Response of Egyptian clover to Nano Clay Flakes in Newly Reclaimed Sandy Soils under Sprinkler Irrigation System

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ABSTRACT

Sandy soils are well known as being droughty, erodible, infertile and non economical. The main objective is to vegetate the desert with a water consumption as low as possible. Two field experiments were conducted during 2012/2013 and 2013/2014 seasons. The experiments were carried out in Developing Agricultural System Project, Sinai Station, Sinai Governorate to study the effect of nanoclay flakes treatments on revegetation of sandy soil under sprinkler irrigation system with Egyptian clover var Gemmiza 1. The experiments were designed as a split-plot design with four replications, the main plots included the source of irrigation dates after 3, 5, 7 and 9 days, the subplot had nanoclay doses (0, 8.5, 17 and 25 kg/plot) at soil depth 10 cm. The results indicated that the water requirement for the Egyptian clover ranged from 2800-3000 m^3/fed of water depending on climate and the growing period, Evaportranspiration increases from establishment of flowering stage (5-7mm/day), the irrigation interval of 7 days showed the highest forage yield, the dose of 17Kg/plot nanoclay flakes had significant effect on forage yield. Also, the obtained results revealed that yield components have been increased due to the nanoclay treatment with high significancy compared to the untreated area by increasing the availability of water for crop use by 50%. Treating the sandy soil with clay nano flakes resulted in significant differences in Egyptian clover yield and led to increasing the nitrogen, phosphorus, potassium, contents and decreasing the water consumption rate by 50%.

Keywords: Egyptian clover, Nanoclay, sandy soil, SDS-PAGE, water consumption.

INTRODUCTION

In the near future, the limitation of water resources will be increased; therefore, it is important to study the effect of using irrigation water of poor quality in agriculture. The challenge in the future will be to maintain or even increase water productivity with less water or with water with poor quality. Water scarcity is the world's major problem. Egyptian clover is considered as one of the most important forage crops in the world. The Egyptian clover in Egypt has a special importance because the local production is not sufficient to supply the annual demand of local requirements. The horizontal extension in Egypt for plant production is carried out in the new by reclaimed areas which suffer from structure, nutritional problems, water scarcity and alkalinity.

Since 1954, intensive efforts have been conduted in Egypt for reclamation of sandy soils. The traditional methods of sandy soils reclamation were based on addition of clay and manures to soils. Applying 150m^3 of Nile-suspended matter gave the most favorable effect for practical use in sandy soils whenever the Nile sediment are available. Water scarcity is one of the most important problems facing the strategic forage production and development. Irrigation water is a limiting factor to increase the Egyptian clover extension areas. Water is an essential resource for life and good health, and lack of water to meet daily needs is a reality today. Globally, the problem is getting worse as cities and populations grow, and the needs for water increases in agriculture, industry and households. This fact highlight consequences of water scarcity, its impact on the international and local development. It urges everyone to be a part of efforts to conserve and protect the resources (WHO, 2010). The water requirement for Egyptian clover is about 5611 and 5734 m^3/fed (Al.Kateeb *et al.,* 2006; and El-Bramawy *et al.,* 2006) and decreasing the amount of used water will help in increasing the reclaimed areas. Therefore, special emphasis should be directed towards the desert, which considered to be95% of the total area. It is well known that the water resources in Egypt are limited. Meanwhile, water demand is continually increasing due to population growth–industrial development. Because of population growth, the per capita share of water has dropped dramatically to be 700m^3 per capita, which, by international standards, is considered the water poverty limit: The value may be decreased to 584m^3 per capita by 2025. According to FAO (2009), Egyptian clover is cultivated on 2.5 million Fadden with a production of 75 million tons fresh forage. An important objective of the Egyptian government is consequently to reduce irrigation water in the new land by a applying proper irrigation systems (sprinkler or drip irrigation). The main constrain to implement these systems is the amount of water available.

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MATERIALS AND METHODS

Experimental set-up

This study was carried out at the Experimental Farm, Sinai Project for Developing Horticultural Crops, Sinai Governorate during 2013-2014 seasons. The aim of this study is to evaluate Nanoclay application to the sandy soil and the effect of this treatment on the Egyptian clover var Gemmiza I production and saving water. The soil is characterized according to Richards (1967). Physical and chemical analyses of soil and irrigation water were presented in Tables (1) and (2), respectively. Nanoclay, the smallest normal occurring clay particle, consists of 1000 to 1200 disks (flakes) on top of each other. Each flake is one nanometer thick and 20 to 100 nanometre in diameter. Desert Control Institute (D.C.I) have been able to splilt mechanically these flakes and adding air – bubbles to both sides of the flakes and with these in water they are flowing free and are stable for up to 3 – 4 days when the water is salt free. This Nanoclay solution can be watered into the sand and of towards the sand will adsorbs water and the water holding capacity is increased by 50%.

Table 1. Some physical and chemical analysis of investigated soils

Parameters	Value
pH*	7.50
EC**, dSm^{-1}	1.45
Soluble cation**, ppm	
Ca^{2+}	130
Mg^{2+}	66
Na^{+}	56
K^{+}	29
Soluble anion**, ppm	
CO_3^{2-}	-
HCO_3^{-}	366
Cl^{-}	336
SO_4^{2-}	96
Sand, %	90.93
Silt, %	8.52
Clay, %	0.55
Textural class	Sandy
Saturation percent, %	24
Total $CaCO_3$, %	0.80
Total N, %	0.15
Available P, ppm	6.0
Available K, ppm	7.00
CEC, Cmol kg^{-1}	3.80
Organic matter, %	0.69

* in soil-water suspension 1: 2.5 - ** in soil paste extract.

Egyptian clover seeds cv. Gemmiza 1 was cultivated / planted in this experiment. Cultivation date was 20th Octber and seeding rate of 30kg/Fed. (inoculations of seeds with nitrogen – fixing) were drilled in rows using dry method of planting called (Afir). All treatments in the field were fertilized with Arate of nitrogen fertilizer 25kg N and this Egyptian clover plants received N at a rate equivalent to 75 kg ammonium nitrate (33.5%) and potassium at 50kg K_2O/fed. Also calcium superphosphate was added at a rate of 50kg P_20_5 / fed in three equal portions during the growth period of clover. At the cutting, a random sample was chosen from four replicates for recording growth attributes, Also, plants of one m^2 were harvested from four replicates of each treatment for calculating yield components/ fed. The obtained data were subjected to the proper statistical analysis. This work was designed as a split polt design with four replications, the main plots included the source of irrigation dates and subplot had nanoclay doses (purched from nanoEgypt company, Alexandria, Egypt). The two factors and their interactions under study were as following :

A- Irrigation frequencies:

A_1 (after 3 days).

A_2 (after 5 days).

A_3 (after 7 days).

A_4 (after 9days)

B- Nanoclay Levels:

B_1 (Zero nanoclay).

B_2 (8. 5kg / plot).

B_3 (17 kg/ P lot).

B_4 (25 kg / plot).

Growth characters:

At each cut, samples of ten guarded plants each were reandomly taken from each plot to determine plant height (cm), number of branches/plant, leaves stems fresh weight ratio and leaves/ stems dry weight ratio- while fresh and dry forage yields/ fed were estimated for the four cuts from the plants of the whole plot area and the yields per fed and calculated. Dry forage yield (ton/fed) estimated from randomly selected oven dried samples at 70℃ until constant weight (estimated by multiplying fresh forage yield XDM%).

The following parameters were recorded on samples of ten plants each from four replicates before cutting:

1- Plant height (cm): it is measured from soil surface.
2- Number of green leaves / plant.
3- Fresh weight.
4- dry matter.

Table 2. The chemical analysis of Nile water, which used for irrigation

Water Source	Cations, meq/L				Anions, meq/L				EC	
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	So_4^{2-}	dSm^{-1}	SAR
Nile water	0.5	0.58	1.78	0.5	-	2.44	1.29	-	0.40	3.29

Chemical Analysis:

Element extraction was conducted on a known weight of the dried samples(0.5gm). Concentrated sulphuric acid(5ml) was added to the sample and the mixture was heated for two hours and then 0.5ml percloric acid was added and heating till a clear solution was developed. The digested solution was transferred to 100 ml volumetric flask using distilled water.

Nitrogen Percentage (N%) and crude protein determinations

Crude protein content (%) was determined in the dry matter by using the modified micro kjeldahal apparatus according to methods by A.O.A. C. (1975). Then, the obtained values were multiplied by 6.25 as used by Tripath *et al*., (1971). Protein yield per fed was also estimated by multiplying the dry forage yield per fed by crude protein content (%).

Nitrgen content was determined by the modified Microkjeldohl method as described by A.O.A.C. (1975).

Phosphorus determination:

Phosphorus was determined calorimetrically using the molybdophosphoric blue color method system as described by Jackson (1967).

Potassium determination:

Potassium was determined using jenway flame photometer according to Brown and Lilliland (1964)

Determination of irrigation requirement by:-

1-
$$\lambda ET = \frac{\Delta(R_n - G) + \rho_a c_p \frac{(e_s - e_a)}{r_a}}{\Delta + \gamma\left(1 + \frac{r_s}{r_a}\right)}$$

Where R_n is the net radiation, G is the soil heat flux, $(e_s - e_a)$ represents the vapour pressure deficit of the air, ρ_a is the mean air density at constant pressure, c_p is the specific heat of the air, Δ represents the slope of the saturation vapour pressure – temperature relationship, γ is the psychrometric constant, and r_s and r_a are the (bulk) surface and aerodynamic resistance. Monteith, (1965).

$$ET_o = \frac{0.408\,\Delta(R_n - G) + \gamma \frac{900}{T + 273} u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34\,u_2)}$$

Where ET_o is the reference evapotranspiration (mm day^{-1}), R_n is net radiation at the crop surface [MJ m^{-2} dan^{-1}], G is soil heat flux density [MJ m^{-2} day^{-1}], T is air temperature at 2 m height [°C], u_2 is wind speed at 2 m height [m s^{-1}], e_s is saturation vapour pressure [kPa], e_a is actual vapour pressure [kPa], e_s-e_a is saturation vapour pressure deficit [kPa], Δ is the slope of the vapour pressure curve [kPa $°C^{-1}$], and γ is the psychrometric constant [kPa $°C^{-1}$]. Allen *et al*., (1994).

SDS-PAGE :

Protein electrophoretic studies:

Sodium dodecyl sulphate Polyacrylamide gel electrophoresis (SDS-PAGE) was performed to distinguish and fragment total soluble protein for fourteen samples according to the methods of Lammli (1970).

Sample Preparation:

Seeds of protein fractions were extracted by grinding 1 g of tissues in 1 ml water- soluble extraction buffer (a ratio of 1:2 w/v) then, vortexes for 2 minutes. Samples were shacked for two hours, then centrifuged for 15 min at 14000 rpm at 4 °C. Supernatant containing water soluble protein was transferred to a new tube.

Stocks solutions used for electrophoresis:

Acrylamide bis - acrylamide solution (30:0.8) was prepared by dissolving 30 g of acrylamide and 0.8 bis-acrylamide in a total volume of 100 ml distilled water. The solution was filtered stocks solutions used for electrophoresis were prepared according to the method of (Bradford, 1976).

Preparation of slab gel:

15% slab gel was prepared according to the method of lammli (1970).

Loading of samples and electrophoresis:

For each sample, after gel polymerization, 30 µg protein was loaded and electrophoresis was performed at 75 volt through stacking gel followed by 125 v during approximately 2 h.

Protein staining:

Gel were stained 0.1 % comassi blue R- 250 for 2 h. Then distained with a solution (1:3:6) of glacial acetic acid; methanol; and water, respectively.

SDS PAGE electrophoresis:

Total protein content was determined in grounded fine powder seeds of each sample by the method described by Bradford (1976) using bovine serum albumin (96%, Sigma Chemical Co, St. Louis, MO, USA), as standard. Then, total soluble proteins was extracted with extraction buffer.

Data and statistical analyses:

Gel documentation system (GelDoc-Ite Imaging System, UVP, England), was applied for data scoring and documentation. Total lab analysis software (Total Lab TL120, v2008) was employed for constructing binary matrix for SDS PAGE data according to presence or absence of a band of each sample which remarked as 1 or 0.

The analysis of variance was done according to the method described by Snedecor and Cochran (1967). The treatment means were compared using the least significant Difference (L.S.D).

RESULTS AND DISCUSSION

Nanoflakes characterization:

The results indicated that the amendment with polymer led to increasing the field capacity. Incorporation of polymer revealed better performance of available water content in sandy soils which is due to low cation exchange capacity of coarse -texture sand soils. Also, use of polymer had increased specific surface area of the sand soils and eventually led to the improvement of it's absorption ability and available water.

Furthermore, it is reported that the water use efficiency increased and water requirement decreased with the addition of such conditioners . The polymer ensured improvement of soil moisture retention for a longer time.

The moisture content (F.C, W.P and AW) was done according to the method described by Kulte, A (1986).

In Fig. (1) Electron Microscopic inspection within enhancement of 1700 to 2000 times, nanoclay flakes look as complete surface of all the individual sand particles. In addition, the binding between the particles are enhanced. So the sand physical properties are completely changed regarding to the attraction of water. Our patent invention, clay nano flakes is homogenising the clay into individual flakes in water. However, the clay conditioner gave no binding of the sand – particles versus sand movement and proved to be too expensive to apply in the field due to the high costs of the mechanical work required (corge amount of clay, intensive plouyhing plus watering) (Olsen, 2010).

Table 3. Moisture content (F.C, W.P and AW) of the Used Polymer, %

AW	W.P	FC	SP
30	35	65	150

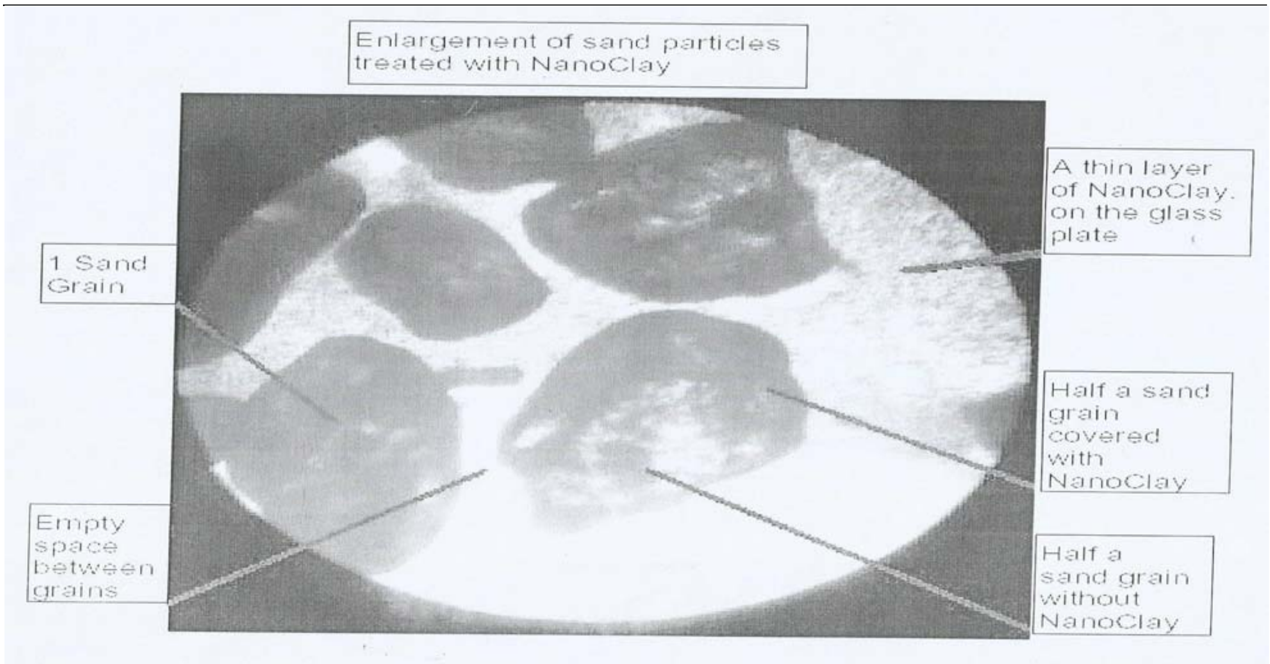


Fig. 1. Delivery the Nanocaly technology installed in the sandy soils (Sinai Station)

Table 4. Effect of irrigation intervals and Nanoclay Doses on plant height (cm) of berseem at the different cuts in the two seasons

Seasons	First cut		Second cut		Third cut		Fourth cut	
Treatments	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
Irrigation intervals, days:								
3	34.14	37.20	47.55	56.20	46.90	55.35	33.15	41.10
5	36.16	40.10	59.75	61.40	58.12	59.85	42.30	41.90
7	42.17	49.98	62.85	65.34	63.50	66.46	38.89	48.60
9	39.20	44.21	60.50	62.77	61.00	63.40	36.40	40.50
Nanoclay doses, Kg/fed:								
Zero	30.11	35.50	50.00	55.70	51.00	56.12	33.12	41.16
8.5	37.26	43.62	55.80	57.80	56.10	57.30	34.15	40.20
17	40.12	50.41	60.52	64.20	61.85	65.45	44.55	48.50
25	38.13	45.18	57.86	59.16	58.43	60.20	38.12	43.14
LSD (5%)	2.26	2.96	3.91	3.81	6.22	6.74	4.28	4.82

Plant heigh:

Data in Table (4) show the effect of nanoclay treatment on plant height (cm). Statistical analysis showed highly significant differences between treated and untreated nanoclay plots with average 30.11 and 65.45cm, respectively. This results were in accordance with the results Abdel Gawad et al., (1999), Khot et al., (2012) and Ma et al., (2010a).

Also statistical analysis showed a high significant difference between 3 and the 9 days treatments with an average 34.14, 66.46, respectively. Similar results were recorded by Lucero *et al.*, (1999) Al-Khateeb (2004), Lin and Xing, (2007).

Number of branches per plant:

Table (5) showed the effect of nanoclay treatment on number of branches / plant. Statistical analysis showed highly significant differences between treated and untreated nanocly plots with an average 1.80, 5.81, respectively. Similar results were observed by Nair *etal* (2010). Concerning irrigation intervals, statistical analysis showed highly significant differences between 3 and 9 days treatments with an average 2.00, 5.81cm. Similar results were detected by Al-Khateeb *et al.*, (2006). Lin and Xing, (2007) and Khot *et al.*, (2012).

Fresh and dry forage yields (ton/fed):

Data in Table (6) showed the effect of irrigation intervals on fresh forage yield (ton / fed). There was a highly significant difference between 3 and 9 days intervals with an average 3-16, 7.45 ton/fed. respectively.

Such observations were recorded by Lin and Xing, (2007).

Concerning nanoclay treatment on fresh forage yield (ton/fed), statistical analysis showed highly significant differences between ton/fed. treated and untreated nanoclay plots with an average 3.12, 7.50 ton/fed. respectively. Similar results were observed by Marchiol, (2012)..

Table 5. Effect of Nanoclay Doses and irrigation intervals on number of branches/plant of berseem at the different cuts in the two seasons

	First cut		Second cut		Third cut		Fourth cut	
Treatments	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
Irrigation intervals, days:								
3	2.00	2.45	2.95	3.04	3.20	3.45	2.66	2.80
5	1.97	2.24	2.77	2.90	3.70	3.84	2.65	2.90
7	2.85	3.40	3.85	3.80	5.80	5.81	4.00	4.50
9	1.95	2.08	2.70	2.80	4.20	4.25	2.60	3.67
Nanoclay doses, Kg/fed:								
Zero	1.80	2.00	2.61	2.70	3.24	3.28	2.63	2.81
8.5	2.30	2.50	2.95	3.00	4.36	4.32	2.60	2.90
17	2.75	3.00	3.30	3.40	5.81	5.77	3.66	3.78
25	1.91	2.09	2.72	2.79	4.18	4.20	2.55	3.60
LSD (5%)	2.01	2.11	2.04	2.32	2.91	2.45	1.62	1.94

Table (7) showed the effect of irrigation intervals on dry forage yield (ton/fed). Also statistical analysis showed highly significant difference between 3 and 9 days intervals with an average 0.463, 1.615 ton/fed. respectively.

The nanoclay treatment significantly increased from dry forage yield Table(7) data showed highly significant differences between the treated and the untreated treatments with an average 0.468 to 1.608 ton/fed. respectively. Haverkamp *et al*., (2007). Similar results were detected by Garadea – Torresdey *et al*., (2002).

Crude protein content (%)& protein yield (kg/fed) :

The effect of nanoclay treatment on crude protein content (%) is shown on Table (8). Results showed highly significant differences between treated and untreated nanoclay treatments with an average 14.20, 24.33, respectively.

Regardless the effect of studied factors, the first cut gave the highest crude protein content (%), then the values decreased gradually, while the lowest value was achieved in the fourth cut. These result were confirmed by the results second season.

The effect of irrigation intervals on protein yield (kg/fed) is shown on Table (9). There were highly significant differences between 3 and 9 days treatments with an average 86.58, 312.02, respectively. Similar results were reported by Leilah *et al*., (2006) and Naire et al., (2010) Also, it is clearly evident from Table (9) that nanoclay treatment had effects on protein yield (kg/fed) where highly significant differences between treated and untreated nanoclay treatments with an average 87.09 and 324.33. Similar results were reported by Nair *et a*l., (2010) and Haverkamp *et al*., (2007).

In general, Irrigation treatments effects on total fresh, dry forage and protein yields in all cuts and both seasons were significantly affected by irrigation intervals. Irrigation every 7 days gave the highest total fresh, dry forage and protein yields in all cuts. Similar results were reported by lucero *et al*., (1999), lin and xing (2007)

Also, Table (10) indicted that the effect of nanoclay treatment on total fresh, dry forage and protein yields. Were significant differences between the nanoclay treatments were highly significant with an average 14.08, 23.53, 2.96, 4.74 and 497.89, 89.80, respectively. Similar results were reported by El.Bramawy *et al* (2006), Marchiol, (2012) and Gardea-Torresdey *et al*,(2002).

Chemical composition:

The effect of Nanoclay treatment on the chemical composition of Egyptian clover variety in 2013 and 2014 seasons are presented in table (11).

Nitrogen percentage:

Results of the effect of nanoclay treatment on nitrogen % during 2013 and 2014 seasons are shown in table (11) nanoclay treatment increased N% in both seasons. Similar results were obtained by Jinghua (2004) and (zhang et al., 2006).

Phosphorus percentage:

The results in table (11) showed that the nanoclay treatment led to increased P% in both seasons similar results were obtained by jinghua (2004) and (Zhang et al., 2006).

Table 6. Effect of irrigation intervals and Nanoclay Doses on fresh forage yield (ton/fed) of berseem at the different cuts in the two seasons

Seasons	First cut		Second cut		Third cut		Fourth cut	
Treatments	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
Irrigation intervals, days:								
3	3.16	3.25	5.47	5.73	4.37	5.62	3.40	3.66
5	3.47	3.42	5.20	5.82	4.50	4.93	3.71	3.84
7	3.77	4.32	6.61	7.45	6.55	7.33	4.20	3.94
9	3.20	3.88	5.85	6.20	4.75	5.22	3.90	3.95
Nanoclay doses, Kg/fed:								
Zero	3.12	3.34	4.31	4.88	3.51	3.44	3.14	3.17
8.5	3.14	3.72	5.63	5.94	5.11	5.08	3.22	3.95
17	3.74	4.25	6.81	7.50	6.60	7.30	4.18	4.48
25	3.33	3.82	5.79	6.35	5.06	5.75	3.26	4.18
LSD (5%)	1.22	1.01	0.16	0.28	0.92	1.02	0.68	0.72

Table 7. Effect of irrigation intervals and Nanoclay Doses on dry forage yield (ton/fed) of berseem at the different cuts in the two seasons

Seasons	First cut		Second cut		Third cut		Fourth cut	
Treatments	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
Irrigation intervals, days:								
3	0.463	0.557	0.990	1.037	1.109	1.228	0.868	0.889
5	0.533	0.587	0.941	1.053	1.194	1.077	0.902	0.933
7	0.572	0.665	1.158	1.406	1.373	1.615	1.00	0.969
9	0.501	0.594	1.059	1.130	1.260	1.140	0.960	0.962
Nanoclay doses, Kg/fed:								
Zero	0.468	0.573	0.780	0.883	0.931	0.912	0.782	0.789
8.5	0.492	0.638	1.019	1.075	1.171	1.164	0.802	0.860
17	0.570	0.648	1.286	1.415	1.384	1.608	1.000	1.072
25	0.498	0.585	1.048	1.199	1.159	1.256	0.856	0.994
LSD (5%)	0.13	0.14	0.26	0.27	0.28	0.29	0.21	0.32

Table 8. Effect of irrigation intervals and Nanoclay Doses on crude protein content (%) of berseem at the different cuts in the two seasons

Seasons	First cut		Second cut		Third cut		Fourth cut	
Treatments	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014
Irrigation intervals, days:								
3	18.70	19.40	18.50	19.20	16.75	17.29	14.40	14.55
5	18.62	19.35	18.31	19.12	16.80	17.30	14.42	14.62
7	21.69	23.84	19.70	21.57	18.37	19.32	16.08	17.16
9	19.60	20.14	18.53	19.82	16.91	17.67	14.54	15.70
Nanoclay doses, Kg/fed:								
Zero	18.61	19.35	18.20	18.51	16.73	17.33	14.46	15.66
8.5	19.50	20.56	18.25	19.13	16.83	17.26	14.20	15.60
17	21.18	24.33	20.06	22.63	18.80	20.17	17.13	17.50
25	20.04	22.65	19.14	20.36	17.21	18.30	14.45	16.17
LSD (5%)	3.68	3.14	9.05	8.76	4.26	4.94	6.41	5.94

Table 9. Effect of irrigation intervals and Nanoclay Doses on protein yield (kg/fed) of berseem at the different cuts in the two seasons

Seasons	First cut		Second cut		Third cut		Fourth cut	
Treatments	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/ 2014	2012/ 2013	2013/20 14
Irrigation intervals, days:								
3	86.58	108.06	183.15	199.68	194.13	212.32	124.99	129.35
5	99.24	113.58	172.30	201.33	200.59	186.32	130.07	136.40
7	124.07	158.54	228.13	303.27	252.22	312.02	160.80	166.28
9	98.19	119.63	196.23	223.97	213.07	201.44	139.58	151.03
Nanoclay doses, Kg/fed:								
Zero	87.09	110.87	141.96	163.44	155.76	158.05	113.08	123.56
8.5	95.94	131.17	185.97	205.65	197.08	200.91	113.88	134.16
17	120.73	157.66	257.97	320.21	260.19	324.33	171.30	187.60
25	99.79	132.50	200.59	244.12	199.46	229.84	123.69	160.73
LSD (5%)	4.71	5.64	9.41	8.42	12.63	16.45	14.17	14.93

Table 10. Effect of irrigation intervals and Nanoclay Doses on total fresh forage yield (ton/fed), total dry forage yield (ton/fed) and total protein yield (kg/fed) of berseem at the different cuts in the two seasons

Seasons	Total fresh forage yield (ton/fed)			Total dry forage yield (ton/fed)			Total protein yield (kg/fed)		
Treatments	**2012/ 2013**	**2013/ 2014**	**Comb.**	**2012/ 2013**	**2013/ 2014**	**Comb.**	**2012/ 2013**	**2013/ 2014**	**Comb.**
Irrigation intervals, days:									
3	16.40	18.26	17.33	3.48	3.71	3.59	588.85	649.41	619.13
5	16.88	18.01	17.44	3.57	3.65	3.61	602.22	637.63	619.92
7	21.13	23.04	22.08	4.10	4.65	4.37	765.22	940.11	852.66
9	17.70	19.25	18.47	3.78	3.83	3.80	647.07	696.07	671.57
Nanoclay doses, Kg/fed :									
Zero	14.08	14.83	14.45	2.96	3.16	3.06	497.89	555.92	526.90
8.5	17.10	18.19	17.64	3.48	3.74	3.61	592.87	671.89	632.38
17	21.33	23.53	22.43	4.24	4.74	4.49	810.19	989.80	899.99
25	17.44	20.00	18.72	3.56	4.03	3.79	623.53	767.19	695.36
LSD (5%)	6.41	5.41	6.72	0.81	0.94	0.77	19.63	20.41	20.54

Table 11. Effect of Nanoclay treatment on Elements status (Nitrogen, Phosphorus and potassium) of Egyptian clover variety

Character	Mean	
	Treated	**Untreated**
Nitrogen (%)	1.83	1.42
Phosphorus (%)	0.45	0.38
Potassium (%)	0.56	0.40
L.S.D (5%)	0.94	1.11

Potasium percentage:

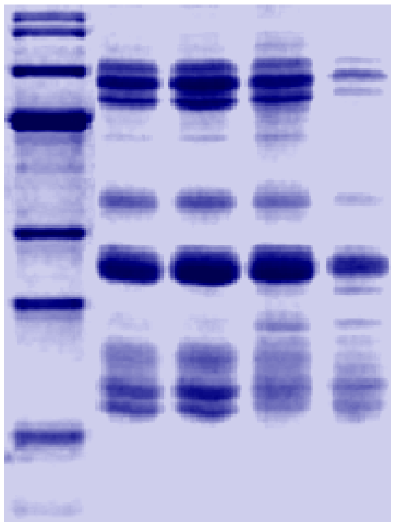
The effect of nanoclay treatment on K% in 2013 and 2014 seasons are presented in Table (11). Application of nanoclay treatment increased K% in both seasons. Similar results were obtained by jinghua (2004) and (Zhang et al., 2006).

Biosafety of nanoclay flakes:

Effect of the lack of Nanoclay could be detected through two levels. The first through presence and absence of protein bands (Figs 2,3&4). Noticeable similarity of protein fractionations for treated (except third treatment) and non treated samples. All previous samples showed seventeen protein bands with 185, 184, 180, 166, 134, 118, 117,109, 106, 103, 96, 90, 87, 82, 70, 65 and 57 KDa. Natar clear of Nanoclay addition was clearly showed through distinguish disappearing of protein fraction with 166 and 134 KDa as a result of extensive addition with 25 Kg of Nanoclay.

Second affect could be notice via protein activities at similar loci. Highly protein activity was recorded for fourteenth protein band with 82 KDa which represent 14 % of each protein fraction contents. Clear effects of Nanoclay addition was noon via the rest of protein fraction activities under different Nanoclay treatment quantities.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE) Pofiles of four studied Egyptian clover under different nanoclay treatments.



SDS – Egyptian Clover.

Fig. 2. Protein electrophoretic patterns for*Egyptian clover* samples under Nanoclayaddition levels

Where:

1- Untreated with Nanoclay
2- 8.5 Kg of Nanoclay addition
3- 17 Kg of Nanoclay addition
4- 25 Kg of Nanoclay addition

Table 12. Protein fractionation pattern parameters for *Egyptian Clover* samples under Nanoclay addition levels

C Control			A			B			C		
Band number	**Molecular weigh**	**Band %**	**Band number**	**Molecular weight**	**Band %**	**Band number**	**Molecular weight**	**Band %**	**Band number**	**Molecular weight**	**Band %**
1	185	8	1	185	10	1	185	8	1	185	4
2	184	5	2	184	4	2	184	6	2	184	0.9
3	180	0.7	3	180	4	3	180	6	3	180	4
4	166	5	4	166	1	4	166	2	4	-	-
5	134	7	5	134	6	5	134	4	5	-	-
6	118	5	6	118	5	6	118	6	6	118	10
7	117	4	7	117	5	7	117	4	7	117	3
8	109	8	8	109	5	8	109	5	8	109	0.7
9	106	1.3	9	106	3	9	106	2	9	106	6
10	103	10	10	103	9	10	103	8	10	103	3
11	96	11	11	96	11	11	96	4	11	96	5
12	90	4	12	90	4	12	90	9	12	90	5
13	87	4	13	87	5	13	87	7	13	87	6
14	82	14	14	82	12	14	82	11	14	82	1.4
15	70	2	15	70	3	15	70	3	15	70	4
16	65	6	16	65	7	16	65	6	16	65	3
17	57	5	17	57	6	17	57	5	17	57	44

A = 8.5 Kg of nanoclay /plot
B = 17 Kg of nanoclay /plot.
C= 25 Kg of nanoclay /plot.
C = Control Zero Kg of nanoclay/plot.
Protein fractionation Pattern Parameters for Egyptain clover sample, under nanoclay addition levels are presented in Table (12).

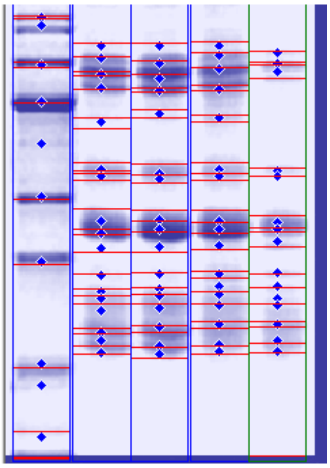


Fig. 3. Computerized protein electrophoretic pattern bands of *Egyptian Clover*samples under Nanoclay addition levels

Where:

1- Untreated with Nanoclay
2- 8.5 Kg of Nanoclay addition
3- 17 Kg of Nanoclay addition
4- 25 Kg of Nanoclay addition

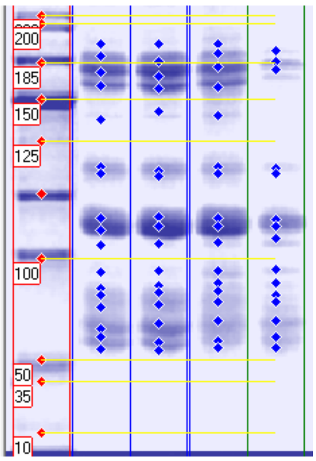


Fig. 4. Computerized molecular weight for *Egyptian Clover*samples under Nanoclay addition levels

Where:

1- Untreated with Nanoclay
2- 8.5 Kg of Nanoclay addition
3- 17 Kg of Nanoclay addition
4- 25 Kg of Nanoclay addition

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الملخص العربي

استجابة البرسيم المصري لرقائق الطين النانومترية في الأراضي الرملية حديثة الاستصلاح تحت نظام الري بالرش

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أجريت تجربتان حقليتان بمزرعة مشروع تطوير النظم الزراعية – محطة البحوث الزراعية بسيناء خلال الموسمين الزراعيين ٢٠١٢ – ٢٠١٣ و٢٠١٣ -٢٠١٤ بهدف دراسة تأثير جرعات رقائق الـ Nanoclay (صفر، ٨،٥، ١٧، ٢٥كجم Nanoclay/فدان) وأيضاً دراسة تأثير فترات الري (٣، ٥، ٧، ٩ يوم) على صفات النمو ومحصول العلف الأخضر والجاف/ فدان والنسبة المئوية للبروتين الخام ومحصول البروتين/فدان على محصول البرسيم المصري صنف جميزة ١ في الأراضي الرملية المستصلحة حديثاً بسيناء تحت نظام الري بالرش وكان التصميم المستخدم هو القطع المنشقة مرة واحدة Split Plot design وأشارت النتائج إلى: أن الري كل سبعة أيام أدى إلى الحصول على أكبر محصول علف طازج وجاف/فدان وأيضاً النسبة المئوية للبروتين الخام ومحصول البروتين/ فدان وأيضاً صفة طول النبات وعدد الفروع/ نبات. استخدام ١٧كجم Nanoclay/فدان أدت إلى زيادة الحصول على أكبر محصول علف طازج وجاف/فدان وكذلك النسبة المئوية للبروتين الخام ومحصول البروتين/فدان وصفات النمو. أظهرت الدراسة أن استخدام مادة الـ Nanoclay تحت ظروف الأراضي الرملية حديثة الاستصلاح ونظام الري بالرش أن الاحتياجات المائية اللازمة لمحصول البرسيم المصري تتراوح من ٢٨٠٠ – ٣٠٠٠ م٣/فدان مع الوضع في الاعتبار الظروف المناخية وفترات النمو المختلفة. أظهرت الدراسة أيضاً الري كل سبعة أيام مع استخدام مادة الـ Nanoclay أعطى أعلى محصول علف طازج وجاف/فدان. استخدام جرعة ١٧كجم Nanoclay/Plot كانت ذات تأثير معنوي على إنتاجية العلف الأخضر والجاف لمحصول البرسيم المصري. أظهرت الدراسة أن تحليل البروتين باستخدام SDS – PAGE لعينات البرسيم المصري المعامل بالجرعات المختلفة من مادة الـ Nanoclay ربما لا يكون لها أي تأثير بيئي ضار وأيضاً ليس لها أي أثر متبقي سواء على محصول البرسيم المصري أوعلى التربة "مادة آمنة بيئياً". مادة الـ Nanoclay تعمل على زيادة قدرة امتصاص الأرض للمياه وذلك بتقليل البخر. زيادة السعة التبادلية الكاتونية. زيادة الشحنات السالبة في التربة مما يزيد من قدرتها على الاحتفاظ بالمياه لفترة أطول مع زيادة حجم جزئيات التربة وقدرة الترابط بينها.

الكلمات الدالة: البرسيم المصري– Nanoclay – التربة الرملية– تحليل البروتين SDS – PAGE - الاحتياج المائي.