Application of Manure and Phosphorus Bio-Solubilizers with Rock Phosphate in Calcareous Soils to Increase Phosphorus Availability and Productivity of Safflower Plant

Attia, M. F., Hassan[1], A. Fawy[1], Rehab H. Hegab[1] and Noha M.M.[1]

ABSTRACT

Field experiments were carried out in Ras Sudr Research Station (29º 32' 29'' N and 32º 39' 27'' E) during two successive seasons of 2013 and 2014. Salinity of irrigation water was 7.36 dSm^{-1} and that of soil paste extract was 7.96 dSm^{-1}. The experimental design was a randomized complete block, with 3 replicates. The treatments included 4 factors as follows: Factor 1: Farmyard manure, 2: treatments, FYM_1 and FYM_2 of 24 and 48 m^3 farmyard manure per hectare, respectively. Factor 2: P is two types, RP "rock phosphate (9.5% P_2O_5)"and SP "superphosphate (15.5% P_2O_5)"Factor 3: P applied at 2 rates of 31 and 62 kg P ha^{-1}, respectively. Factor 4: Biofertilizers applied at 4 treatments as follows: none, VAM (Vesicular-arbuscular mycorrhiza), PS "P-solubilizing bacteria" (*Pseudomonas fluorescence* and *Bacillus megaterium*) and VAM+PS. Thus, the total number of treatments in combinations were 32 (2 FYM, 2 P-type, 2 P rates and 4 biofertilzations). An extra treatment was performed involving no application of fertilizers nor biofertilizers (control). The biofertilizer was a mixture of inoculate of the P-solubilizing bacteria of *Pseudomonas fluorescence* and *Bacillus megaterium*. VAM was added at 20 kg ha^{-1}. The results showed that plant yield and N, P and K contents and uptake increased by applying bio-fertilizers as well as farmyard manure and p-mineral fertilizers. The most effective combinations are as follows: RP2 + (VAM) +FYM1 < SP2 + VAM + FYM1 < SP2 + (PS)+FYM1 < SP2 + (VAM +PS) + FYM1< SP2 + (VAM +PS) + FYM2. The Integration between mineral and bio-P-fertilizers with farmyard manure (FYM) application produced the most effective treatment (SP2+ VAM +PS+FYM2) which achieved the highest safflower yields which recorded 6.59, 3.36 and 36 for stalk (ton ha^{-1}), seeds (ton ha^{-1}) and oil (%) respectively.

Keywords: Rock phosphate, Superphosphate, Bio-P-fertilizers, farmyard manure, safflower, sandy loam soil Ras Sudr

INTRODUCTION

Negative effect of salinity for agricultural activities is common in the newly reclamation areas of Sinai-Egypt, especially in soils with high salinity water irrigation of Ras Sudr regions. Ras Sudr soils are affected by irrigation with saline water, which increased soil salinity (Hergert and Knudsen 2004). They reported that the water of EC<0.75 dSm^{-1} has no detrimental effect, 0.75 - 1.50 dSm^{-1} has detrimental effects on sensitive crops, 1.50 - 3.0 dSm^{-1} required careful management practices, and 3.0-7.5 dSm^{-1} was used only for salt tolerant plants. Safflower (*Carthamus Tinctorius* L.) is a herbaceous plant cultivated for its seed oil, and is grown in soils of arid regions which included many saline soils, since it is moderately tolerant to salinity (Oelke *et al*., 1992). Application of antioxidants can alleviate the adverse effect of salinity (Farouk, 2011). Phosphorus could be involved in such alleviation and presence of enough P in the rhizosphere can augment plant resistance to salinity (Ceulemans *et al.,* 2011 and Lambers *et al.,* 2014).

Concerning to farmyard manure and mineral P fertilizer on yield components and nutrients content in safflower plants; Kizil *et al*., (2008) stated that the seed yield and fatty oil percentage of the stalk cultivars ranged from 1706 to 3111 kg ha^{-1} and 26.1 to 35.1%, respectively. Rabie *et al*., (2010) reported that the combination of high concentration of compost (20 ton/fed) and rock phosphate (1000 kg/fed) recorded the highest values of yield parameters and good quality of safflower oil by increasing unsaturated / saturated fatty acids ratio. Ali and Mahmoud (2012) stated that the highest yield components and nutrient uptake were obtained when safflower was fertilized by 18 ton farmyard manure along with 130 kg N ha^{-1}. Ghasemi *et al*. (2012) obtained highest seed yield of safflower (3512 kg ha^{-1}) by application of biofertilizers and mineral fertilizers. Raju *et al*. (2013) applied 50 % of recommended N as inorganic forms and 50% as organic form to safflower and obtained the highest seed yield and N, P and K uptake. Hamza (2015) obtained the highest seed yield and oil yield of 2890 and 927 kg ha^{-1} with plant density of 240000 plant ha^{-1}.

Regarding to the P bio-fertilizer effect on the yield parameters and nutrients contents; Yasin *et al*., (2012) stated that P-biofertilization is important for safflower. Neetu *et al*. (2012) obtained maximum yield by inoculating plants with *Arbuscular Mycorrhizal* Fungi (AMF) i.e. Vesicular-arbuscular mycorrhiza "VAM" and *Pseudomonas fluorescens*. They also reported that inoculation with *Azotobactor* and *Azosprillum* to fix atmospheric nitrogen had positive effects. El Mokadem and Sorour (2014) reported that *Azospirillum* + P

[1]Soil Fertility and Microbiology Dept.,
Desert Research Center (DRC), Cairo
Received December 14, 2016, Accepted December 29, 2016

dissolving bacteria + foliar spray of nutrients produced the highest growth and yield parameters. Amin and Moayedi (2014) reported that the combination of using mineral N and P with P Bio-fertilizers increased growth parameters and yield of safflower. The objective of the current work therefore, was to evaluate the effect of farmyard manure, mineral phosphorus and P-bio-solubilizes on the growth performance of safflower under conditions of salinity at Ras Sudr-Sinai, Egypt.

MATERIALS AND METHODS

Field experiment was carried out in Ras Sudr Research Sation (located at 29º 32' 29" N and 32º 39' 27 " E) of Desert Research Center during two successive seasons (2013 and 2014). The salinity of irrigation water was 7.36 dSm^{-1} and salinity of soil paste extract was 7.96 dSm^{-1} (Tables, 1 and 2). Seeds of safflower (*Carthamus Tinctorius* L.) cv Giza 1 were sown on 15th of November 2013, 20 cm between seeds and 70 cm between rows at about 3 cm depth. Final plant density was 71400 plants ha^{-1} (30000 plant Egyptian $feddan^{-1}$).

Available P was determined according to Olsen *el al*., (1982). Available potassium was extracted by ammonium acetate. Available nitrogen was extracted by potassium chloride and determined by Kjeldahl method. Available micronutrients were extracted by DTPA and measured by Atomic Absorption Spectrometry. Soil physical and chemical analyses were determined according to the methods of Page *et al. (*1982) and Klute (1986) and the results obtained are shown in Table 1 and 2. The experimental design was a randomized complete block with 3 replicates. There were 3 factors as follows; Factor 1: Farmyard manure; 2 treatments, FYM_1 and FYM_2 of 24 and 48 m^3 farmyard manure per hectare, respectively, Factor 2: two types of P, RP "rock phosphate (9.5% P_2O_5)" and SP "superphosphate (15.5% P_2O_5)", Factor 3: P applied at 2 rates of 31 and 62 kg P ha^{-1}, respectively, Factor 4: Biofertilizers applied at 4 treatments as following; none, VAM (Vesicular-arbuscular mycorrhiza), PS "P-solubilizing bacteria" (*Pseudomonas fluorescence* and *Bacillus megaterium*) and VAM+PS. Thus, the total treatments combinations were 32 (2 FYM, 2 P-type, 2 P rates and 4 biofertilizers). An extra treatment was performed involving no application of fertilizers nor biofertilizers (non-treated). The biofertilizer was a mixture of inoculates of the P-solubilizing bacteria of *Pseudomonas fluorescence* and *Bacillus megaterium* which added at one rate of 2L/200L. VAM was added at a rate of 20 kg ha^{-1}. All treatments received 170 kg N ha^{-1} (as urea: 460 g N kg^{-1}) + 100 kg K ha^{-1} (as K-Sulphate: 480 g K_2O kg^{-1}), in 3 equal splits 40, 80, 120 days after seeding.

Isolates of bacteria used as bio-fertilizers were purified and identified according to (Bergey's Manual of Determinative Bacteriology, 1994). The selected isolates of *B. megaterium and P. fluorescence* were subjected to different biochemical tests for screening their hormonal and enzymatic activity (Rizzolo *et al.* 1993). They produce biochemical and hormonal substances (Table 4) that could result in beneficial effects in the field (El-Saidy *et al*., 2011).

Table 1. Chemical and physical properties of the experimental soil

Depth (cm)	pH	E.C (dSm^{-1})	OM	$CaCO_3$	Particle size distribution			Texture	C.E.C ($cmol\ kg^{-1}$)
			%		Sand	Silt	Clay		
					%				
0-30	7.87	7.96	2.28	27.2	79.5	9.72	10.78	L.S.	6.18
30-60	7.65	7.58	1.82	28.3	78.26	11.35	10.39	L.S.	6.96
				Soluble cations and anions in soil ($mmol\ L^{-1}$)					
	Na	K	Ca	Mg	HCO_3^-		Cl^-	SO_4^{2-}	
0-30	43.6	8.5	22.3	5.2	8.3		48.2	23.1	
30-60	40.5	12.5	15.4	4.1	3.8		45.3	23.4	
				Available nutrients in soil ($mg\ kg^{-1}$)					
	N	P	K	Fe	Mn		Zn	Cu	
0-30	38.5	5.35	51.3	4.32	2.23		1.36	0.64	
30-60	23.8	3.96	57.5	4.73	2.39		1.42	0.69	

Table 2. The main chemical composite of groundwater of irrigation ($mmol\ L^{-1}$)

pH	EC	Na	K	Ca	Mg	HCO_3^-	Cl^-	SO_4^{2-}
7.87	7.36	43.9	2.6	19.5	7.6	6.7	44.3	22.6

Table 3. Fertilizers treatments during the two seasons

Fertilizer Treatment	Mineral fertilizer			
	Superphosphate		Rock phosphate	
	kg P ha^{-1}	kg SP ha^{-1}	kg P ha^{-1}	kg RP ha^{-1}
Phosphorus 1	31	461	31	752
Phosphorus 2	62	921	62	1504
N & K_2O	167 and 100 kg $ha^{-1}$of N and K respectively(applied for all plots)			
FYM	24 and 48 m^3 ha^{-1} added during seeds bed time			
Bio-fertilizer 1	P solubilizing bacteria (PSB) , *B. megaterium* & *P. fluorescence* applied at 2L/200L			
Bio-fertilizer 2	*(VAM) fungi (19 kg ha^{-1}) added to soil at the time of P applied.			

*(VAM) Vascular Arbiscular Mychorrhiza**.**

Table 4. The biochemical activities of microbial isolates (according to Rizzolo *et al.* 1993)

Excreted hormone (µg mL^{-1})	*B. megaterium*	*P. fluorescens*
IAA	0.29	10.2
GA3	1.81	1.95
Cytokinine	14.92	18.39
Amylase	+	-
Phosphatase	++	-
Protease	++	-

Fresh liquid cultures of *Azotobacter chrococcoccum*, *B. megaterium* and *P. fluorescence* were applied to soil inoculation at the rate of 108 colony forming unit (cfu/ml). Rhizosphere soil samples were collected after plant harvest. For mycorrhizal inoculation, mycorrhizae spores were isolated from soil pre-inoculated with mycorrhiza (*Glomus macrocarpium*) by wet-sieving and decantation method as described by Gerdeman and Nicolson (1963). The samples were analyzed for total counts of microorganisms according to Nautiyal (1999). Counting of the growing phosphate dissolving bacteria was carried out by using Pikovskaya's agar medium (PVK) according to Goenadi *et al*. (2000). Counting and growing Azotobacter was done by modified Ashby's media (Hill, 2000). Pseudomonas, CO_2 evolution was determined by Kings media according to Anderson (1982). Plant samples were collected at harvest. Plant height, weight of seeds $plant^{-1}$, weight of straw $plant^{-1}$, yields of seeds straw ha^{-1}. Seed oil content was determined using the soxhlet extraction method with hexane as described in AOAC (1990). Stalk defoliated plants were collected for retting process as described by Sanio *et al*. (1995). The plant samples were first washed by tap water then by distilled water and oven-dried, ground and wet digested by H_2SO_4 and H_2O_2 as reported by Carter (1993) to determine plant content of N, P and K according to Cottenie *et al*. (1982). The data were statistically analyzed according to (Snedecor and Cochran 1980) with the aid of CoStat computer program (version 6.4) for statistics. Differences among treatments were tested with LSD at a 5% level of significance.

RESULTS AND DISCUSSION

Safflower yield components:

Table (5) showed that yield components of safflower plants increased with increasing of farmyard manure, SP (superphosphate), RP (rock phosphate) application rates and with using two types of bio-P fertilizers applications under saline condition of Ras Sudr soil during the studied two growth seasons. RP_2 treatments showed higher significant increases of yield parameters than RP_1 treatments indicating relative increases of 2.7, 12.4 and 9.9 for stalk (kg/fed), seeds (kg/fed) and oil%, respectively, while SP_2 treatments caused higher increases than SP_1 by 1.9, 9.1 and 7.0%.

The SP showed relative increases of yield components as 29.0, 26.8 and 26.6% for stalk, seeds and oil content, respectively, over the RP. The bio-PS increased these parameters by 11.7, 11.2 and 3.1% over the bio-VAM. FYM_2 increased yield components over FYM_1 by 11.0, 15.3 and 19.7%, respectively. These results agree with those obtained by Rabie *et al*. (2010), Ghasemi *et al*. (2012), Ali and Mahmoud (2012) and Amin and Moayedi (2014).

Yield and its components increased with combination of mineral and bio-fertilizers and FYM application (Figs 1 to 3). The effect of treatments on yield could be arranged as follows: RP2 + (VAM) + FYM1 < SP2 + (VAM) + FYM1 < SP2 + (PS) + FYM1 < SP2 + (VAM +PS) + FYM1< SP2 + (VAM +PS) + FYM2. The (SP2+VAM+PS+FYM2) treatment produced the highest yield. These results agree with those reported by Neetu *et al*. (2012), El-Nagdy *et al*. (2010), Yasin *et al*. (2012) and Amin and Moayedi (2014).

Nutrients concentrations and uptake of safflower plant:

Data in Tables 6 and 7 showed that the RP_2 surpassed RP_1 for nutrients contents in stalk and seeds and SP_2 surpassed SP_1. The SP recorded higher relative increases of nutrients contents and uptake in

stalk than RP by 29.9, 29.6 and 28.9 % for N, P and K respectively, and 20.2, 32.6 and 27.8% respectively in seeds. These results agree with those found by El-Nagdy *et al*. (2010), Yasin *et al*. (2012) and Amin and Moayedi (2014). The VAM treatment caused lower content of nutrients in stalk than *P*- bio-fertilizers by relative values of 3.9, 9.7 and 3.8% for N, P and K respectively and 2.1, 7.4 and 2.6% respectively in seeds. The FYM treatment increased nutrients contents in stalk by relative increases of 5.7, 12.6 and 5.9 % for N, P and K respectively, and 3.7, 10.4 and 4.4% in seeds.

Table 5. Effect of fertilizers treatments on the yield and yield component of safflower

FYM	P Type	P Rates	Bio	Plant height (cm)	No. branches /plant	W. stalk / plant (g)	W. seed/ plant (g)	W.1000 seed (g)	Seed Oil (%)	Stalk yield (ton/ha)	Seed yield (ton/ha)
FYM_1		Control		49	3.4	28.7	15.3	13.6	15.1	2.05	1.09
	RP	P_1	Bio_0	75	5.2	44.9	23.5	20.9	23.1	3.21	1.68
		P_1	VAM	85	6.3	55.4	27.6	23.8	24.9	3.95	1.97
		P_1	PS	93	7.1	65.0	32.3	26.4	25.8	4.64	2.31
		P_1	VAMPS	105	8.2	70.9	35.8	30.4	27.4	5.07	2.56
		P_2	Bio_0	84	5.86	51.1	26.2	22.9	25.1	3.65	1.87
		P_2	VAM	97	7.29	65.5	32.3	27.2	27.0	4.68	2.30
		P_2	PS	102	7.8	71.7	35.0	29.1	27.7	5.12	2.50
		P_2	VAMPS	112	8.63	77.6	37.7	32.1	28.8	5.54	2.69
	SP	P_1	Bio_0	94	6.29	56.3	28.4	25.3	28.0	4.02	2.03
		P_1	VAM	106	7.62	69.3	33.4	28.8	30.1	4.95	2.39
		P_1	PS	116	8.59	78.5	39.1	31.9	31.2	5.61	2.79
		P_1	VAMPS	120	9.08	84.0	40.4	33.4	31.9	6.00	2.89
		P_2	Bio_0	103	7.03	63.5	31.4	27.4	30.2	4.54	2.25
		P_2	VAM	113	8.09	74.6	35.7	30.6	31.7	5.33	2.55
		P_2	PS	124	9.23	87.6	41.5	34.1	33.0	6.25	2.96
		P_2	VAMPS	132	9.93	94.5	43.8	36.7	34.0	6.75	3.12
FYM_2		Control		56	3.91	33.6	19.9	17.7	19.2	2.40	1.42
	RP	P_1	Bio_0	79	5.53	41.7	24.9	21.9	24.1	2.98	1.78
		P_1	VAM	91	6.8	56.6	29.9	25.5	26.0	4.04	2.14
		P_1	PS	98	7.45	66.9	33.7	27.8	26.7	4.78	2.40
		P_1	VAMPS	108	8.42	75.7	36.7	31.2	28.1	5.41	2.62
		P_2	Bio_0	92	6.52	50.4	29.0	24.9	27.2	3.60	2.07
		P_2	VAM	108	8.29	71.2	36.9	30.5	29.2	5.09	2.64
		P_2	PS	111	8.51	76.1	37.7	31.8	29.5	5.44	2.69
		P_2	VAMPS	118	9.06	72.3	39.6	33.6	30.3	5.16	2.83
	SP	P_1	Bio_0	103	7.03	57.4	31.4	27.4	30.2	4.10	2.25
		P_1	VAM	113	8.09	70.7	35.7	30.6	31.7	5.05	2.55
		P_1	PS	124	9.23	78.0	41.5	34.1	33.0	5.57	2.96
		P_1	VAMPS	132	9.93	82.7	43.8	36.7	34.0	5.91	3.12
		P_2	Bio_0	113	7.76	67.2	34.5	29.6	32.4	4.80	2.46
		P_2	VAM	120	8.55	76.2	38.0	32.5	33.3	5.45	2.71
		P_2	PS	132	9.86	85.8	43.9	36.3	34.7	6.13	3.14
		P_2	VAMPS	144	10.78	92.3	47.1	40.0	36.0	6.59	3.36

Table 5. Continue ($LSD_{5\%}$)

Variables / $LSD_{0.05}$	Plant height (cm)	No. branches /plant	W. stalk / plant (g)	W. seed/ plant (g)	W.1000 seed (g)	Seed Oil (%)	Stalk yield (ton/ha)	Seed yield (ton/ha)
FYM	0.67	0.05	0.12	0.21	0.17	0.15	0.010	0.019
P Types	1.28	0.08	0.81	0.35	0.29	0.32	0.057	0.025
P rates	0.52	0.04	0.37	0.17	0.14	0.11	0.028	0.012
Bio	1.27	0.10	0.91	0.41	0.34	0.26	0.068	0.029
FYM x PT	0.80	0.05	0.51	0.22	0.18	0.20	0.035	0.016
FYM x PR	0.73	0.06	0.53	0.24	0.19	0.15	0.015	0.017
FYM x Bio	0.44	0.10	0.91	0.41	0.34	0.09	0.068	0.029
PT x PR	0.90	0.07	0.20	0.29	0.24	0.19	0.048	0.021
PT x Bio	1.27	0.10	0.91	0.41	0.34	0.26	0.068	0.029
PR x Bio	1.27	0.10	0.91	0.41	0.34	0.26	0.068	0.029
FYM x PT x PR	1.27	0.10	0.91	0.41	0.34	0.26	0.021	0.029
FYM x PT x Bio	1.27	0.10	0.91	0.41	0.34	0.13	0.068	0.029
FYM x PR x Bio	0.63	0.05	0.91	0.20	0.17	0.13	0.068	0.014
PT x PR x Bio	1.27	0.10	0.91	0.41	0.34	0.26	0.068	0.029
FYM x PT x PR x Bio	0.89	0.07	0.91	0.29	0.24	0.19	0.068	0.020

Notes: Treatment designations are as follows: OM1 and OM2 farmyard manure at 24 and 48 m^3 ha^{-1} , SP & RP at rates 31 & 62 kg P ha^{-1} respectively ; PS: biofertilization with P-solubilizing bacteria *B. megaterium* & *P. fluorescence* Bio_0 : no biofertilization; VAM : biofertilization using VAM (Vesicular-arbuscular mycorrhiza).

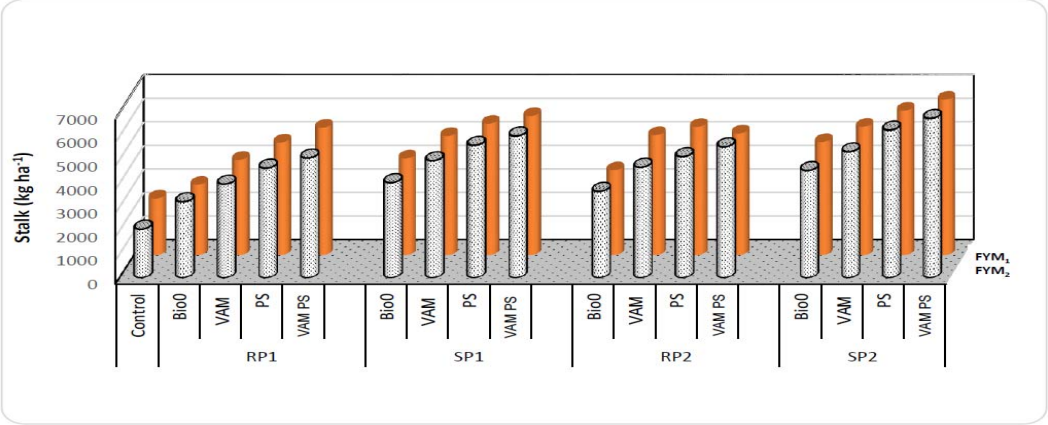

Fig.1. The relation between the studied treatments and the stalk yield of safflower plant

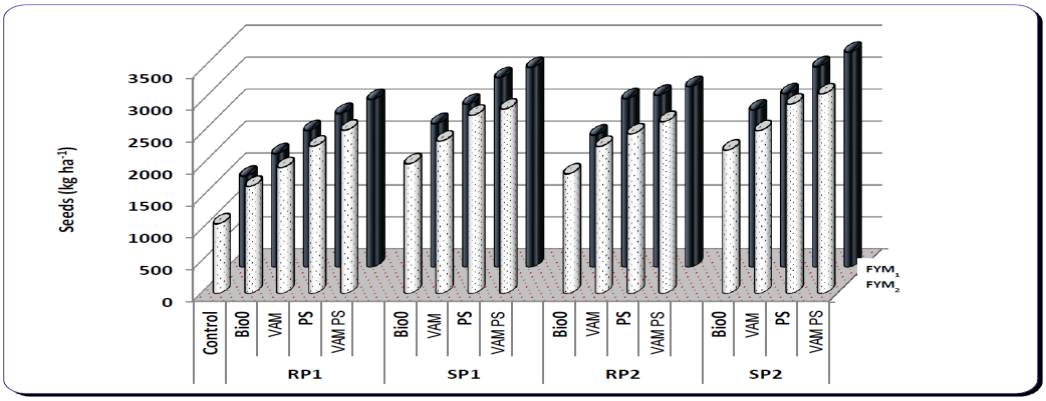

Fig.2. The relation between the studied treatments and the seeds yield of safflower plant

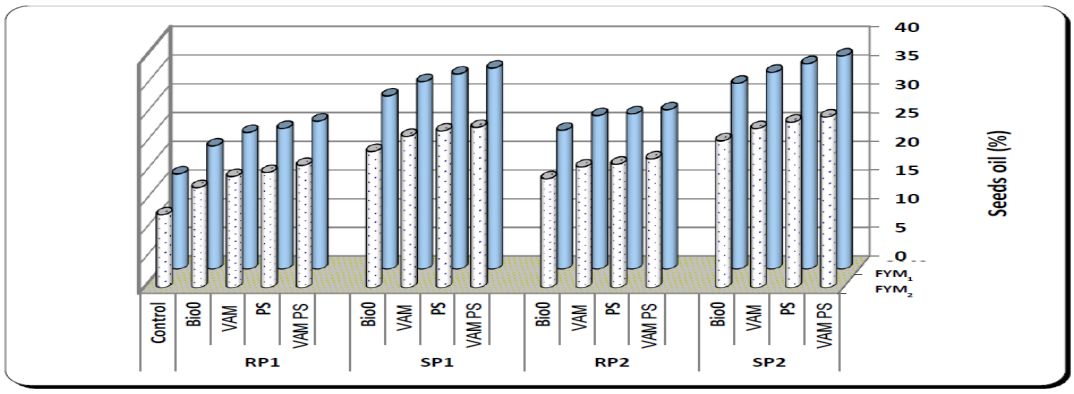

Fig.3. The relation between the studied treatments and oil% of safflower seeds

Table 6. Effect of fertilizers treatments on nutrients concentrations in stalks and seeds of safflower plants

FYM	P Type	P Rates	Bio	Stalk nutrients concentration			Seeds nutrients concentration		
				N %	P%	K%	N %	P%	K%
FYM_1		Control		0.61	0.17	0.47	1.07	0.21	0.81
	RP	P_1	Bio_0	0.92	0.27	0.73	1.65	0.32	1.25
		P_1	VAM	0.95	0.31	0.76	1.69	0.36	1.29
		P_1	PS	0.98	0.34	0.81	1.73	0.39	1.33
		P_1	VAMPS	1.08	0.39	0.87	1.81	0.46	1.39
		P_2	Bio_0	0.98	0.32	0.81	1.74	0.39	1.33
		P_2	VAM	1.03	0.35	0.83	1.78	0.43	1.37
		P_2	PS	1.07	0.39	0.86	1.84	0.47	1.41
		P_2	VAMPS	1.15	0.45	0.92	1.92	0.55	1.50
	SP	P_1	Bio_0	1.17	0.33	0.95	1.86	0.44	1.56
		P_1	VAM	1.21	0.36	0.98	1.89	0.48	1.59
		P_1	PS	1.25	0.41	1.02	1.93	0.51	1.63
		P_1	VAMPS	1.32	0.49	1.1	1.99	0.59	1.72
		P_2	Bio_0	1.25	0.39	0.97	1.95	0.52	1.64
		P_2	VAM	1.31	0.45	1.03	1.98	0.56	1.68
		P_2	PS	1.37	0.51	1.05	2.02	0.59	1.73
		P_2	VAMPS	1.43	0.59	1.15	2.08	0.67	1.82
FYM_2		Control		0.65	0.19	0.51	1.15	0.26	0.86
	RP	P_1	Bio_0	0.97	0.29	0.78	1.73	0.36	1.29
		P_1	VAM	1.01	0.35	0.81	1.76	0.39	1.33
		P_1	PS	1.05	0.40	0.86	1.79	0.44	1.37
		P_1	VAMPS	1.12	0.43	0.91	1.89	0.51	1.46
		P_2	Bio_0	1.05	0.37	0.85	1.82	0.46	1.41
		P_2	VAM	1.09	0.42	0.88	1.85	0.49	1.45
		P_2	PS	1.16	0.46	0.91	1.89	0.53	1.49
		P_2	VAMPS	1.22	0.52	0.99	1.97	0.61	1.57
	SP	P_1	Bio_0	1.22	0.38	0.99	1.91	0.49	1.60
		P_1	VAM	1.26	0.43	1.02	1.95	0.53	1.64
		P_1	PS	1.31	0.47	1.05	1.99	0.57	1.67
		P_1	VAMPS	1.39	0.55	1.14	2.07	0.66	1.83
		P_2	Bio_0	1.34	0.48	1.06	2.02	0.57	1.71
		P_2	VAM	1.39	0.52	1.11	2.06	0.61	1.75
		P_2	PS	1.43	0.55	1.15	2.09	0.65	1.79
		P_2	VAMPS	1.5	0.64	1.21	2.17	0.73	1.88

Table 6. continue (LSD 5%)

Variables	Stalk nutrients concentration			Seeds nutrients concentration		
$LSD_{0.05}$	N %	P%	K %	N%	P %	K%
FYM	0.007	0.005	0.005	0.007	0.005	0.007
P Types	0.017	0.006	0.013	0.012	0.008	0.019
P rates	0.005	0.004	0.003	0.005	0.004	0.005
Bio	0.012	0.010	0.008	0.012	0.010	0.012
FYM x PT	0.010	0.004	0.008	0.007	0.005	0.012
FYM x PR	0.007	0.006	0.005	0.003	0.006	0.007
FYM x Bio	0.012	0.010	0.008	0.004	0.010	0.012
PT x PR	0.009	0.007	0.006	0.003	0.007	0.009
PT x Bio	0.012	0.010	0.008	0.012	0.010	0.012
PR x Bio	0.012	0.010	0.008	0.012	0.010	0.012
FYM x PT x PR	0.004	0.010	0.008	0.012	0.010	0.012
FYM x PT x Bio	0.006	0.010	0.004	0.006	0.005	0.006
FYM x PR x Bio	0.006	0.010	0.004	0.006	0.005	0.012
PT x PR x Bio	0.006	0.010	0.008	0.006	0.005	0.006
FYM x PT x PR x Bio	0.009	0.010	0.008	0.008	0.007	0.009

See footnotes of Table 5 for treatment designations

Nutrients contents in stalk and seeds of safflower plants increased as a result of combining more than fertilization treatments (Table 6). The bilateral, triple and tetra interaction between studied treatments assured significant effects on the studied parameters. The SP treatments showed significant increases nutrients content than RP treatments. Also, bio-PS fertilizer treatments were superior for nutrients contents as compared with bio-VA treatments (Table 6). The P-bacterial biofertilizers treatments were superior VAM .The SP_2+VAM+PS+FYM_2 gave the highest nutrients content during the studied two sequence seasons. The results agree with those obtained by Neetu *et al*. (2012), Yasin *et al*. (2012) and El Mokadem and Sorour (2014).

The N, P and K uptake increased with increasing of farmyard manure, RP and SP rates with the biofertilizers. The SP treatments gave higher increases than RP treatments FYM_2 caused higher increases of nutrients uptake (Table 7) than FYM_1. Nutrient uptake by plants increased with combination of mineral and bio P fertilizers with FYM application. The bilateral, triple and tetra interaction between studied treatments assure that SP treatments showed higher significant increases for nutrients uptake by safflower plant than RP treatments. On the other side, bio-PS fertilizer treatments were superior for nutrients uptake when compared with bio-VAM treatments. The combination of farmyard manure, mineral P-fertilizers and bio-fertilizers applications produced the most effective treatment (SP_2+VAM+PS+FYM_2) which achieved the highest nutrients uptake by stalk and seeds of safflower plant when compared with the other studied treatments. The current results agreed with those obtained by Rabie *et al*. (2010), Ali and Mahmoud (2012), Weisany *et al*. (2013) and Raju *et al*. (2013).

Microbial activities in rhizosphere:

Initial total microbial counts in Ras Sudr soil of Sinai were 30×10^5 cfu/g dry soil. Data in Table (8) showed that total microbial counts in the rhizosphere tended to increase by treatments receiving fertilizers. The highest counts were obtained with FYM_2, RP and SP applications. Microbial respiration (CO_2 evolution) increased after long term from P addition; Microbial activity increase in the presence of P and allowed rapid transformation of soil organic matter. The carbon dioxide (CO_2) is an indication of the biological activity in the rhizosphere. The treatment combining farmyard manure, mineral and bio-fertilizer gave the highest CO_2 evolution. Data of CO_2 evolution were in harmony with those of total microbial counts. These results agree with those found by Visser and Dennis (1992), Gilliam *et al*. (2011) and Liu *et al*. (2012). The control treatment or the non-treated treatment showed the lowest value, the bio-fertilizers and mineral fertilizers treatments showed the highest positive counts with additions of FYM2, RP and SP at the high rates. These results agree with those obtained by Yasin *et al*. (2012), Neetu *et al*. (2012) and El Mokadem and Sorour (2014). The results obtained showed that combining bio-fertilizers with mineral fertilizers could be useful to obtain safflower yield increases. It is also clear that application of VAM and *B. megatherium* increased both the amounts of the available nutrients in soil, plant growth, soil fertility and counts of microbial communities. These results agree with those obtained by Yadav *et al*. (2007) and Daneshmandi *et al*. (2012).

Table 7. Effect of fertilizers treatments on the nutrients uptake by safflower plants

FYM	P type	P Rates	Bio	stalk nutrients uptake (kg ha^{-1})			Seeds nutrients uptake (kg ha^{-1})		
				N	P	K	N	P	K
FYM_1		Control		12.5	3.5	9.6	11.7	2.3	8.8
	RP	P_1	Bio_0	29.5	8.7	23.4	27.7	5.4	21.0
		P_1	VAM	37.5	12.2	30.0	33.3	7.1	25.4
		P_1	PS	45.5	15.8	37.6	40.0	9.0	30.7
		P_1	VAMPS	54.8	19.8	44.1	46.3	11.8	35.6
		P_2	Bio_0	35.8	11.7	29.6	32.5	7.3	24.9
		P_2	VAM	48.2	16.4	38.8	40.9	9.9	31.5
		P_2	PS	54.8	20.0	44.0	46.0	11.8	35.3
		P_2	VAMPS	63.7	24.9	51.0	51.6	14.8	40.4
	SP	P_1	Bio_0	47.0	13.3	38.2	37.8	8.9	31.7
		P_1	VAM	59.9	17.8	48.5	45.2	11.5	38.0
		P_1	PS	70.1	23.0	57.2	53.8	14.2	45.5
		P_1	VAMPS	79.2	29.4	66.0	57.5	17.1	49.7
		P_2	Bio_0	56.8	17.7	44.0	43.9	11.7	36.9
		P_2	VAM	69.8	24.0	54.9	50.5	14.3	42.8
		P_2	PS	85.6	31.9	65.6	59.8	17.5	51.2
		P_2	VAMPS	96.5	39.8	77.6	64.9	20.9	56.8
FYM_2		Control		15.6	4.6	12.2	16.3	3.7	12.2
	RP	P_1	Bio_0	28.9	8.6	23.2	30.8	6.4	23.0
		P_1	VAM	40.8	14.1	32.7	37.7	8.3	28.5
		P_1	PS	50.2	19.1	41.1	43.0	10.6	32.9
		P_1	VAMPS	60.6	23.3	49.2	49.5	13.4	38.3
		P_2	Bio_0	37.8	13.3	30.6	37.7	9.5	29.2
		P_2	VAM	55.5	21.4	44.8	48.8	12.9	38.3
		P_2	PS	63.1	25.0	49.5	50.8	14.3	40.1
		P_2	VAMPS	63.0	26.8	51.1	55.8	17.3	44.4
	SP	P_1	Bio_0	50.0	15.6	40.6	43.0	11.0	36.0
		P_1	VAM	63.6	21.7	51.5	49.7	13.5	41.8
		P_1	PS	73.0	26.2	58.5	58.9	16.9	49.4
		P_1	VAMPS	82.1	32.5	67.4	64.6	20.6	57.1
		P_2	Bio_0	64.3	23.0	50.9	49.7	14.0	42.1
		P_2	VAM	75.8	28.3	60.5	55.8	16.5	47.4
		P_2	PS	87.7	33.7	70.5	65.6	20.4	56.2
		P_2	VAMPS	98.9	42.2	79.7	72.9	24.5	63.2

Table 7. continue (LSD 5%)

Variables	stalk nutrients uptake (kg ha^{-1})			Seeds nutrients uptake (kg ha^{-1})		
$LSD_{0.05}$	N	P	K	N	P	K
FYM	0.30	0.23	0.26	0.45	0.19	0.37
P Types	1.52	0.54	1.21	0.78	0.32	0.87
P rates	0.57	0.32	0.42	0.34	0.16	0.31
Bio	1.41	0.77	1.02	0.84	0.40	0.75
FYM x PT	0.95	0.34	0.76	0.49	0.46	0.55
FYM x PR	0.81	0.45	0.59	0.49	0.23	0.43
FYM x Bio	1.41	0.77	1.02	0.29	0.40	0.75
PT x PR	1.00	0.55	0.72	0.60	0.09	0.17
PT x Bio	1.41	0.77	1.02	0.84	0.40	0.75
PR x Bio	1.41	0.77	1.02	0.84	0.40	0.75
FYM x PT x PR	1.41	0.24	1.02	0.84	0.40	0.75
FYM x PT x Bio	1.41	0.77	1.02	0.84	0.40	0.75
FYM x PR x Bio	1.41	0.77	1.02	0.42	0.20	0.37
PT x PR x Bio	1.41	0.77	1.02	0.84	0.40	0.75
FYM x PT x PR x Bio	0.99	0.77	1.02	0.59	0.28	0.52

See footnotes of Table 5 for treatment designations

Table 8. Effect of the studied treatments on microbial activities in rhizosphere soil of safflower plants

FYM	P Type	P Rates	Bio	Total microbial Counts ($\times10^2$cfu/g D.S)	PDB counts ($\times10^2$cfu/g D.S)	Azotobacter densities ($\times10^3$cells/g D.S)	Ps counts ($\times10^2$cfu/g D.S)	CO_2 mg/100g D.S /24hr
FYM_1		Control		24	3.20	5.90	1.20	4.80
	RP	P_1	Bio_0	30	4.30	6.40	2.10	6.10
		P_1	VAM	70	6.90	8.50	3.70	7.00
		P_1	PS	89	7.00	8.60	2.90	7.10
		P_1	VAMPS	108	7.50	8.90	4.10	7.40
		P_2	Bio_0	31	4.60	6.30	2.20	6.15
		P_2	VAM	87	7.55	8.20	3.85	7.15
		P_2	PS	101	7.95	8.30	3.00	7.55
		P_2	VAPS	127	8.20	8.50	4.35	7.80
	SP	P_1	Bio_0	32	4.40	6.60	2.40	6.40
		P_1	VAM	85	7.20	8.90	4.00	7.50
		P_1	PS	97	7.90	9.10	2.90	7.80
		P_1	VAMPS	128	8.20	9.70	4.20	7.90
		P_2	Bio_0	35	4.80	6.55	2.35	6.60
		P_2	VAM	102	7.60	8.65	4.25	7.65
		P_2	PS	116	8.40	8.75	3.10	7.90
		P_2	VAMPS	147	8.75	9.30	4.50	8.10
FYM_2		Control		28	3.70	6.20	1.60	5.30
	RP	P_1	Bio_0	31	4.75	6.25	2.25	6.18
		P_1	VAM	95	7.88	8.05	3.93	7.23
		P_1	PS	106	8.43	8.15	3.05	7.78
		P_1	VAMPS	136	8.55	8.30	4.48	8.00
		P_2	Bio_0	31	4.90	6.20	2.30	6.20
		P_2	VAM	103	8.20	7.90	4.00	7.30
		P_2	PS	112	8.90	8.00	3.10	8.00
		P_2	VAMPS	145	8.90	8.10	4.60	8.20
	SP	P_1	Bio_0	37	5.00	6.53	2.33	6.70
		P_1	VAM	110	7.80	8.53	4.38	7.73
		P_1	PS	125	8.65	8.58	3.20	7.95
		P_1	VAMPS	156	9.03	9.10	4.65	8.20
		P_2	Bio_0	38	5.20	6.50	2.30	6.80
		P_2	VAM	118	8.00	8.40	4.50	7.80
		P_2	PS	134	8.90	8.40	3.30	8.00
		P_2	VAMPS	165	9.30	8.90	4.80	8.30

Table 8. continue (LSD 5%)

Variables / $LSD_{0.05}$	Total microbial Counts ($\times10^2$ cfu/g D.S)	PDB counts ($\times10^2$cfu/g D.S)	Azotobacter densities ($\times10^3$cells/g D.S)	Ps counts ($\times10^2$cfu/g D.S)	CO_2 mg/100g D.S /24hr
FYM	1.22	0.058	0.019	0.022	0.028
P Types	0.86	0.019	0.030	0.013	0.024
P rates	0.52	0.022	0.011	0.006	0.009
Bio	1.27	0.055	0.026	0.016	0.021
FYM x PT	1.22	0.027	0.019	0.018	0.034
FYM x PR	0.73	0.032	0.015	0.009	0.012
FYM x Bio	1.27	0.055	0.026	0.016	0.021
PT x PR	0.89	0.039	0.006	0.011	0.015
PT x Bio	1.27	0.055	0.026	0.016	0.021
PR x Bio	1.27	0.055	0.026	0.016	0.021
FYM x PT x PR	1.27	0.055	0.008	0.005	0.021
FYM x PT x Bio	1.27	0.055	0.013	0.016	0.021
FYM x PR x Bio	1.27	0.027	0.013	0.008	0.011
PT x PR x Bio	0.63	0.027	0.013	0.008	0.021
FYM x PT x PR x Bio	0.89	0.038	0.018	0.011	0.015

See footnotes of Table 5 for treatment designations

CONCLUSION

Safflower responded positively to application of FYM, superphosphate, rock phosphate and bio fertilizer. Application of VAM fungi and *B. megatherium* increased yield and N, P and K uptake, oil content in seeds and yield. The most effective combination treatment on yield parameters, nutrient content and uptake were RP_2 + (VAM) +FYM_1 < SP_2 + (VAM) + FYM_1 < SP_2 + (PS) + FYM1 < SP_2 + (VAM+PS) + FYM_1< SP_2 + (VAM +PS) + FYM_2. The combination of mineral fertilizers P and bio-fertilizers with farmyard manure gave 6647 kg stalk ha^{-1}, and 3390 kg seeds ha^{-1} and oil content of 360 g oil kg^{-1} seeds. The bilateral, triple and tetra interaction between the studied treatments assured that SP treatment significant increase yield components, nutrients concentrations and uptake of stalk and seeds of safflower plants grown in loamy sand soil in Ras Sudr.

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الملخص العربي

اضافة السماد العضوى ومذيبات الفوسفور الحيوية مع الصخر الفوسفاتى في الأراضى الجيرية لزيادة تيسر الفسفور وانتاجية نبات القرطم

محرم فؤاد عطية، حسن عبد العاطى فاوى، رحاب حلمى حجاب، نهى موسى محمد

اقيمت دراسة حقلية لنبات القرطم بمحطة راس سدر (29º 32'29" N and 32º 39' 27" E) - مركز بحوث الصحراء. كانت ملوحة مياه الرى ٧,٣٦ ديسيمنز/م. وملوحة التربة لمستخلص عجينة التربة المشبعة كانت ٧,٩٦ ديسيمنز/م. زرعت بذور القرطم صنف جيزة ١ فى ١٥ نوفمبر على مسافة ٢٠ سم بين النباتات ٧٠ سم بين الخطوط لعمق ٣ سم وكثافة النباتات كانت ٧,١٤ نبات/م^{٢} (بحيث كانت ٣٠,٠٠٠ نبات/فدان).

الهدف من الدراسة هو تقييم تأثير كل من التسميد العضوى والفوسفور المعدنى(سوبر فوسفات: ١٥,٥% فو٢أ٥ وصخر الفوسفات: ٩,٥٦% فو٢أ٥) مع الاسمدة الحيوية المذيبة للفوسفور على انتاجية وجودة نباتات القرطم النامي فى أرض جيرية ملحية وعلى محتوى الزيت تحت ظروف ملوحة مياه الرى والتربة فى راس سدر - سيناء.

توضح النتائج المتحصل عليها ان القياسات المحصولية وتركيز المغذيات (نيتروجين، فوسفور، بوتاسيوم) والممتص منها بواسطة القش والبذور لنبات القرطم قد زادت مع زيادة معدلات اضافة المادة العضوية والسوبر فوسفات وصخر الفوسفات مع اضافة الاسمدة الحيوية المذيبة للفوسفور Vascular Arbiscular Mychorrhiza (VAM) و B. *megatherium* (PSB) خلال موسمى الدراسة. وقد زادت القياسات المحصولية وتركيز المغذيات والممتص مع التكامل بين المعاملات تحت الدراسة. وتؤكد التفاعلات الثنائية والثلاثية والرباعية بين المعاملات وجود زيادات معنوية لقياسات المحصول وتركيز المغذيات والممتص منها فى كل من القش والبذور. وقد وجد ان معاملات السماد الحيوى (PS) كانت متفوقة لنفس القياسات السابقة عند المقارنة مع السماد الحيوى(VAM). ويمكن ترتيب تأثير التداخل بين المعاملات على القياسات المحصولية وتركيز المغذيات والممتص منها بواسطة القش والبذور لنبات القرطم تصاعديا كالاتى:

$RP_2 + (AV)+FYM_1 < SP_2 + (AV)+ FYM_1 < SP_2+ (PS)+FYM_1 < SP_2 + (AV+PS) + FYM_1 < SP_2 + (AV+PS) + FYM_2$.

وقد أعطى التكامل بين الاسمدة المعدنية والحيوية للفوسفور مع اضافة المادة العضوية المعاملة الاكثر تأثيراً (SP_2+VAM+PS+FYM_2) والتى أدت الى الحصول على اعلى محصول للقرطم حيث كان ٦,٥٩ و٣,٣٦ طن/ هكتار للقش والبذور على التوالى كما كانت نسبة الزيت ٣٦%.