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Effect of Microbial Inoculation and Bentonite Amendments on Growth, Enzyme Activity and Yield of Cowpea Cultivated in Sandy Soil

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A FIELD experiment was conducted twice at the farm of Environmental Studies and Research Institute, Sadat City University, Egypt, during the two consecutive seasons of 2015 and 2016 to study the effect of different additives of bentonite (i.e., 0, 3, 6 and 9 t fed^{-1}), and microbial inoculation with *Bradyrhizobium* sp. and *Azospirillum brasilense* either alone or mixed on vegetative parameters, enzyme activity and yield of cowpea plants. Results revealed that co-inoculation treatment and addition of 9 Mg fed^{-1} bentonite significantly enhanced plant height, number of branches, fresh and dry weight of plant in both seasons compared with the control at 60 days from sowing and this increase reflected on enzyme activity which attained 25.56, 27.66 µmol/ C_2H_4/h/g dry nodules and 50.93, 54.57 µg TPF/g dry soil/h for nitrogenase (N_2-ase) and dehydrogenase in the two growing seasons, respectively. On the other hand, the highest total seed yield of cowpea was recorded from use the co-inoculation with 9 ton ton/fed which was 0.679 and 0.682 Mg fed^{-1} for seed yield and 0.316, 0.324 Mgfed^{-1} for straw yield during 2015 and 2016 seasons, respectively. Consequently, it is to recommend that mixture inoculate with *Bradyrhizobium* sp. and *Azospirillumbrasilense* and addition of 9 Mg fed^{-1} bentonite could be improving crop growth of cowpea and improve sandy soil fertility.

Keywords: Cowpea, Microbial inoculation, Bentonite, Enzyme activity, Yield.

Introduction

Cowpea (*Vignasinensis*) is an individual of fabaceae family and one of the most essential vegetable crops in Egypt. Seeds represent a chief source of protein and carbohydrates content. It globally has a considerable rank in agriculture with about 270 million tons harvested of 12.2 million planted area ha^{-1} as a potential production. In Egypt, Cowpea consider as a main crop, where its planted area is about 0.178 million ha^{-1} with production is about 4.80 million tons (FAO 2013).

Sandy soils are known to have a low productivity due to their poor physical and chemical properties. Incorporation of soil conditioners to these soils was reported to create a favorable environment for plant growth, such as sufficient aeration, suitable retention of water and nutrients as well as restricting the formation of surface crust (Asady et al.1985; Stone,1985).

Likewise, organic manures, composts and bentonite have been used in Egypt as natural soil conditioners which for reclaiming sandy soils. Bentonite is a rock containing mainly 2:1 clay mineral montmorillonite, a member of the semectite family which has been recognized in many countries as a good amendment to improve such infertile sandy soil Benkhelifa et al. (2008). Czaban et al. (2013) found that, the addition of bentonite at rates of 3,6 and 12kg/$m^2$of soil significantly improved soil structure and increased the amounts of nitrogen and organic matter in sandy soils. According to Mi et al. (2017), the addition bentoniteas amendment on soil hydraulic parameters and millet crop performance in a semi-arid region significantly increased above-ground biomass and grain yield and improved water use efficiency. They stated that increases ranges were 2 to 39, 3 to 20 and 0 to 29% for above-ground biomass increases, grain yield and water use efficiency. They add that the application of 24 Mg ha^{-1} bentonite recorded the greatest effect over five years with maximum rate of 30 Mg ha^{-1}

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in the fifth year of the experiment which showed the greatest effect.

An excellent combination of traits useful in disease control and plant growth promoting rhizobacteria (PGPR) was first defined by Kloepper and Schroth (1978) where the soil bacteria colonize the roots of plants. Tilak et al. (2005) documented that PGPRs, belong to diverse genera of *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Enterobacter*, *Erwinia*, *Acinetobacter*, *Bacillus, Burkholderia*, *Flavobacterium, Pseudomonas, Rhizobium* and *Serratia,* are able to exert beneficial effects on plant growth.

According to Xie et al. (2009), Tejada et al. (2011) and Omara et al. (2017), the assessment of soil dehydrogenase activity (DHA) indicated the microbial activity as indicator of soils quality as well as for determining the various factors that affect the microbiological quality of soils such as different application of pollutants like pesticides or excessive fertilizers usage. Also in such context, the determination of dehydrogenase activity DHA in the soil samples could potentially give us useful and large amount of information about biological characteristic of the soil because it is influenced by various other factors present in the soil environment, like moisture, availability of oxygen, reduction-oxidation potential, pH, content of organic matter, the soil profile depth, temperature, season of the year and heavy metal contamination (Wolińska and Stępniewska 2012).

Therefore, the objectives of the current study were to investigate the effect of three levels of bentonite and microbial inoculation either alone or mixed on cowpea vegetative growth characteristics, enzyme activity and yield.

Materials and Methods

Two field experiments were executed at the farm of Environmental Studies and Research, Institute, Sadat City University during the two consecutive summer seasons of 2015 and 2016, under system of drip irrigation, to study the effect of bentonite rate amendments (0,3,6 and 9 Mg/fed) as a soil conditioner and bacterial inoculation *Bradrhizobium* sp. and *Azospirillumbasilenes* either alone or mixed on vegetative growth, yield and microbial activity in the rhizosphere of cowpea cv. Kareem 7 under sandy soil conditions.

The site is located (Latitude 30°2′41.185″N; Longitude 31°14′8.1625″E), which is characterized by a semi-arid climate with moderate cold winters and warm summers. Physical and chemical properties of experiment soil used in experiment were determined according to the methods of Page et al. (1982) and their data are presented in Table 1. The experiment was performed in a spilt plots design in three replications.

TABLE 1. Analytical data of the soil used.

Physical properties					
$CaCO_3$ (g 100 g^{-1})	Organic matter (g 100 g^{-1})	Particle size (%)			Texture class
		Sand	Silt	Clay	
19.0	3.0	88.6	4.8	6.6	Sandy

Chemical Properties									
PH*	EC** dS m^{-1}	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	K^+	Na^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
7.63	1.82	0.36	0.32	0.14	0.56	-	0.41	0.36	0.61

*In the 1:2.5 Soil: water suspension. **In the soil paste extract.

Main plot consists of four levels of bentonite amendment (0, 3, 6 and 9 Mg/fed.), the bentonite had a chemical composition on a weight basis of : 73.25% SiO_2, 11.4% Al_2O_3, 0.31% Na_2O,2.67% CaO,1.05% MgO,2.58% K_2O,0.29% Fe_2O_3 and the practical size was 75 µm. Bentonite was adding before planting, while bacterial inoculation in subplots with un-inoculated (control), *Bradrhizobium* sp, *A. basilenes* and co-inoculation. Seeds were treated with microbial inoculant suspension before sowing for one hour and shade dried for one hour. Seeds were planted during 14th of April in the two years in plot area 9.0m^2.

All treatments received the recommended dose of superphosphate (15.5% P_2O_5) at a rate of 200 kg / fed., potassium sulfate (48% K_2O) at a rate of 50 kg/fed before sowing (as one dose). Nitrogen fertilizer application added as a form of ammonium nitrate (33.5% N) at rate 60 Kg/fed (full dose) to control only. The rest of the treatments added half of the recommended dose of nitrogen fertilizer. Added at two equal doses, the first at three weeks after sowing, the second after 15 from the previous one.

Data recorded

Vegetative growth character

Three plants were taken randomly from each replicate at flowering (60 days after sowing) to measure the following vegetative characters i.e. plant height (cm), number of branches, fresh and dry weight/plant (g).

Yield component

Weight of 100 seed, seed yield (ton/fed) and straw yield (ton/fed) were determined.

Enzyme activities

Fresh samples (soil and roots) were collected to determine enzyme activities.

- Dehydrogenase activity (DHA) was determined as described in Friedel et al. (1994) after soil incubation at 37°C for 24h with 2,3,5-triphenyl-tetrazolium chloride (TTC) and the red color of triphenyl formazan (TPF) absorbance was measured at 546 nm using spectrophotometer.

- Nitrogenase activity (N_2ase) was measured according to (Turner and Gibson, 1980).

Statistical analysis

All recorded data were subjected to ANOVA to identify significant treatments and/or interaction effects by 'F test' using the SAS program (SAS Systems for Windows, release 9.2, SAS Institute, Cary, NC, SAS, 2003). Mean separation between the significant treatments was calculated by L.S.D.

Results and Discussion

Vegetative parameters of cowpea plants

Data in Table, 2 and 3 show the effect of bentonite amendment and bacterial inoculation and their interaction on plant height, number of branches and fresh and dry weight/plant. All the studied vegetative growth characters showed significantly increases with adding bentonite level up to 9 t fed^{-1} which scored significantly highest increase with all tested parameters. The highest level 9 t/fed scored the tallest plants in both seasons but they were non-significant with using 6 t fed^{-1}. Also, the highest level of bentonite amendment gave the high number of branches and weight of plants compared with zero amendment.

In this respect, Shalaby et al. (2005) found that, application of soil conditioners amendments such as chicken manure at rate of 10 m^3 fed^{-1} bentonite at rate 8 ton fed^{-1} and iron ore at rate of 200 Kg fed^{-1} combined with mineral fertilizers at rates 75kg fed^{-1} N, 22kg fed^{-1} P_2O_5 and 72kg fed^{-1}K_2O gave the highest vegetative growth components including plant height, number of

TABLE 2. Effect of bentonite amendments and microbial inoculation on some vegetative growth of cowpea cultivated in sandy soil.

Treatments	Plant height (cm)		No.branches/plant		Fresh weight (g/plant)		Dry weight (g/plant)	
Bentonite rates(ton/fed.)	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
0	66.61 c	67.75 b	2.83 c	3.04 b	125.87d	118.85d	56.74 d	61.30 d
3	71.72 b	67.70 b	3.08 b	3.52 a	144.19c	148.19c	66.18 c	67.05 c
6	74.64 a	75.23 ab	3.27ab	3.35 a	163.97b	173.23b	76.34 b	78.41 b
9	75.93 a	76.53 a	3.46 a	3.56 a	181.23a	189.07a	80.51 a	83.64 a
LSD at 5%	2.18	8.47	3.16	0.237	7.49	6.43	2.45	2.61
Biofertilizers	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Control	69.66 c	70.19 c	2.68 d	2.77 d	128.65 c	129.62c	60.53 c	61.38 c
B	71.45 b	72.60 b	3.03 c	3.08 c	148.63 b	157.02b	66.78 b	68.73 b
A	73.96 a	74.75 a	3.15 b	3.36 b	152.32 b	161.71b	68.72 ab	70.36 ab
Mixture	70.03 bc	71.15 b	3.38 a	3.47 a	174.15 a	182.81a	70.57 a	72.62 a
LSD at 5%	1.526	1.239	0.079	0.0608	6.634	4.908	2.448	2.514

In a column means followed by a common letter are not significantly different at 5% level by DMRT. **B**: inoculation with *Bradyrhizobium* sp.; **A**: inoculation with *Azospirillum basilenes* branches per plant**.**

Concerning the effect of microbial inoculant results in Table 2 exhibited that, mixture inoculation was significantly increased number of branches and fresh and dry weight of plant compared to other treatment, *Azospirillum* inoculum scored tallest plants. On the other hand, the lowest recorded of the previous characters achieved from un-inoculated control. Similar results were reported by Hungria et al. *(*2010) for maize and wheat crops individually inoculated with *Azospirillum* strains which recorded an increase ranged from 24 to 30%and 13–18% in grain yields of maize and wheat as compared with non-inoculated control. In field experiments, the yield of maize and wheat raised up by 27 and 31%, respectively by liquid and peat-based inoculants carrying a combination of *A. brasilense* strains. Such effects, duo to inoculation, could be attributed to general increments in uptake of various macro and micronutrients rather than to biologically fixed nitrogen. According to Turan et al. (2014), treated cabbage seedlings with PGPRs stimulate the growth in terms of fresh and dry weights of shoot and root, stem diameter, seedling height, chlorophyll values and leaf are as eedlings as compared with the un-inoculated control. Also, Ojeda-Quintana et al. (2016) found that inoculation of *Megathyrsusmaximus* seeds with *Azospirillumlipoferum* increased dry matter. They concluded that this biopreparation can be an alternative to mineral fertilization.

Data of Table 3 represent the interaction effect between bentonite amendments and bacterial inoculation on plant height, number of branches, fresh and dry weight of cowpea plants cultivated in sandy soil. There were significant interactions between treatments for above mentions traits. The highest characters obtained with adding 9 ton bentonite with mixture inoculation compared to individual inoculation by *bradyrhizobium* or *Azospirillum*. The promotive effect of mixture inoculation demonstrated by many investigators (El-Howeity 2008 and El-Howeity et al. 2009)

TABLE 3. Effect of interaction between bentonite amendments and microbial inoculationon some vegetative growth of cowpea cultivated in sandy soil.

Treatments	Bio.	Plant height (cm)		No. branches/plant		Fresh weight (g/plant)		Dry weight (g/plant)	
Bentonite (ton/fed.)		1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
0	C	59.22l	65.52i	2.75g	2.77h	109.89i	118.90g	54.04i	57.75j
	B	69.45j	70.91h	2.69g	2.75h	126.04g	132.19f	59.11i	62.69hi
	A	74.87ehfg	76.75ef	2.85fg	3.04g	160.15e	165.27d	59.29h	61.72i
	M	72.71ihg	75.98efg	3.03f	3.08g	148.63f	157.02e	65.02fg	68.94de
3	C	66.33 k	66.66i	2.66g	2.85gh	118.79h	128.56f	57.77h	63.60ghi
	B	74.41ihfg	77.94de	3.32e	3.36f	159.97e	164.86d	63.60g	65.55fg
	A	76.34efd	78.23edc	3.62d	3.65e	162.40e	164.44d	63.60g	64.45gh
	M	79.86ab	80.64b	3.68cd	3.80ed	173.93d	180.52c	65.76efg	67.52ef
6	C	72.22i	74.26g	3.78bcd	3.85cde	179.34c	188.40b	68.77bcd	70.41cd
	B	72.56ih	75.11fg	3.55ed	3.80ed	174.78d	179.67c	68.34cde	70.29cd
	A	75.97ef	77.56de	3.71cd	3.89cde	179.30c	181.48c	66.53def	69.17de
	M	78.44bcd	80.33cb	4.03ab	4.14ab	185.15b	188.34b	71.15ab	73.42b
9	C	75.04efg	77.75de	3.74cd	3.95bcd	182.78bc	189.33b	69.51bc	70.74bcd
	B	77.15ecd	79.85bcd	3.89bc	4.04abc	184.42b	189.53b	70.69abc	72.54bc
	A	79.28bc	81.23b	4.02ab	4.10abc	186.85ab	190.84b	69.77bc	72.78bc
	M	82.11a	83.77a	4.20a	4.28a	190.70a	201.86a	72.84a	77.47a
LSD at 5%		2.443	2.294	0.274	0.245	4.461	4.839	2.618	2.704

In a column means followed by a common letter are not significantly different at 5% level by DMRT. **C:** Control; **B**: inoculation with *Bradyrhizobium* sp.; **A**: inoculation with *Azospirillum basilenes;* **M**: Mixture.

Considering the mixture incubation, Parmar and Dufresne (2011) mentioned that the positive effect of combined inoculation of *Azotobacter*and *Rhizobium* sp. showed significantly improve the nodulation status. The synergistic interactions between host and *Azotobacter* sp. was enhanced due to increasing nitrogen content in roots and shoots of respiring/metabolizing plant cells which improves various conditions within the rhizosphere. In open field conditions, the improvement of growth yields in various soil mineral compositions was shown by both *Azotobacter* and *Azospirillum* which declare a mutualistic relationship established between *Azotobacter* and *Azospirillum* to interact with *Rhizobium* leading to enhancement in chick pea yields. Also, Mishra et al. (2012) pointed out that, co-inoculation of PGPRs with *Rhizobium leguminosarum* significantly increased nodulation and plant biomass by 156.2 and 57.1%, respectively. Furthermore, co-inoculation enhanced total chlorophyll content, available iron, total iron, leghaemoglobin, N, P and K uptake of shoots by 31.5, 106.7,95.9, 17.5, 66.3, 23.3 and 47.1%, respectively, as compared with uninoculated control.

Nitrogenase and dehydrogenase activities

Nitrogen fixation (N_2-ase) of cowpea root nodules and dehydrogenase activity in the rhizosphere as affected by bentonite amendments and microbial inoculation and their interactions are illustrated in Fig. 1,2 and 3. Figure 1 showed significant increases with increasing bentonite amendments which stimulated microbial activity in soil, the highest activity of N_2-ase and DHA were found by adding 9t / fed in two seasons. It recorded 15.65 and 16.13 µmol/ C_2H_4/h/g dry nodules for N_2-ase and 38.07and 40.40 µg TPF/g dry soil/h for DHA, during 2015 and 2016 growing seasons, respectively. On the other hand, the lowest values were scored with control (0 bentonite). Incorporation bentonite improved physical and chemical soil properties. Results illustrated in Fig. 3 show the relationship between incorporation bentonite and biofertilizers with microbial activities DHA and N_2-ase activity

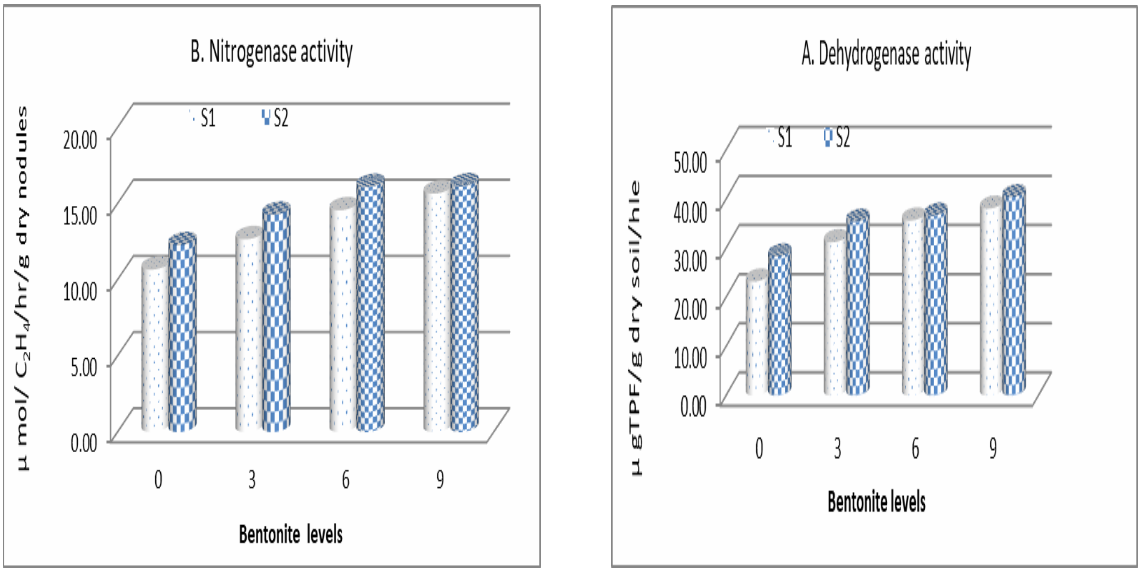

Fig. 1. Effect of bentonite amendments on DHA in soil (A), and N_2-ase activity in root nodules of cowpea plants (B).

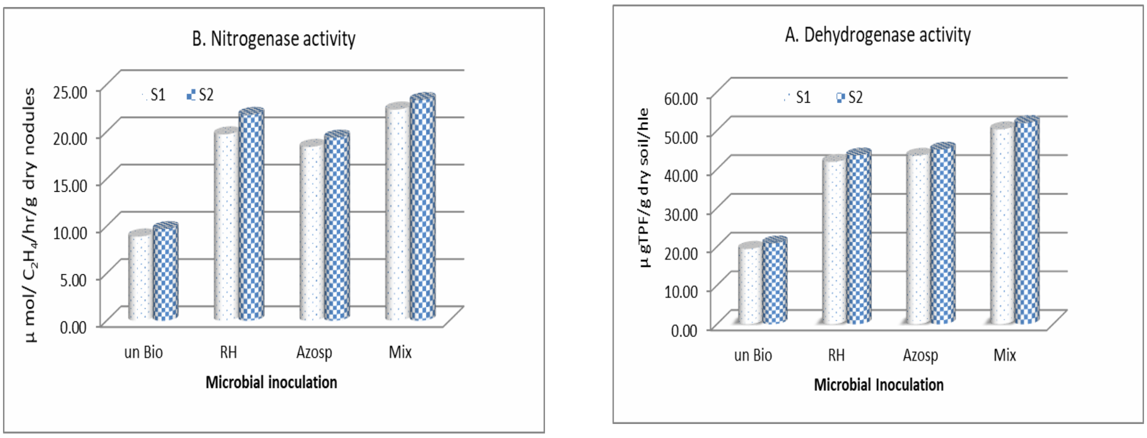

Fig. 2. Effect of microbial inoculation on DHA in soil (A), and N_2-ase activity in root nodules of cowpea plants (B). RH: inoculation with *Bradyrhizobium* sp.; Azosp: inoculation with *Azospirillum basilenes*.

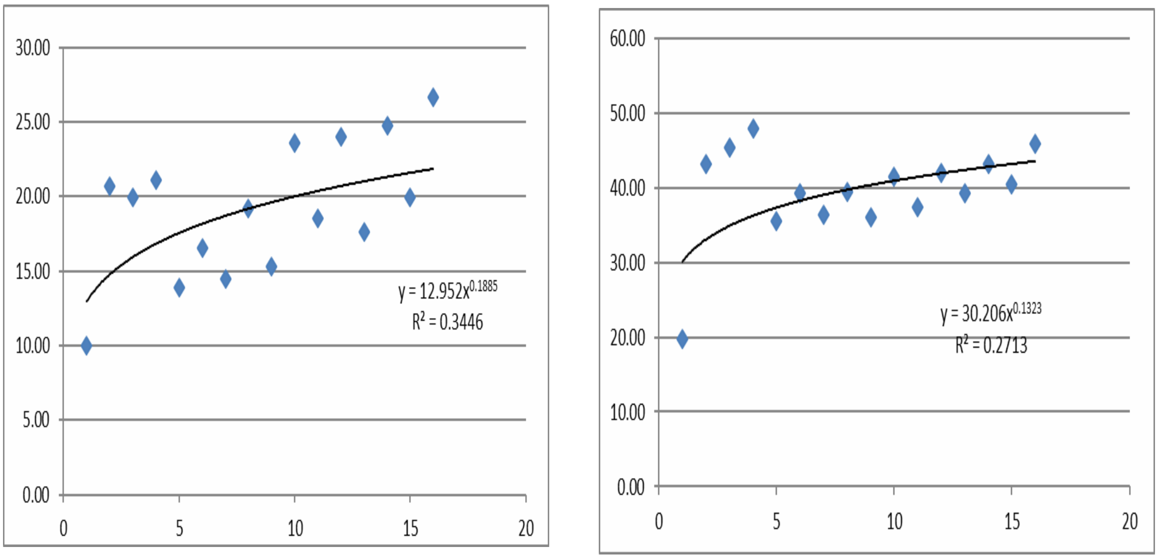

Fig. 3. Effect of interaction between treatments on DHA activity in the rhizosphere of cowpea plants (A); and N_2-ase activity in root nodules of cowpea plants (B).

in the rhizosphere. The obtained coefficients of determination (R^2) were liner relationships where R^2 values ranging 0.27 and 0.34 with DHA and N_2-ase activity, respectively.

These results are in harmony with those obtained by Umer and Rajab (2012) were found that, application of organic matter to soil enhancement soil aggregate and caused positive correlation between soil respirations, microbial biomass with soil aggregate stability. Also, Mi et al. (2017) show bentonite amendments significantly increased above-ground biomass from 2 to 39% and grain yield from 3 to 20%, as well as improved water use efficiency 29 % through five years.

Concerning the effect of microbial inoculation on enzyme activities are presented in Fig. 2, inoculation by *bradyrhizobium* sp. or *Azospirillum* significantly increased soil enzyme activity. The highest activity was significantly with the treatment co-inoculation compared to single inoculation and uninoculated. Increases above control with co-inoculation were 150.4, 140.86, 158.76 and 149.35 % for N_2-ase and DHA activity in two seasons, respectively. Concerning the effect of interaction between bentonite and biofertilizers, there were significant interactions between treatments for two enzyme activity. The best interaction was found with co-inoculation and 9 ton bentonite amendment, which gave 25.56, 27.66µmol/ C_2H_4/h/g dry nodules and 50.93, 54.57 µgTPF/g dry soil/h for N_2- ase and DHA in two seasons, respectively. Also, Fig. 3 shows the correlation positive between soil amendment and enzyme activity in the rhizosphere. Similar results were also reported by Tallai (2011) who studied the effect of bentonite and zeolite on microbial activity in sandy soil. Who found that, adding bentonite at rate 10 t/ha^{-1} and zeolite at rate 5 t/ha^{-1} increased total microbial count and stimulated enzyme activities, urease, phosphatase in sandy soil. Jarvan et al. (2014) showed that addition of organic matter increased DHA in soil by 22.7% over control. In this concern, El-Howeity and Abdel Gawad (2017) found that, mixed inoculation with Bradyrhizobium and cyanobacteria improved dehydrogenase activity in the rhizosphere of soybean plants under field conditions.

Yield of cowpea plants

The response of cowpea yield and its attributes to bentonite amendments and microbial inoculation by *Bradyrhizibiom* sp. or *Azospirillum* sp. and co-inoculation are illustrated in Tables 4 and 5. Results showed that cowpea seed and straw yield and 100 seed weight were significantly affected by bentonite amendment. Increasing bentonite rates from 3 to 6 up to 9 t/fed caused

TABLE 4. Effect of bentonite amendments and microbial inoculation on yield of cowpea cultivated in sandy soil.

Straw yield (Mg/fed)		Seed yield (Mg/fed)		100 seed weight		Treatments
2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	Bentonite rates (ton/fed.)
0.208 b	0.203 b	0.387 d	0.381 d	15.46 d	15.37 d	**0**
0.243 a	0.229 a	0.460 c	0.451 c	15.89 c	15.75 c	**3**
0.269 a	0.262 a	0.567 b	0.562 b	16.60 b	16.29 b	**6**
0.246 a	0.262 a	0.682 a	0.667 a	17.03 a	16.88 a	**9**
0.0284	0.0592	0.0191	0.0171	0.217	0.283	**LSD at 5%**
2nd Season	**1st Season**	**2nd Season**	**1st Season**	**2nd Season**	**1st Season**	**Biofertilizers**
0.207 b	0.203 b	0.396 b	0.390 b	15.48 c	15.31 c	**Control**
0.235 a	0.210 a	0.428 a	0.403 ab	16.41 a	15.96 ab	**B**
0.235 a	0.210 a	0.416 a	0.402 ab	15.79 cb	15.63 cb	**A**
0.238 a	0.225 a	0.431 a	0.414 a	16.21 ab	16.11 a	**Mixture**
0.012	0.061	0.0.172	.0199	0.572	0.457	**LSD at 5%**

In a column means followed by a common letter are not significantly different at 5% level by DMRT. **B**: inoculation with *Bradyrhizobium* sp.; **A**: inoculation with *Azospirillum basilenes*.

TABLE 5. Effect of interaction between bentonite amendments and microbial inoculation on yield of cowpea cultivated in sandy soil.

Straw yield (Mg/fed)		Seed yield (Mg/fed)		100 seed weight		Bio.	Treatments
2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season		Bentonite (ton/fed.)
0.187 g	0.219 d	0.387 j	0.381 i	15.48 h	15.31 h	**C**	**0**
0.235 f	0.210 d	0.428 i	0.409 hi	15.59 h	15.53 gh	**B**	
0.235 f	0.210 d	0.420 i	0.407 hi	15.63 h	15.61 g	**A**	
0.245 ef	0.222 d	0.429 i	0.416 hi	16.17 f	16.02 f	**M**	
0.243 ef	0.229 cd	0.460 h	0.451 gh	15.89 g	15.75 g	**C**	**3**
0.289 bcd	0.285 ab	0.552 f	0.515 f	16.36 f	16.04 ef	**B**	
0.300 abc	0.286 ab	0.510 g	0.500 gf	16.24 f	16.00 f	**A**	
0.315 abc	0.310 a	0.573 e	0.569 cd	16.66 e	16.26 ed	**M**	
0.269 de	0.262 bc	0.567 ef	0.562 ed	16.74 e	16.30 cd	**C**	**6**
0.303 abc	0.304 a	0.601 d	0.586 cd	17.07 d	16.91 b	**B**	
0.290 bcd	0.286 ab	0.592 d	0.513 f	16.86 de	16.52 c	**A**	
0.308 abc	0.308 a	0.682 a	0.667 a	17.65 b	17.40 a	**M**	
0.246 ef	0.262 bc	0.628 c	0.617 bc	17.03 d	16.88 b	**C**	**9**
0.316 ab	0.308 a	0.662 b	0.652 ab	17.35 c	17.10 b	**B**	
0.288 bcd	0.313 a	0.662 b	0.659 ab	17.32 c	17.01 b	**A**	
0.324 a	0.316 a	0.682 a	0.679 a	18.02 a	17.39 a	**M**	
0.0283	o.0335	0.0174	0.048	0.241	0.246		**LSD at 5%**

In a column means followed by a common letter are not significantly different at 5% level by DMRT. **C:** Control; **B**: inoculation with *Bradyrhizobium* sp.; **A**: inoculation with *Azospirillum basilenes;* **M**: Mixture

significant increases in seed yield (t/fed) and 100 seed weight. In the other hand, increases in straw yield were significant compared to control but insignificant increases between bentonite levels.

Increases in seed yield may be introduction bentonite to soil improve physical, chemical soil properties for cowpea plants and bentonite contain organic matter 1.12%, total nitrogen 0.064% and other trace elements (Table 1) which enhancement plant growth and increase the yield of cowpea. These results are in harmony with those obtained by Junzhen et al. (2017) mentioned that, adding bentonite increased plant growth and grain yield. In this respect, Arafa et al. (2015) found that, application of bentonite at rate 12 ton/fed significantly increased all vegetative growth characters and yield of cowpea cultivars grown in sandy soil.

The response of cowpea yield and its attributes to inoculation with *Bradyrhizobium* and *Azospirillum*individually and co-inoculation is listed in Table 4. Results showed that cowpea yield and its attributes were significantly affected by microbial inoculation. Co-inoculation with *Bradyrhizobium* and *Azospirillum* caused highly significant increases in seed yield (ton/fed), straw yield (ton /fed) and 100 seed weight(g) in the 2^{nd} season. Results of the interaction effect between bentonite amendment and microbial inoculation on the above characters of cowpea are presented in Table 5. The highest total seed yield of cowpea was recorded from use the co-inoculation with 9Mg/fed which was 0.679, 0.682, 0.316, 0.324 Mg/fed for seed and straw yield in the two growing seasons, respectively. However, these increases were pronounced with adding 6 ton bentonite with co-inoculation gave 0.667, 0.682, 0.308 and 0.308 Mg/fed for seed and straw yield in two seasons, respectively. In this concern, El- Howeity (2008) found fresh and dry weights of shoot and root of phaseolus plants as well as improvement the ability for water absorption increased due to *Azospirillum* inoculation. Zahir (2011) referred that co-inoculation increased lentil growth, nodulation and yield compared to single inoculation under pot and field conditions. Also, El-Howeity (2012) found that the application of yeast and Aminokem as bio-organic amendments with or without mineral fertilizers showed increases in N, P, K contents and Ca in leaves of Valencia orange seedlings. Abdel-Aziz (2013) reveled that using microbial inoculation with *Azospirillum* sp., *Trichoderma* sp. *Bacillus megaterium* and *Pseudomonas fluorescens* as mixtures enhanced cowpea yield and its attributes compared to single inoculation and control. This result agreed with the study of Chatterjee and Bandyopadhyay (2017) in that the inoculation of cowpea with (*Rhizobium* + PSB) significantly increased the growth and yield compared to uninoculated plants.

Conclusion

Microbial inoculation with mixture of *Bradyrhizobium* and *Azospirillum* as well as addition of 9 ton/fed bentonite as a soil amendment significantly increased vegetative growth, enzymes activity and yield of cowpea plants cultivated in sandy soil in both growing seasons compared to control. The over-all results of the present study suggest that microbial inoculation and bentonite amendment could be improving crop growth and improve sandy soil fertility.

References

Abdel Aziz M.A. and Salem M.F. (2013) Effect of microbial inoculation on reduction of cowpea chemical fertilizers under newly reclaimed soils condition in Egypt. *J. Plant Production, Mansoura Univ.*, **4**(5), 745-761.

Arafa M.M., Darwish W.M. and El-Howeity M.A. (2015) Effect of water use efficiency and bentonite levels on growth, yield and chemical composition of seeds for cowpea (*Vignaunguiculata* L.) cultivars grown in sandy soil. *Annals of Agric. Sci. Moshtohor*, **53**(4), 667-678.

Asady G.H., Smucker A.J.M. and Adams M.W. (1985) Seedling test for the quantitative measurement of root tolerances to compacted soil. *Crop Sci.*, **25**, 802-1985.

Benkhelifa M., Belkhodje M., Daoud Y. and Tessier D. (2008)Effect of Maghnian bentonite on physical properties of sandy soils under semi-arid Meditrranean climate.*Pak. J. Biol. Sci.*, **11**(1), 17-25.

Chatterjee R. and Bandyopadhyay S. (2017)Effect of boron, molybdenum and biofertilizers on growth and yield of cowpea (*Vignaunguiculate* L. Walp.) in acid soil of eastern Himalayan region. *J. of the Saudi Soc. of Agric. Sci*., **16**, 332-336.

El-Howeity M.A. (2008)Diazotrophy and growth of beans (*Phaseolus vulgaris*) genotypes inoculated with rhizobia and lactic acid bacteria. *Minufiya J. Agric. Res.*, **33**(1), 211-230.

El-Howeity M.A. (2012)Utilization of bio-organic compounds and mineral fertilizer to improve growth, nutrient content in leaves of Valencia orange seedlings grown in desert land. *Egypt. J. Hort.*, **39**(1), 31-43.

El-Howeity M.A. and Abdel-Gawad Sh. A. (2017) Response of soybean plants to inoculation with rhizobia and cyanobacteria. *Menoufia J. Soil Sci.*, **2**, 135 – 144.

El-Howeity M.A., Abdalla A.A., Abo-Kora H. A.

and El-Shinnawi M.M. (2009) Response of faba bean plants to inoculation with *Rhizobium leguminosarium* and other Rhizobacteria under three nitrogen levels in newly reclaimed soil. *J. Agric. Sci. Mansoura Univ.*, **34**(6), 7259-7272.

FAO (2013) Agriculture Rome, (2015) Available in: http: //faostat3. fao.org /faostat /collections? Subset=agriculture. Accessed at: June. 96, 9, 1275–1284.

Friedel J.K., Molter K. and Fischer, W.R. (1994) Comparison and improvement of methods for determining soil dehydrogenase activity by using triphenyltetrazolium chloride and iodo nitro tetrazolium chloride. *Biol. Fertil. Soils*, **18**, 291-296

Gzaban J., Siebielec G., Czyz E. and Niedzwieck J. (2013) Effect of bentonite addition on sandy soil chemistry in a long –term plot Experiment (1): effect on organic carbon and total nitrogen. *Pol. J. Environ. stud.*, **22**(6), 1661- 1667.

Hungria M., Campo R.J., Souza E.M., Pedrosa F.O. (2010) Inoculation with selected strains of *Azospirillumbrasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. *Plant Soil*, **331**,413–425

Järvan M., Edesi L., Adamson A., Võsa T. (2014) Soil microbial communities and dehydrogenase activity depending on farming systems. *Plant Soil Environ.,* **60** (10), 459–463.

Junzhen Mi., Edward G., Shengtao, Xu. and Neil M. (2017) Effect of bentonite amendment on soil hydraulic parameters and millet crop performance in a semi-arid region. *Field Crops Research*, **212**,107-114.

Kloepper j.andSchroth M.N. (1978). Plant growth-promoting rhizobacteria on radishes. Pages 879-882 in *Proc. IV[th] international conference on plant pathogenic bacteria .2, Angers,* France.

Mi J., Edward G., GregorichShengtaoXu., McLaughlin N.B., Ma B., Liu J. (2017) Effect of bentonite amendment on soil hydraulic parameters and millet crop performance in a semi-arid region. *Field Crops Research*, **22**, 107-114.

Mishra P.K., Bisht S.B., Mishra S., Selvakumar G., Bisht J.K., and Gupta H.S. (2012) Coinoculation of *Rhizobium Leguminosarum* Pr1 With A cold tolerant *Pseudomonas* Sp. improves iron acquisition, nutrient uptake and growth of field pea (*Pisumsativum* L.). *J. of Plant Nutrition*, **35**, 243–256.

Ojeda-Quintana L.J., Toledo-Vazquez L., Hernández-Rodríguez C., Machado-DíazY..and Gómez E.F. (2016). The influence of application of *Azospirillumlipoferum* in Megathyrsusmaximusvc. guineatobiatá in Pardo Grisáceo soil. *Pastos y Forrajes,* **39** (1),27-32.

Omara A., Hauka F., Afify A., Nour El-Din M., Kassem M. (2017) The Role of Some PGPR Strains to BiocontrolRhizoctoniaSolani in Soybean and Enhancement The Growth Dynamics and Seed Yield. *Environ. Biodiv.& Soil Security,* **1**, 47 – 59.

Page, A.L., Miller R.H. and Keeney D.R. (1982) Methods of soil analysis; 2.Chemical and microbiological properties, 2.Aufl. 1184 S., American Soc. of Agronomy (Publ.), Madison, Wisconsin, USA, gebunden 36 Dollar.

ParmarN., and Dufresne J. (2011) Beneficial Interactions of Plant Growth Promoting Rhizosphere Microorganisms. In: A. Singh et al. (eds.), *Bioaugmentation, Biostimulation and Biocontrol.* Springer Heidelberg Dordrecht London New York.

Shalabey O.E., Araf, M.M. and Bayourai N.A. (2005) Utilization some soil amendments a source of micronutrients in soil and their effect on yield of cowpea plants (*Vignaunguiculata* L.) in newly reclaimed soil. *Annals of Agric. Sc., Moshtohor*, **43**(3), 1357-1373.

Stone J.A. (1985). Poorly drained conditions and root development of eight indeterminate soybean cultivars. *Agron.J.*, **77**,787-789.

Tallai M. (2011). Effect of bentonite and zeolite on characteristics and change of microbial activity of acidic humic sandy soil. *Pol. J. Environ. Stud.,* **26**, 1-8.

Tejada, M., Gomez, I., Garcia-Martinez A., Osta P. and Parado J. (2011)Effects of prochloraz fungicide on soil enzymatic activities and bacterial communities. *Ecotoxicology & Environmental Safety*, **74**, 1708-1714.

Tilak K. V., Ranganayaki, B. R., Saxena, A. K.,Nautiya C. S., Shilpi M., Tripathi A. K. and Johri B. N. (2005). Diversity of plant growth and soil health supporting bacteria. *Current Science*, **89**(1), 126-149.

Turan M., Melek E., Ertan Y., Adem G., Kenan K., Recep K., Atilla D. (2014). Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. *Turk. J. Agric. For.*, **38**: 327-333.

Turner G.L. and Gibson A.H. (1980) Measurement of nitrogen fixation by indirect means. Pp. 111–138 in *'Methods for Evaluating Biological Nitrogen Fixation',* ed by F.J. Bergersen. John Wiley & Sons Ltd: Chichester, New York.

Umer M. I. and Rajab S.M. (2012) Correlation between aggregate stability and microbiological activity in two Russian soil types. *Eurasian J. Soil Sci.*, **6**, 45-50.

Wolińska A. and Stępniewska Z. (2011) Microorganisms abundance and dehydrogenase activity as a consequence of soil reoxidation process, In: *Soil Tillage & Microbial Activities,* M. Miransari, (Ed.), 111-143, Research Singpost, Kerala, India.

Xie W., Zhou J., Wang H., Chen X., Lu Z., Yu J. and Chen X. (2009) Short-Term Effects of Copper, Cadmium and Cypermethrin On Dehydrogenase Activity and Microbial Functional Diversity. In Soils After Long-Term Mineral or Organic Fertilization. *Agriculture, Ecosystems & Environment,* **129**, 450-456.

Zahir A. Z., Zafar-ul-Hye M., Sajjad S. and Naveed M. (2011) Comparative effectiveness of Pseudomonas and *Serratia* sp. containing ACC-deaminase for coinoculation with *Rhizobium leguminosarum* to improve growth, nodulation, and yield of lentil. *Biol Fertil. Soils*.**47**, 457–465.

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