25.16	30.88	30.90	26.60
	40 Kg N fed^{-1}+bio-fertilizer	23.86	27.24	29.71	31.14	32.05	28.80
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	21.70	23.53	25.07	29.95	---	25.06
	40 Kg N fed^{-1}	22.46	25.43	26.16	30.38	---	26.11
	60 Kg N fed^{-1}	23.82	25.78	26.47	29.47	---	26.39
	20 Kg N fed^{-1}+bio-fertilizer	22.11	24.20	25.89	30.05	---	25.56
	40 Kg N fed^{-1}+bio-fertilizer	23.69	25.61	26.38	30.65	---	26.58
L.S.D 0.05		0.144	0.118	0.163	0.110	0.184	0.160

Data presented in Figs (1,2) showed the total digestible nutrients (TDN) and digestible energy (DE) in the five taken cuts of Egyptian clover and Ryegrass pure stands. Results revealed that the highest values of TDN and DE were obtained with Egyptian clover pure stand in the five cuts and were significantly higher than Ryegrass. Results also demonstrated the positive effect of 40 Kg N fed^{-1}+ bio-fertilizer on the values of TDN and DE over the five taken cut. While, the DE was not significantly affected by the investigated nitrogen and bio-fertilizer in 1st, 2nd and 4th cuts. Similar finding was noticed with the TDN in the 4th and 5th cuts. In respect to the effect of pure stand and nitrogen levels and bio-fertilizer treatments interaction on TDN and DE in the five taken cuts, results in Table (10) revealed that the highest values were recorded with 100% Egyptian clover and 20 Kg N fed^{-1} followed by 100% Egyptian clover and 40 Kg N fed^{-1}. While, the lowest value was obtained by 100% Ryegrass and 20 Kg N fed^{-1}. These results could be used in upgrading quality of the assigned forage mixture through selecting the appropriate association of botanical components. Similar results obtained by **Lithourgidis et al., (2006)** and **Dordas et al., (2012)**.

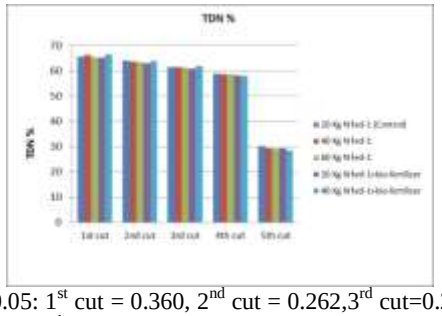


L.S.D $_{0.05}$: 1st cut = 1.02, 2nd cut = 1.85,3rd cut=1.06, 4th cut= 1.14, 5th cut= 1.06

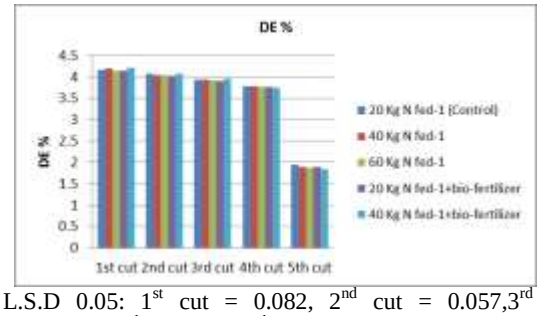


L.S.D 0.05: 1st cut = 0.11, 2nd cut = 0.18,3rd cut=0.10, 4th cut= 0.13, 5th cut= 0.11

Fig. (1): Effect of pure stand treatments on total digestible nutrients (TDN) and digestible energy (DE) in the five taken cuts (combined over two seasons).



L.S.D 0.05: 1st cut = 0.360, 2nd cut = 0.262,3rd cut=0.287, 4th cut= 0.911,5th cut= 0.381



L.S.D 0.05: 1st cut = 0.082, 2nd cut = 0.057,3rd cut=0.013, 4th cut= 0.71,5thcut= 0.016

Fig. (2): Effect of nitrogen levels and bio-fertilizer treatments on total digestible nutrients (TDN) and digestible energy (DE) in the five taken cuts (combined over two seasons).

Table (10): Effect of pure stand and nitrogen levels and bio-fertilizer treatments interaction on total digestible nutrients (TDN) and digestible energy (DE) in the five taken cuts (combined over two seasons).

Mixing ratio	Nitrogen level and bio-fertilizer	1st cut	2nd cut	3rd cut	4th cut	5th cut	Mean
TDN%							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	68.62	66.88	64.43	62.42	60.43	64.56
	40 Kg N fed^{-1}	67.69	65.04	62.74	60.71	58.65	62.97
	60 Kg N fed^{-1}	65.91	64.43	61.87	59.92	58.29	62.08
	20 Kg N fed^{-1}+bio-fertilizer	66.43	64.16	62.16	60.37	58.65	62.35
	40 Kg N fed^{-1}+bio-fertilizer	67.86	65.11	62.82	58.18	57.00	62.19
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	62.62	61.58	58.73	55.47	---	59.60
	40 Kg N fed^{-1}	64.70	62.41	60.21	56.80	---	61.03
	60 Kg N fed^{-1}	65.02	62.51	60.66	57.32	---	61.38
	20 Kg N fed^{-1}+bio-fertilizer	64.18	62.00	59.70	56.25	---	60.53
	40 Kg N fed^{-1}+bio-fertilizer	65.33	62.74	61.27	57.93	---	61.82
L.S.D 0.05		0.151	0.064	0.048	0.210	0.014	0.102
DE %							
Egyptian clover 100%	**20 Kg N fed^{-1} (Control)**	4.32	4.22	4.09	3.98	3.87	4.10
	40 Kg N fed^{-1}	4.27	4.12	4.00	3.89	3.77	4.01
	60 Kg N fed^{-1}	4.17	4.09	3.95	3.84	3.75	3.96
	20 Kg N fed^{-1}+bio-fertilizer	4.20	4.07	3.96	3.87	3.77	3.97
	40 Kg N fed^{-1}+bio-fertilizer	4.28	4.13	4.00	3.75	3.68	3.97
Ryegrass 100%	**20 Kg N fed^{-1} (Control)**	3.99	3.93	3.78	3.60	---	3.82
	40 Kg N fed^{-1}	4.10	3.98	3.86	3.67	---	3.90
	60 Kg N fed^{-1}	4.12	3.98	3.88	3.70	---	3.92
	20 Kg N fed^{-1}+bio-fertilizer	4.08	3.96	3.83	3.64	---	3.88
	40 Kg N fed^{-1}+bio-fertilizer	4.14	4.00	3.92	3.73	---	3.95
L.S.D 0.05		0.065	0.052	0.080	0.034	0.090	0.063

Conclusion

In general, from the obtained results, it could be concluded that mixing 75% Egyptian clover with 25% Ryegrass and fertilized with 40 Kg N fed^{-1} supported with bio-fertilizer could be recommended for better forage quantity and quality. Thus, it is beneficial in future to increase areas of such forage mixing ratio under the combined fertilizers for many reasons which include improving soil properties as a result of Egyptian clover cultivation, and obtaining better forage in quantity and quality for animal feeding.

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