J.Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 9(9): 393 - 401, 2018

Effect of Mineral, Nano and Bio Nitrogen Fertilization on Nitrogen Content and Productivity of *Salvia officinalis* L. Plant

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ABSTRACT

Nanotechnology is a novel discovery being explored in almost all the fields and is benefitted too; it may provide keener solutions for the current problems in the field of agriculture. Nanotechnology and bio fertilization are represented the most important tools in modern agriculture and anticipated to become a driving economic force in the near future. A field experiment was carried out at Baloza Research Station of Desert Research Center, North Sinai (located at 31° 3′ 0″ N, 32° 36′ 0″ E), to study the effects of application for nitrogen fertilizer (urea), nano urea and biofertilization (*Azotobacter chroococcum*), on the chemical composition and productivity of sage plant (*Salvia officnalis* L.). Two mineral fertilizers, nano urea, urea and biofertilizer (*A. chroococcum*) were applied to the soil. Split split plot design with three replications per each treatment was used, during 2017. Results showed that yield components of sage plants increased with increasing of nano urea and urea application rates during both cuts. The mineral treatments with bio N fertilizer gave the highest yield values. The most effective treatment (Nano 500ppm with bio N fertilizer) gave the highest significant values of yield components amounted to 64.4, 2.77, 11249 and 4395 for plant height (cm), oil (%), herb fresh and dry weight (kg/fed), respectively in first cut. In second cut yield components were higher than first cut. Nutrients contents and then uptake showed almost trend. The N-efficiency parameters; the highest values of nitrogen use efficiency (NUE) and nitrogen uptake efficiency (UPE) were obtained by the application of nano urea. The values of nitrogen utilization efficiency (UTE) and NUE decreased with increasing fertilizer application while that of UPE increased with increasing nitrogen. The superior treatment Nano 500 with bio N fertilizer highly increased the available nutrients in the studied soil. The same effect was observed on the values of dehydrogenase activity and *Azotobacter* densities in rhizosphere of *Salvia officnalis*.

Keywords: Nano urea, Urea, *A. chroococcum*, Salvia officnalis plant production, nutrient content, oil %

INTRODUCTION

Salvia officinalis L. (sage) is one among the economically necessary species in lamiaceae family. Sage could be a perennial woody sub-shrub and blue to purple flowers. It was used for several functions in ancient time. Leaves of Salvia officinalis L. are consumed as foods, drugs and perfums and that they have antioxidative properties. Additionally, other significant benefits are antibacterial, fungistatic, virustatic, astringent, symptom and cytotoxic activities. (Cigdem and Emine 2017).

Nitrogen is considered as one of the essential macronutrients needed by the plants for growth, development and yield Singh *et.al.,(*2003). Element (N) fertilizer use has contend a big role in increasing crop yield. (Modhej *et. al.,* 2008 and Aminifard *et. al.,* 2010).

Regarded to impact of mineral and biofertilizers on yield components of sage plant. Abdel Kader *et. al.,* (2014) detailed that the soil application of bio-fertilizer (VAM organisms and phosphorine) and foliar garlic extract improved the profitability vegetative growth, essential oil % and uptake of N, P and K content of sage (salvia officinalis L.) plant. While, Nadjafi *et. al.,(*2014) stated that the results of two years study demonstrated that biofertilizers (N-fixer bacteria, phosphate solubilizing bacteria) had no significant impact on growth, yield, essential oil and chemical components in thyme (Thymus vulgaris L.) and Sage (Salvia officinalis L.) plants. Ghoushchi *et. al.,* (2015) stated that the integration between chemical and bio fertilizer was obtaining higher yield components of sage plant. It may be possible replacement of high doses of chemical fertilizers to bio and thus reduce the need for chemical fertilizers. Abaas (2014) reported that the highest values of all growth parameters and volatile oil percentage of sage plant leaf were obtained at (230 kg N ha^{-1} with 80 kg P ha^{-1}).

Concerning the effect of nano fertilizer on available nutrients and yield parameters of sage plants, Subbaiya *et. al.,*(2012) reported that the urea modified hydroxyapatite particles (nano) have been employed in agriculture, because of their higher NUE and slow release of the nitrogen to the soil. They concluded that Hydroxyapatite molecule shows the good utilization of nitrogen source. So it has the better yield than other conventional fertilizer; Urea modified HA particle treated seed of green gram show the good germination of 100% towards the yield whereas urea alone gives only 70% of seed germination. Nitrogen release was estimated by using nitrate estimation. Nitrate estimation of HA particle was calculated as 100μg/ml whereas urea treated was calculated as 50μg/ml. Manjili *et. al.,(*2014) indicated that the foliar application of nano chelate molybdenum fertilizer at four levels (3, 2, 1, 0 grams/ liter) with the soil application of nitrogen fertilizer in three levels (60, 30, 0 kg/ha), the highest yield of peanut is obtained 2320 and 3715 kg per hectare in manure treatment of 3 g of molybdenum per liter with 60 kgN/ha. Also, the highest yield of pods and seed is obtained 2320 and 3715 kg per hectare in manure treatment of 3 g of molybdenum per liter. Abyaneh and Varkeshi (2014) reported that the soil nitrate during the growing season of potato in Nano- Nitrogen Chelate (NNC), Sulphur Coated Nano- Nitrogen Chelate (SNNC), and Sulphur Coated Urea (SCU) fertilizers were 10.36%, 29.92% and 23.95 % more than Urea (U) fertilizer, respectively. Ekinci *et. al.,* (2014) stated that the foliar application of nano fertilizers (Nanonat and Fer-banat) significantly improved the yield compared to control. The highest yield (149.17 t ha^{-1}) occurred in Ferbanat 4.0 L ha^{-1} application. The foliar applications of liquid fertilizer could improve the plant growth and yield of cucumbers. Mahmoodi *et. al.,(*2014) reported that the urea fertilizer could significantly improve growth traits in terms of dry and fresh weights and also plant height. Nano urea interestingly increased essential oil production. Davarpanah *et. al.,* (2017) indicated that the two foliar applications of nN (0.25 and 0.50 g N/L, equivalent to ’1.3 and 2.7 g N/tree or 0.9 and 1.8 kg N/ha; nN1 and nN2, respectively) and urea (4.6 and 9.2 g N/L, equivalent to ’24.4 and 48.8 g N/tree or 16.3 and 32.5 kg N/ha; U1 and U2, respectively) were applied at full bloom and 1 month later. The highest fruit of Pomegranate yields (17.8 and 21.9 kg/tree) and number of fruits per tree (62.8 and 70.1/tree) were obtained with the treatment nN2 (1.8 kg N/ha).

Regarding the effect of bio fertilizer on yield and nutrients content in sage plant; Azotobacter spp. are known as Gram negative, free-living, most common in soil. *A chroococcum* is a symbiotic nitrogen fixer, heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year (Kizilkaya, 2009), consume atmospheric nitrogen gas for their cell protein synthesis, after death, Azotobacter are mineralized in soil and release available nitrogen to crop plants. Also, Azotobacter has beneficial role in: biosynthesis of biologically active substances (Chen, 2006). Azotobacter makes availability of certain nutrients like carbon, nitrogen, phosphorus and sulphur through accelerating the mineralization of organic residues in soil. Azotobacter is an important alternative of chemical fertilizer because it provides nitrogen in the form of ammonia, nitrate and amino acids without situation of over dosage, which might be one of the possible alternatives of inorganic nitrogen source (eg. Urea). It also helps in uptake of macro and certain micro nutrients which facilitates better utilization of plant root exudates itself Lévai *et. al.,*(2008). Azotobacter are regarded as Plant Growth Promoting Rhizobacteria (PGPR) which synthesize growth regulators that enhances plant growth and development and secreting antibiotic. It also helps in nutrient uptake and produces some biochemical substances such as protein, amino acids etc. Azotobacter improves seed germination and has beneficiary response on Crop Growth Rate (CGR). It helps to increase nutrient availability and to restore soil fertility for better crop response. It is an important component of integrated nutrient management system due to its significant role in soil sustainability (Jnawali *et. al.,* 2015).

The objective of this work is to study the effect of different mineral nitrogen sources, Nano urea and bio fertilizer on yield components and nutrients content of sage plant (*Salvia officnalis)*, and the impact of each on soil fertility status**.**

MATERIALS AND METHODS

A field experiment was carried out at Baloza Research Station of the Desert Research Center, located at 31° 3′ 0″ N, 32° 36′ 0″ E. Sage plants *(Salvia officnalis L.)* were cultivated during 2017 season to study the effects of different nitrogen sources as soil and foliar applications of nitrogen fertilizer (urea), nanourea (Nurea) and bio fertilization (*Azotobacter chroococcum*) on chemical composition and productivity of Sage plant *(Salvia officnalis L.)*. Nano urea was prepared by reducing its size using some of the reducing agents like Tri Sodium Citrate (TSC) according to Corradini *et .al.,*(2010). The experimental design was split split plot with three replications including 10 treatments. All treatments were applied as soil and foliar applications. Two mineral sources of nitrogen fertilizers were urea (40 and 80 kg N/fed) and Nano urea (250 and 500 ppm) applied as soil application in three doses with the different stages of sage growth. The foliar applications were 500 ppm for Nano N and 0.75 kg urea/600L (575ppm)/fed, which applied for two soil application rates of the same class. Bio N fertilizer was applied *as (Azotobacter chroococcum)*in two rates, The foliar application was done a week after soil application.

Yield parameters were recorded for two cuts during 2017. The experimental field irrigated by drip irrigation system. Sage seedlings were cultivated in April in rows spacing 50 cm apart and 15 cm between seed hills (56000 plant/fed). All treatments were received a basal dose of potassium by the rate of 50 kg K_2O/fed as potassium sulfate was applied in two equal doses, Phosphorus (45 kg P_2O_5 /fed) as super phosphate during soil preparation. The other agricultural practices were done as recommended by Ministry of Agriculture and Land Reclamation. Sage plant was harvested twice in June and September. Soil samples were collected from each plot from two depths (0-30 and 30-60 cm) for physical and chemical analysis. (Tables 1A and 1B).

Table 1A. Some chemical and physical properties of the experimental soil

Chemical properties												
Depth	**pH***	**EC****	**Soluble ions in soil extract(1:1) (meq/L)**							**Available nutrients mgkg^{-}**		
cm		**dS/m**	**Na**	**K**	**Ca**	**Mg**	**Cl**	**HCO_3**	**SO_4**	**N**	**P**	**K**
0-30	8.20	1.37	5.13	0.54	3.65	4.40	3.30	3.85	6.57	25	1.47	32
30-60	8.06	1.21	2.84	3.91	4.89	0.48	3.12	3.54	5.47	17	1.16	24

Physical properties				
Depth	Particle size distribution (%)			Texture class
cm	Sand	Silt	Clay	
0-30	89.12	6.34	4.54	Sandy
30-60	90.73	5.56	3.71	Sandy

*** pH in saturated soil paste, ** Electrical conductivity in soil extract(1:1).**

Table 1B. Chemical analysis of irrigation water

Samples	**pH**	**EC (ppm)**	**S.A.R**	**Soluble cations (meq/l)**				**Soluble anions (meq/l)**			
				Ca^{++}	**Mg^{++}**	**Na^{+}**	**K^{+}**	**CO_3^{-}**	**HCO_3^{-}**	**$SO_4^{=}$**	**Cl^{-}**
	7.45	1456	4.9	2.90	3.20	8.60	0.60	--	5.60	2.10	7.50

Particle size distribution

Both fertilizer (urea and nano urea) were sieved through 40 µm sieves and the particle sizes were measured using by dynamic light scattering (DLS, Malvern Zetasizer Nano-ZS Nano Series). About 0.1 mg of sample was dispersed in 10 ml of water and sonicated for 30 min with 10 seconds on-off cycle. The samples were dispersed in water. The size measurements were performed at 25°C at 90º/173º scattering angle.

Soil analyses were done according to Page *et. al.,* (1982) and Klute (1986). Plant samples were analyzed for N, P and K according to Cottenie *et. al.,* (1982). At maturity, yield parameters of Sage plant such as plant height(cm), herb fresh and dry weights (kg/fed), while essential oil in fresh herb of sage were isolated using a Clevenger-type apparatus according to British Pharmacopoeia.

The collected plant samples were dried in an oven at 70 ºC for up till reaching to a stable weight and ground. The samples were wet digested in H_2SO_4 - H_2O_2 mixture for N, P and K analysis. The N-efficiency parameters of nitrogen use efficiency (NUE), nitrogen uptake efficiency (UPE), and nitrogen utilization efficiency (UTE) were calculated for each treatment as follows (Aghaalikhani *et. al.,* 2012):

NUE (kg/kg) =YE/FN
UPE (kg/kg) =LN/FN
UTE (kg/kg) =YE/LN

Where YE is sage yield, FN is N applied, and Ln is N uptake by plant.

Isolation, Purification of bacterial isolates from study area: Different soil samples were collected from different sites of North Sinai for isolation of *Azotobacter* Purification trials were carried out and purified isolates were maintained for further study. Screening biochemical activity and identification of selected of microbial isolates. Obtained isolates were examined for N_2 fixation according to modified Keldahl method after (Page, *et. al.,*1984). for Azotobacter. The selected isolates were subjected to different biochemical tests for screening their activities toward production of Phytohormones by Rizzolo (1993). Enzymes, (Ladjama *et. al.,* 2007). Then Identification of highly active isolates using 16S rRNA genes sequencing according to Sambrook and Russel (2001) at Sigma company for scientific services.

Preparation of inoculum for field experiment: Fresh liquid culture of *Azotobacter chroococcum* was used for plants inoculation at the rate of 10^8 colony forming unit (cfu/ml). The biofertilizer was added as a soil drench after 45, 90 and 135 days of planting date. The addition of biofertilizer was repeated after the first cut for one time.

Microbiological determinations:-

a- Total microbial counts in rhizosphere soil:-

Rhizosphere soil samples were collected at 1^{st} and 2^{nd} cuts for both seasons and analyzed for total counts of microorganisms according to Nautiyal (1999). For counting and growing Azotobacters, modified Ashby's media (Hill, 2000), Dehydrogenase activity according to method described by (Casida *et al*.,1964). Nitrogenase activity was determined according to (Haahtela *et. al.,* 1981).

The analysis of variance (ANOVA) was used to determine the effects of treatments on the generated data. Least significant difference (LSD) was used to test the differences between means at probability < 0.05 using Mstat (1987) software package according to the methodology described by Snedecor and Cochran (1990).

RESULTS AND DISCUSSION

Characterization of Nano urea

Pertaining to particle size distribution of fertilizer urea and nano urea shown in Fig(1) The average data on Particle size distribution indicated that particle size of urea is 1673.2nm and nano urea is 59.2 nm. These results were due to Particle size of materials can be reduced by collapsing the solvent. Particle size can be tailored by varying the concentration of the solution, amplitude and the number of cycles, reduction of particle size and increasing their bioavailability .The results are in agreement with Subbaiya *et. al.,* (2012) and Ruby Celsia and Mala (2014).

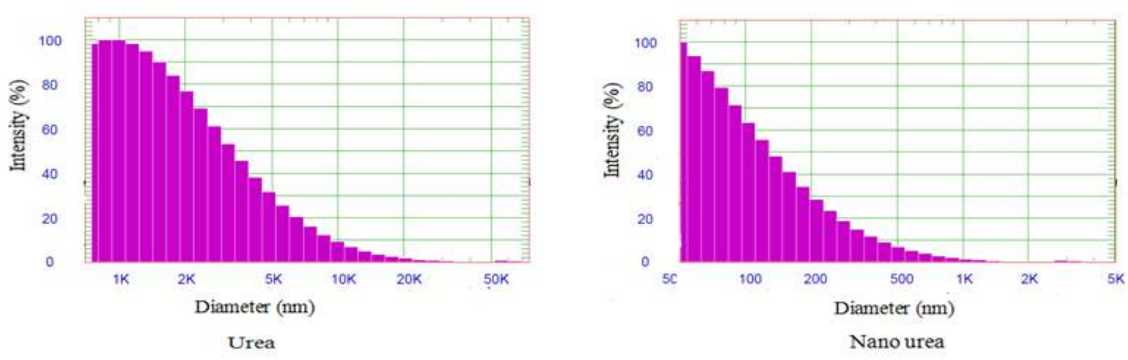

Fig.1. Particle size distribution (in nm) of urea and Nano urea fertilizers

Growth and Yield parameters of Sage plant in two cuts

Data in Table (2) showed that yield components of sage plants increased with increasing of Nano urea, urea application rates during both cuts. The mineral treatments with bio fertilizer gave greater yield values than without applied bio fertilizer. The most effective treatment (Nano N2 with bio N fertilizer) gave highest significant values of yield components amounted to 64.4, 11249 and 4395 for plant height (cm), herb fresh and dry weight (kg/fed) , respectively. The highest oil of 2.77% was obtained by superior treatment (Nano N2 with bio N fertilizer).

Table 2. Effect of application of Nano urea, urea and bio fertilization on yield parameters and oil% in two cuts of sage plant

treatments	N Types	Rates	plant height cm Cutting 1st	plant height cm Cutting 2nd	fresh weight kg/fed Cutting 1st	fresh weight kg/fed Cutting 2nd	Dry weight kg/fed Cutting 1st	Dry weight kg/fed Cutting 2nd	Oil in leaves % Cutting 1st	Oil in leaves % Cutting 2nd
Without bio fertilizer	Control		36.2	34.2	4136	4031	1210	1115	1.89	1.82
	urea	N1	50.7	54.3	8033	10018	2775	2853	2.37	2.44
		N2	55.1	60.7	9864	10687	3036	3337	2.58	2.64
	Nano	N1	54.0	57.2	8224	10452	2847	2993	2.53	2.58
		N2	57.1	63.1	10209	11292	3432	4360	2.69	2.74
With bio fertilizer	Control		39.0	37.9	4449	4423	1306	1213	2.01	1.93
	urea	N1	51.7	55.5	8133	10086	2775	2925	2.43	2.47
		N2	56.5	61.5	10000	10897	3111	3424	2.64	2.69
	Nano	N1	54.4	57.9	8286	10402	2903	3036	2.56	2.65
		N2	59.2	64.4	10770	11249	3478	4395	2.74	2.77
LSD $_{0.05}$ for Bio N			0.346	0.386	50	32	11	16	0.013	0.014
N types			0.500	0.526	73	83	44	109	0.016	0.018
Rates			0.505	0.676	148	183	52	69	0.019	0.021
Bio N x N types			0.351	0.369	51	58	31	77	0.007	0.008
Bio N x Rates			0.714	0.956	142	176	50	66	0.026	0.030
N types x Rates			0.714	0.956	209	259	74	97	0.026	0.030
3 factors			0.686	0.919	201	249	71	93	0.025	0.029

In comparison between the first and second cutting, yield components of sage plant was higher in the second cut than the first cut. This result may be due to the residual effect in the second cut and the probable utilization of N in the form of N urea as N released slowly for the entire growth period leading to higher photosynthetic rate and finally more accumulation of biomass yield.

The previous results agree with those obtained by Junrungreang *et al.,* (2002), Khan *et. al.,* (2008), Manikandan &Subramanian (2016) and Sohrab *et. al.,* (2017).

Nutrients content *of* Sage plant in two cuts.

Data at Table (3) showed that the average values of N content of Sage herbs during the two cuts increased with increasing the application of Nano urea, urea rates and bio fertilizer application for both cuts. P and K showed little increases with rates of Nano urea and urea fertilizers compared with N content increases. The treatment of Nano N2 with bio fertilizer recorded highest significant increases for N, P and K contents when compared with other studied treatments.

Table 3. Effect of application of Nano urea, urea and bio fertilization on the nutrients content (%) in two cuts of Sage plant

Treatments	N Types	Rates	N % 1st cut	N % 2nd cut.	P % 1st cut	P % 2nd cut.	K % 1st cut	K % 2nd cut.
Without bio fertilizer	Control		0.305	0.305	0.086	0.123	1.056	0.978
	urea	N1	0.453	0.461	0.165	0.235	2.254	2.327
		N2	0.647	0.667	0.254	0.363	2.621	2.672
	Nano	N1	0.503	0.516	0.168	0.24	2.483	2.545
		N2	0.807	0.834	0.266	0.38	2.716	2.756
With bio fertilizer	control		0.305	0.305	0.095	0.135	1.166	1.107
	urea	N1	0.461	0.500	0.172	0.245	2.307	2.347
		N2	0.683	0.691	0.263	0.375	2.654	2.732
	Nano	N1	0.511	0.549	0.174	0.249	2.568	2.584
		N2	0.824	0.844	0.304	0.434	2.731	2.781
LSD $_{0.05}$ for Bio N			0.003	0.004	0.001	0.001	0.014	0.013
N types			0.009	0.01	0.003	0.008	0.022	0.02
Rates			0.009	0.01	0.004	0.006	0.041	0.045
Bio N x N types			0.004	0.004	0.001	0.008	0.009	0.008
Bio N x Rates			0.009	0.01	0.004	0.009	0.04	0.043
N types x Rates			0.013	0.014	0.006	0.009	0.058	0.063
3 factors			0.013	0.013	0.005	0.013	0.056	0.061

The application of bio N fertilizer gave higher nutrients content in Sage plant. Urea N2 with bio N fertilizer ranked the second level of nutrients content. Comparison effect between the first and second cutting on nutrients content, the second cutting was the best. These results were due to the Nano particles can be adsorbed by soil colloids, thereby reducing losses and there after released into the soil solution slowly. The above results agreed with those obtained by Junrungreang *et. al.,* (2002); Manikandan and Subramanian (2016) and Parizad *et. al.,*(2017).

Nutrients uptake by Sage plant in two cuts

The previous results from yield and nutrients content were assured in results of nutrients uptake in Table

(4) showed that the superior treatment (Nano N2 with bio N fertilizer) gave higher nutrients uptake by Sage plant during two cuts than other studied treatments. Urea N2 with bio N fertilizer ranked the second level of nutrients uptake after the superior treatment. The application of Bio N fertilizer recorded higher significantly increases of nutrients uptake by Sage plant than non-applied.

Table 4. Effect of application of Nano urea, urea and bio fertilization on nutrients uptake (kg/fed) in two cuts of Sage plant

treatments	N Types	Rates	N uptake		P uptake		K uptake	
			Kg/fed					
			1st cut	2nd cut.	1st cut	2nd cut.	1st cut	2nd cut.
Without bio fertilizer	Control		3.69	3.40	1.04	1.37	12.8	10.9
	urea	N1	12.57	13.16	4.56	6.70	62.6	66.4
		N2	19.65	22.26	7.71	12.11	79.6	89.2
	Nano	N1	14.32	15.44	4.78	7.18	70.7	76.2
		N2	27.70	36.37	9.13	16.57	93.2	120.2
With bio fertilizer	Control		3.98	3.70	1.23	1.64	15.2	13.4
	urea	N1	12.80	14.63	4.76	7.17	64	68.7
		N2	21.24	23.67	8.17	12.84	82.6	93.6
	Nano	N1	14.85	16.65	5.06	7.56	74.6	78.5
		N2	28.67	37.11	10.57	19.07	95	122.2
LSD 0.05 for Bio N			0.1	0.14	0.04	0.04	0.5	0.54
N types			0.45	0.75	0.16	0.51	1.5	2.66
Rates			0.38	0.49	0.15	0.28	1.88	2.37
Bio N x N types			0.19	0.31	0.07	0.21	0.63	1.11
Bio N x Rates			0.37	0.47	0.14	0.4	1.81	2.28
N types x Rates			0.54	0.7	0.21	0.4	2.66	3.35
3 factors			0.52	0.67	0.2	0.57	2.55	3.22

The N-efficiency parameters

Table (5) presented a comparison of the average values of NUE, UPE and UTE in each of the treatments. Also, It showed that the highest values of NUE and UPE were obtained by the application of Nano urea. The values of UTE and NUE decrease with increasing fertilizer application while that of UPE increases with increasing nitrogen. Therefore, using Nano urea rates (250 and 500 ppm) would help in reducing the quantity of fertilizers application and hence farmers' profitability. Thus, nanotechnology can be successfully exploited to increase food production, minimize costs, and protect the environment. Comparison of N-efficiency parameters showed that the highest NUE value belonged to the first nitrogen level and its lowest belonged to the third nitrogen level of urea and nano-fertilizers are more efficient in cultivations with nitrogen requirements. The findings by Zareabyaneh and Bayatvarkeshi (2015). also confirm our conclusion.

Table 5. Effect of application of Nano urea, urea and bio fertilization on N-efficiency parameters for average of two cuts of Sage plant

Treatments	N Types	Rates	NUE (kg/kg)	UPE (kg/kg)	UTE (kg/kg)
Without bio fertilizer	control				
	urea	N1	140.70	0.64	218.72
		N2	79.66	0.52	152.06
	Nano	N1	648.89	3.31	196.29
		N2	472.24	3.88	121.61
With bio fertilizer	control				
	urea	N1	117.77	0.57	207.80
		N2	73.93	0.51	145.49
	Nano	N1	341.32	1.81	188.53
		N2	316.18	2.64	119.70

Available nutrients in soil at the end of the experiment

Table (6) shows the available N, P and K in soil after completing the experiment (Harvest stage). The application of Nano urea, urea and bio N fertilizer increased the available nutrients in the studied soil. When comprising the effect between the previous studied amendments on available nutrients; Nano N2 with bio N fertilizer treatment gave higher significantly increases of available nutrients in the studied soil. Also, soil application of bio N fertilizer gave the higher values of available nutrients than non-application. The superior treatment (Nano N with bio N fertilizer) gave higher significant increases on available nutrients in soil at layer 0-30 cm by (88, 1.73 and 99 mg/kg) for available N, P and K, respectively.

The inference of this finding is that the N supply from Nano source remains available long time compared with conventional fertilizer. The previous results seem to be supported by those obtained by Banishwal *et. al.,* (2006); Li *et.al., (*2013) and Anjuman *et. al.,* (2017).

Table 6. Effect of application of Nano urea, urea and bio N fertilizer on available nutrients in soil after harvest stage of sage plant

treatments	N Types	Rates	Available nutrients in soil mg/kg N	P	K
Without bio fertilizer	control		25	1.47	32
	urea	N1	56	1.56	73
		N2	75	1.64	91
	Nano	N1	64	1.59	79
		N2	83	1.67	96
With bio fertilizer	control		36	1.52	37
	urea	N1	63	1.61	78
		N2	82	1.69	96
	Nano	N1	69	1.65	85
		N2	88	1.73	99
LSD $_{0.05 \text{ for}}$ Bio N			2.17	0.015	1.385
N types			2.19	0.015	1.385
Rates			0.78	0.005	0.492
Bio N x N types			0.95	0.007	0.600
Bio N x Rates			1.08	0.006	0.508
N types x Rates			1.09	0.008	0.696
3 factors			1.13	0.008	0.718

Microbial community in rhizosphere soil during two cuts of Sage plant

Total microbial counts:

a-Total microbial count

Initial total microbial count in Baloza soil was 74.1×10^5 c.f.u./ gm dry soil. Table (7) showed that the change in the count which tend to increase in all treatments refers to control. Total microbial count showed marked increase in second cut ,total microbial counts were affected by mineral fertilizer type (Urea and Nano Urea), Nano urea at second level recorded significant increase in total microbial counts. Inoculation with Azotobacter chroococcum Nano urea at second level recorded the highest increase in total microbial count compared to other treatments. Abd El-Gawad (2014) reported that microbial inoculants increase number and biological activities of beneficial microorganisms and improve the soil fertility in the root zone.

b-Azotobacter densities .

The initial count of N_2 fixing *A. chroococcum* in Baloza soil was 10×10^4 cells/ gm dry soil. Data recorded in Table (7) showed that, the count recorded marked increase in first cut and increase gradually in second cut. The counts under Azotobacter chroococcum inoculation showed the highest counts Azotobacter densities in rhizosphere of Saliva officenalis significantly affected by mineral types and rates. Nano urea at second level (N2) recorded higher value of microbial counts than urea at same rate. Also, the superior treatment was obtained by mixed application of Azotobacter chroococcum + Nano urea at second level (N2) which achieved highest A.chroococcum densities. It was clear from the obtained results that N2 fixers Azotobacter chroococcum enrich the soil by nitrogen fixation which increase soil fertility. The promoting effect due to application of Azotobacter chrococcum not only due to nitrogen fixation but also, production of plant growth promoting substances, production of antimicrobial substances as well, which increase soil fertility, microbial community and plant growth (Jnawali *et. al.,*2015).

Table 7. Effect of application of Nano urea, urea and bio fertilization (*Azotobacter chroococcum)* on microbial determinations in rhizosphere soil during two cuts of Sage plant

treatments	N Types	Rates	Total microbial count $\times10^5$ c.f.u./ gm dry soil 1st cut	2nd cut.	Azotobacter Densities 10^4 cells/ gm dry soil 1st cut	2nd cut	Dehydrogenase 1st cut	2nd cut	Nitrogenase 1st cut	2nd cut
Without Bio fertilizer	control		62.9	58.6	6.2	5.5	0.34	0.41	0.12	0.11
	urea	N1	84.2	86.1	11.8	12.6	0.59	0.62	0.15	0.17
		N2	86.4	88.5	13.4	14.1	0.68	0.75	0.19	0.23
	Nano	N1	85.9	87.6	12.9	13.7	0.61	0.71	0.18	0.20
		N2	89.1	91.7	14.5	15.4	0.75	0.79	0.22	0.25
With bio fertilizer	control		93.4	98.6	15.5	16.3	0.81	0.86	8.16	8.22
	urea	N1	117.6	119.6	17.2	19.5	1.24	1.28	10.7	11.2
		N2	121.5	123.5	19.5	21.8	1.46	1.51	12.1	12.6
	Nano	N1	119.3	121.3	18.6	20.2	1.35	1.48	11.8	12.1
		N2	123.7	125.7	20.9	22.3	1.67	1.74	12.9	13.3
LSD $_{0.05}$ for Bio N			9.40	10.30	2.00	2.35	0.16	0.17	2.97	3.05
N types			9.43	10.32	2.02	2.37	0.19	0.19	3.00	3.08
Rates			3.35	3.67	0.72	0.84	0.07	0.07	1.07	1.10
Bio NxN types			4.09	4.48	0.87	2.37	0.18	0.19	3.00	3.08
Bio NxRates			4.70	5.16	1.00	1.17	0.09	0.08	1.48	1.52
NtypesxRates			4.74	5.19	1.01	1.19	0.10	0.10	1.51	1.55

Enzymatic activities: enzymatic activities of soil samples are critical index of soil fertility because enzymes play an important role in nutrient cycles (Dick *et. al.,* (1996) and Anwesha *et. al.,* (2012), data in Table (7) showed the determination of enzymatic activity in rhizosphere area of some forage crops plants.

Dehydrogenase enzyme: Data in Table 7 show the determination of enzymatic activities in rhizosphere of some fodder crops. Dehydrogenase activity (DHA) represents the energy transfer, therefore, it is considered as an index of overall microbial activity in the soil. Represented data recorded that mineral fertilizer types and rates affected dehydrogenase activity in soil, Interaction treatment of organic A.chroococcum with mineral fertilization types (urea and nano urea) and rates(N1,N2) recorded the highest DHA activity. This may be due to that the A.chroococcum played important role as plant growth promoting rhizobacteria via N2 fixation (El-Howeity *et. al.,* 2003) and Muthukumar and Udaiyan 2006). This might led to accumulate available nutrients and stimulate the microorganisms in soil rhizosphere.

Nitrogenase activity: It increased with *A.chroococcum* inoculation. The highest mean values for nitrogenase enzyme was recorded with the interaction treatments (bio+ Nano urea level 2). Many investigators demonstrated the positive effect of inoculation with N2-fixer on N2-ase activity (El- Komy 2005).

CONCLUSION

Yield components of sage plants increased with increasing of Nano urea, urea application rates during both cuts. The mineral treatments with bio fertilizer gave greater yield values than without applied bio fertilizer. The most effective treatment (Nano N2 with bio N fertilizer) gave highest significantly values of yield components by 64.4, 11249 and 4395 for plant height (cm), herb fresh and dry weight (kg/fed) , respectively in first cut, while in second cut was higher significantly increases of yield components than first cut. The nutrients content and uptake take the same trend in yield parameters. The N-efficiency parameters; the highest values of NUE and UPE obtained by the application of Nano urea, The values of UTE and NUE decreased with increasing fertilizer application while that of UPE increased with increasing nitrogen. Nano N2 with bio N fertilizer treatment gave higher significantly increases of available nutrients in the studied soil. Also, soil application of bio N fertilizer gave the higher values of available nutrients than non-application. The superior treatment (Nano N with bio N fertilizer) gave higher significant increases on available nutrients in soil at layer 0-30 cm by (88, 1.73 and 99 mg/kg) for available N, P and K, respectively. The superior treatment was obtained by mixed application of Azotobacter chroococcum + Nano urea at second level (N2) which achieved highest A.chroococcum densities. It was clear from the obtained results that N2 fixers Azotobacter chroococcum enrich the soil by nitrogen fixation which increase soil fertility. This study concluded that using Nano sources of fertilizers can reduce the amount of wasted chemicals and hence minimizes the pollution hazard.

ACKNOWLEDGEMENT

I express my deepest thanks to the head of soil fertility unit Dr. Hassan A. Fawy for providing the facilities to carry out the research work.

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تأثير التسميد النيتروجينيى المعدنى والنانوى والحيوى على محتوى النيتروجين وانتاجية نبات المريمية

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النانو تكنولوجى يعتبر من أهم الاكتشافات الجديدة المفيدة فى العصر الحديث والذى دخل فى جميع المجالات، ويعتبر النانوتكنولوجى والتسميد الحيوى من أهم الوسائل المستخدمة حاليا فى الزراعة الحديثة . أقيمت تجربة حقلية فى محطة بحوث بالوظة التابعة لمركز بحوث الصحراء بشمال سيناء لدراسة تأثيراضافة السماد النيتروجينى يوريا و نانو يوريا والسماد الحيوى (الأزوتوباكتر) على التركيب الكيميائى وانتاجية نبات المريمية . أقيمت تجربة حقلية فى محطة بحوث بالوظة التابعة لمركز بحوث الصحراء بشمال سيناء لدراسة تأثيراضافة السماد النيتروجينى يوريا و نانو يوريا والسماد الحيوى (الأزوتوباكتر) على التركيب الكيميائى وانتاجية نبات المريمية . حيث اجريت المعاملات كالاتى اضافة نوعين من السماد المعدنى النيتروجينى نانو يوريا بمعدل 250-500 جزء فى المليون، ويوريا بمعدل 40- 80 كجم/ فدان مع اضافة وبدون السماد الحيوى الأزوتوباكتر باستخدام تصميم احصائى قطع منشقة مرتين وعدد 10 معاملات و 3 مكررات لكل معاملة للحشتين خلال موسم زراعى 2017. اوضحت النتائج المتحصل عليها ان انتاجية نبات المريمية زادات بزيادة اضافة معدلات نانو يوريا ويوريا فى كلتا الحشتين. واضافة التسميد الحيوى أدى الى زيادة الانتاجية بالمقارنة بعدم الاضافة. أعطت المعاملة نانو يوريا (ن2) مع التسميد الحيوى أعلى زيادة معنوية فى قياسات المحصول وكانت الزيادة حوالى4395 and 11249 ,2.77 ,64.4 لكل من ارتفاع النبات (سم) والوزن الطازج والجاف (كجم/فدان) على التوالى فى الحشة الأولى بينما فى الحشة الثانية أعطت قيم معنوية أكبر من الحشة الأولى. ولوحظ أيضا أن المحتوى البيوكيميائى ومحتوى العناصر الغذائية الممتص لنبات المريمية سلك نفس الاتجاة. اوضحت الدراسة أن سماد النانو يوريا أدى الى زيادة كفاءة استخدام السماد بالمقارنة باليوريا حيث حقق المعدل العالى من التسميد النيتروجينى معدل أقل لكل من كفاءة استخدام النيتروجينىNUE وكفاءة استغلال النيتروجين UTE بينما زاد معدل كفاءة امتصاص النيتروجين UPE بزيادة معدل التسميد. نانو يوريا 500جزء فى المليون مع السماد الحيوى هى المعاملة الأكثر تأثيرا حيث أعطت زيادة معنوية فى الكمية الميسرة من العناصر فى التربة، وأيضا أدتالى زيادة نشاط انزيم الديهيدروجينيزو زيادة عدد الازوتوباكتر فى منطقة الريزوسفيرلنبات المريمية.