Effect of Organic and Bio-fertilizers on Phosphorus and Some Micronutrients Availability in a Calcareous Soil

Nader R. Habashy, Amal W. Abou El-Khair and Raafat N. Zaki

Soil, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt.

Abstract: Poor performance of particularly phosphorus and micronutrients availability is one of the major factors related to crop productivity in the soil calcareous in nature. This investigation was, therefore, carried out on a calcareous sand clay loam soil under field conditions of Bangar El-Sukar area, El Nubariya region, Egypt to evaluate the efficiency of organic manure (composted rice straw) and biofertilizer (arbuscular mycorrhizal fungi, AM) for improving phosphorus (P) and some micronutrients availability in a calcareous soil cultivated with maize. To achieve this target, P was applied at the rates of 0 (P₀, inert phosphorus content), 15 (P₁) and 30 (P₂) kg P₂O₅/fed, in combination with mycorrhiza inoculum (AM multiplied in peat : vermiculite : perlit, 1:1:1) or organic compost at the rate of 10 ton/fed added as solely or combined treatments. All applied treatments received the recommended doses of nitrogen and potassium fertilizers. The results obtained clearly show that the combined treatment of (AM + organic compost) significantly increased the released P and micronutrients in the soil. This may be due to lowering soil pH and favourable air-water balance, which considerably showed a positive reflection on maize yield. This beneficial effect was more pronounced for the combined treatment in case of applying 15 kg P₂O₅/fed plus AM inoculum, with insignificant difference for the applied rate of 30 kg P₂O₅/fed. That was true, since the pronounced increases in mycorrhizal root colonization percent and number of spores/g soil led to easily mobility of phosphorus and micronutrients, and then their uptake by maize plants as compared to the control treatment. Moreover, the data obtained reveal that integrated effect of AM fungi in combination with organic compost was the best treatment as compared with the solely ones, such finding is emphasized by phosphorus recovery.

Key words: Arbuscular mycorrhizal fungi (AM infection), organic compost, phosphorus, micronutrients and calcareous soil.

INTRODUCTION

The role of arbuscular mycorrhiza (AM) in the acquisition of nutrients from the soil has been long recognized and well documented. Particularly with regard to the more immobile plant nutrition in soil, such as P, Zn, and Cu, however, the role of AM is often essential^[8,31]. Three principal mechanisms have been postulated as being responsible for the effect of AM, i.e., i) the greater exploitation of the soil volume by the hyphae network, thus reducing the diffusion pathway by extending the active absorption surface and enabling access to sites normally not penetrable by roots^[19,17]; ii) the higher affinity of hyphae fore phosphate as expressed in the Michaelis-Menten equation by a lower Km-value[9] and the ability to absorb P at lower solution concentrations than the root themselves, as expressed by a lower Cm-value[16] and iii) changes in the rhizosphere by AM, such as exudation of acids[40] or chelates[11]. Several factors

influence the sorption of P by components of soils and sediments. These include the amount and nature of the soil components involved, other ions, pH of system, time of reaction, concentrations of P, ionic strength of the background solution, solid: solution ratio, and organic compounds^[23]. Besides other factors, lower soil P may be one of the reasons for poor harvests since 90 % of the Egyptian soils suffer from moderate to severe P deficiency^[3,4]. Phosphorus fertilization is, therefore, very essential; for exploiting maximum yield potentials of different crop plants[38] Arbuscular mycorrhizal fungi occur in most soils and from a symbiotic relationship with the roots of most cultivated crops. Such relationship helps plants acquire mineral nutrients. In soils where some elements may be deficient or otherwise less available, i.e., phosphorus, mycorrhizal fungi increased efficiency of mineral uptake, resulting in enhanced plant growth^[45]. Mohmoud et al.^[30] and Pacovsky et al.[35] concluded that inoculation with mycorrhizal significantly increased plant N and P uptake. Kawaia and Yamamoto^[22] added that mycorrhizal plant had higher uptake of P in their stems and leaves than thats of non-mycorrhizal ones.

Habashy and Abo-Zide^[18] showed that the availability of micronutrients (Fe, Mn and Zn) was positively affected by inoculation with AM fungi when compared to the uninoculated treatments. DTPA extractable Fe and Mn were slightly affected by AMfungi inoculation than that uninoculated one. In addition, the DTPA extractable Zn was also increased in the soil treated with AM.

In general, majority of the calcareous soils showed poor hydrophysical and fertility characteristics. So addition of organic materials is of vital importance to improve physical and chemical characteristics as well as fertility status of these soils.

The pH value was the soil property modified to the greatest depth by organic amendments. Tester^[44] and Park *et al.*^[37] concluded that application of organic wastes from food processing factories increased the contents of organic matter in the soils. Abdel-Aziz *et al.*^[1] and Basyouny^[7] reported that increasing the rates of applied organic manure to calcareous soil, in general, resulted in an increase for soil organic matter content as well as a decreases of soil pH.

Hence, the present work was undertaken to verify the fertilizing efficiency of organic manure and AMfungi on phosphorus, some micronutrients availability and its recovery by maize crop in a calcareous soil as well as to evaluate their performance in reducing the recommended chemical P fertilizer rates.

MATERIALS AND METHODS

A field experiment was conducted on a calcareous sand clay loam soil cultivated with maize plants (Single cross 10 hybrids) during summer growing season of 2007 at Banger El-Sukar area, El-Nubaria region, Egypt. The previous crop was wheat, which received 30 kg P_2O_5 /fed. Some physical and chemical properties of the investigated soil were determined according to the methods described by Richards^[39] and Jackson^[20], as shown in Table (1).

Preparing Arabuscular Mycorrhizal (AM) Fungi Spores and Count: The AM spores were isolated from the rhizosphere of maize and Egyptian clover, and multiplied in peat: vermiculite: perlit with ratios 1:1:1^[6]. Maize seeds were inoculated with mycorrhizal inoculum before planting.

Spores were extracted blending the 10g of each soil sample in 100 ml of tap water for 20 sec to release interadical spores. These samples were wet sieved onto a $37~\mu m$ sieve (Kormanik and Mcgraw,

1982), resuspended in deionized water and extracted at the interface of a 70% sucrose/water gradient following centrifugation at 1700 r.p.m for 3 min. Mycorrhizal spores were the recovered from the supernatant, which was poured through a 45 µm sieve, thoroughly rinsed with tap water and transferred to filter paper for counting and storage at 4°C. No attempt was made to identify spores to taxonomic levels. Total spores number was counted in nematode counting dish under the low power of dissecting microscope.

Preparing Composted Rice Straw: Rice straw was composted at the farm according to the method described by Abou El-Fadle^[2] as follows: 400 kg of rice straw was cut to small pieces and arranged in 10 equal layers to enhance the aeration and decomposition processes. To activate the decomposition process, an activator mixture of 15 kg ammonium sulphate, 8 kg rock phosphate and about 40 kg of farmyard manure were added to the composted materials. Some characteristics of used compost are shown in Table (2).

Field Experiment: The current experiment was designed in randomized complete blocks with three replicates. The experimental plot included 4 rows 6 m long with 70 cm between rows, and it has an area of 16.8 m². Maize seeds were sown on 15 April, 20 days after plant seedlings were thin to one per hill resulting 100 plants each plot.

Phosphorus treatments were inert phosphorus content (P_0) , 15 (P_1) and 30 kg P_2O_5 /fed. P was added in the form of superphosphate (15 P_2O_5 %) during soil preparation before planting. Nitrogen fertilizer in the form of ammonium nitrate (33.5 N%) was added at the rate of 105 kg/fed into two equal doses (30 and 60 days after planting). Potassium fertilizer was added in the form of potassium sulphate (48 % K_2O) at the rate of 24 kg K_2O /fed with the second nitrogen dose. Normal cultural practices were carried out throughout the growth period.

Sampling: Soil samples were taken at a depth of 0-15 cm, and air dried, ground, sieved through a 2 mm sieve then analyzed for available P^[34] and DTPA extractable Fe, Mn, Zn and Cu^[28] using the standard methods described by Page *et al.*^[36]. Active acids was extracted from manure composts, and then determined according to Kononova^[24].

The plant samples were collected at 70 days after planting, dried in an oven at 70 C° to a constant weight, ground in Wiley's mill and one gram of ground material was digested in acid mixtures of HNO₃ and HClO₄ prepared in 5:11 ratio. The digested material was analyzed for P using Ascrobic acid method and measured by spectrophotometer. Nitrogen was

Table 1.	Some physico-chen	ical properties	and nutrianta	status of the	avnarimental co	. ;1
Table 1:	Some physico-chen	ncai propernes	and numents	status of the	experimental sc	111

ics	Value	Soil ch	Soil characteristics				
tribution%:		Soluble cations (soil paste $m \ mol_c \ L^{-1}$):					
	66.93		Ca ²⁺		9.75		
	11.70		Mg ²⁺		4.61		
	21.37		Na ⁺		13.20		
	Sandy clay loam K ⁺				0.75		
roperties:		Soluble	e anions (soil paste, m n	ıol _c L ⁻¹):			
pH (1:25 soil water suspension)			CO ₃ ²⁻		0.00		
% 18.39			HCO ₃ -				
%	0.98		Cf		16.80		
paste extract)	2.82		SO ₄ ² -				
	Available macro &	micronutrients (mg/kg))				
P	K	Fe	Mn	Zn	Cu		
3.91	276.42	3.83	0.86	0.58	0.49		
ne characteristics of con	mposted rice straw.						
		Characte	ristics		Value		
Characteristics Value Bulk density (g/cm ⁻³) 0.72			Total N %				
nt %	8.17	C/N ratio			15.58		
)	1.91	Chemically available macronutrients(µg/g):		ents(μg/g):			
pH (1:10 suspension) 7.56		Phoenho	489				
	roperties: ater suspension) paste extract) P 3.91 ne characteristics of contem ⁻³) at %	######################################	Soluble		stribution%: Soluble cations (soil paste m mol, L^{-1}): 66.93 Ca^{2+} 11.70 Mg^{2+} 21.37 Na^{+} Sandy clay loam K^{+} roperties: Soluble anions (soil paste, m mol, L^{-1}): ater suspension) 8.09 CO_3^{-2} 3.99 CO_3^{-2} 40.98 CI 3.98 CI 4.098 CI 4.098 CI 4.098 CI 3.91 2.82 5.004 CI 4.098 CI		

Potassium

Extracted active organic acids %

determined by the Kijeldahl methods. The concentrations of P obtained were used for calculating P-uptake and P-recovery. The data obtained were subjected to statistical analysis according to Snedecor and Cochran^[42].

Organic matter %

Organic carbon %

45.01

26.17

RESULTS AND DISCUSSION

Effect of Organic Compost and Mycorrhizal Inoculation under Different P Levels on Soil pH and Available P in the Studied Calcareous Soil: Data in Table (3) show that the available P and pH values of the studied soil were more affected at the combined treatment of (AM + organic compost) than each one when applied alone. Interestingly, the higher soil available P was obtained from soil treated with AM + organic compost at 15 (P_1) and 30 kg P_2O_5 /fed (P_2), with insignificant difference as compared to inert phosphorus content in the soil (P_0). That means applying 15 kg P_2O_5 /fed in combination with AM fungi and organic compost is satisfactory from the

economical view of point. The increase of available P could be explained by the production of CO₂ and forming H₂CO₃ during organic matter decomposition, which enhancing phosphate solubility. In this connection, It was reported that water extracts of decomposed plant materials were effective in dissolving phosphate soil. Datta and Shrivastav^[10] and Sinha^[41] explained the mechanism involved, that during the decomposition of compost, organic acids are produced, which may influence the pH and available phosphorus, or they may form complexes or chelates with the other cations and thus releasing the phosphorous.

480

28.52

Soil pH is the major function of changes in the soil conditions. Data presented in Table (3) indicated that the inoculation with AM + organic compost had a favorable effect than each one alone on the soil pH, especially at treatment of 15 kg P_2O_5/fed (P_1). This finding is expected to be due to the beneficial effect of organic acids that produced during organic matter decomposition. Similar results were obtained by El-Fayoumy and Ramadan^[12].

Table 3: Effect of organic	compost and	mycorrhizal	inoculation	under	different I	P levels	on	soil pH	and	available P of	the studied
calcareous soil											

Treatment (T)	pH value			Available P (mg kg ⁻¹ soil)					
	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂	Means		
Control	8.18	8.08	8.13	5.07	6.27	6.44	5.93		
AM fungi	8.07	8.09	8.11	7.09	8.25	8.91	8.08		
Compost	7.91	8.00	8.05	7.50	7.80	7.71	7.67		
AM fungi + compost	7.85	7.77	7.79	8.21	9.30	9.87	8.79		
Means				6.97	7.91	8.23	7.70		
LSD at 0.05				T		P	$T \times P$		
				1.02		1.00	0.50		

Effect of Organic Compost and Mycorrhizal Inoculation under Different P Levels on Availability of Micronutrients (Fe, Mn, Zn and Cu) of the Studied Calcareous Soil: Data in Table (4) indicate that the concentrations of Fe, Mn, Zn and Cu were significantly increased in cases of applied organic compost as well as AM + organic compost. On the other hand, there was an increase for each of the studied micronutrients in case of treated soil with AM fungi, such increases were significant as compared to the control treatment. In this concerning, Habashy and Abo-zaid[18] showed that the availability of micronutrients (Fe, Mn, Zn and Cu) were positively affected by inoculation AM fungi when compared to the uninoculated treatments. DTPA extractable Fe and Mn were slightly affected by mycorrhizal inoculation than that uninoculed one. In addition, the DTPA extractable Zn and Cu were also increased in the soil treated with AM fungi.

Effect of Organic Compost and Mycorrhizal Inoculation under Different P Levels on N and P Uptake: Data in Table (5) show that the addition of P increased N and P uptake by maize plants with a pronounced effect, with a parallel trend for their increases in the studied calcareous soil. Mawardi *et al.* indicated that phosphate mobility in calcareous soil was much lower, probably due to the higher pH and CaCO₃ values.

Data also indicated that phosphorous uptake by maize plants, in general, increased when organic matter was added to the studied calcareous soil, such beneficial effect may be attributed to enhancing the applied organic matter for releasing phosphate in soil and make it to be more available for plants^[33]. Also, addition of organic manures improved the physical properties of the soil, and increased the supplying power of available nutrients to plants. Increasing P level to 30 kg P₂O₃/fed increased N uptake, may be due to the effect of P on plant metabolism^[46].

Table 5 also shows that mycorrhiza was more effective as compared to the applied organic manure on

N and P uptake by maize plants. Mahmoud *et al.*^[30] and Pacovsky *et al.*^[35] concluded that inoculation with mycorrhiza significantly increased N and P uptake by plant. Kawai and Yamamoto^[22] added that mycorrhizal plants higher of P in their stem and leaves than that of non-mycorrhizal ones.

Effect of Organic Compost and Inoculation of Mycorrhizal Fungi on Grain Production: Table (6) showed maize grain production as affected by biofertilizer application and inoculation of mycorrhyzal fungi. The lower mean values were noticed for compost with inert phosphorus content (P_0). On the other hand, the higher mean values were obtained for AM + organic compost in combination with 15 (P_1) and 30 kg P_2O_5 /fed (P_2).

Inoculation of maize plants with AM fungi and their combination AM + organic compost resulted in increasing the maize grain yield. Statistically analysis (Table 6) showed higher yield mean values that were obtained with inoculation by AM + organic compost at 15 (P_1) and 30 kg P_2O_5/fed (P_2) with insignificant differences. The lowest grain yield was obtained with uninoculation under lower P level (P_0).

The relatively increase of grain yield (32.8%) was recorded with inoculation by AM + organic compost at 15 kg P_2O_5 /fed (P_1), while the usefulless increase of grain yield (12.6%) was observed with inert phosphorus content (P_0) at composted treatment. Faber *et al.*^[15] Elwan and El-Sharawy^[14] and Koreish *et al.*^[25] reported that corn grain yield was increased with inoculation by AM fungi.

Effect of Organic Compost and Mycorrhizal Inoculation on Mycorrhizal Root Infection, Spore Numbers and Phosphorus Recovery under Different P Levels:

AM Root Colonization (%): The results of mycrrohyzal colonization percent shown in Table 7, and revealed that a gradual increase with inoculation by AM fungi and AM + organic compost, while it showed insignificantly increased with organic compost

Table 4: Effect of organic compost and mycorrhizal inoculation under different P levels on availability of micronutrients (Fe, Mn, Zn and

Transment (T)	Fe (ppm)				Mn (ppm)					
Treatment (T)	P ₀	P ₁	P_2	Means	P ₀	P ₁	P ₂	Means		
Control	3.91	3.44	3.42	3.59	0.89	1.00	1.10	1.00		
AM fungi	4.51	4.89	5.03	4.81	0.94	1.63	1.69	1.24		
Compost	4.89	5.00	5.50	5.13	1.18	1.68	1.71	1.52		
AM fungi + compost	5.43	5.62	5.75	5.60	1.41	1.73	1.77	1.64		
Means	4.69	4.72	4.93	4.78	1.11	1.51	1.57	1.35		
LSD at 0.05	T 0.90		P 22	T x P 0.55	T 0.		P 0.09	T x P 0.17		
	Zn (ppm)				Cu (ppm)					
Control	0.61	0.64	0.69	0.65	0.51	0.53	0.54	0.53		
AM fungi	0.64	0.71	0.75	0.70	0.54	0.60	0.67	0.60		
Compost	0.79	0.85	0.90	0.85	0.64	0.71	0.79	0.71		
AM fungi + compost	1.03	1.07	1.12	1.07	0.69	0.83	0.88	0.80		
Means	0.77	0.80	0.84	0.82	0.60	0.67	0.72	0.66		
LSD at 0.05	T 0.04		P 08	T x P 0.12	T 0.0		P 0.07	T x P 0.08		

Table 5: Effect of organic compost and mycorrhizal inoculation under different P levels on N and P uptake by maize plant.

_	N uptake (kg fed ⁻¹⁾			P uptale (kg fed ⁻¹)				
Treatment (T)	P ₀	P,	P,	Means	P ₀	P,	P,	Means	
Control	13.4	17.4	20.1	17.0	3.43	3.50	3.55	3.49	
AM fungi	21.3	24.5	26.2	24.0	5.37	5.56	5.63	5.52	
Compost	22.0	26.5	29.6	26.0	4.18	4.29	4.37	4.28	
AM fungi + compost	24.5	31.9	30.6	31.0	6.46	7.02	7.89	7.12	
Means	20.3	25.1	28.1	24.5	4.86	5.09	5.24	5.10	
LSD at 0.05	T	Р		ТхР	Т		P	ТхР	
	3.2	4.4		3.5	3.01	1.	.00	1.58	

	Grain yield (t	•			on yield and increasing relative yield of m Relative increase %			
Treatment (T)	P ₀	P ₁	P,	Means	P ₀	P ₁	P,	
Control	3.56	4.00	4.32	4.04				
AM fungi	4.04	4.77	5.19	4.67	13.5	19.3	20.1	
Compost	4.01	4.90	5.00	4.36	12.6	22.5	14.9	
AM fungi + compost	4.16	5.31	5.47	4.98	16.9	32.8	26.6	
Means	3.94	4.75	5.05	4.51				
LSD at 0.05	T	P	1	TxP				
	0.31	0.4	4	0.52				

alone comparing to the control treatment. The colonization percent was highly increased as 15 (P_1) and 30 kg P_2O_5 /fed (P_2) were added as compared to inert phosphorus content (P_0). Manyumath $et\ al.^{[29]}$; El-Sharawy $et\ al.^{[13]}$ and Koreish $et\ al.^{[25]}$ reported that

mycorrhizal root infection significantly increased by application of rock phosphate. In contrast, Kothari *et al.*^[27] found that colonization rate did not differ appreciably among the various treatments of P and micronutrients supply.

Table 7: Effect of organic compost and mycorrhizal inoculation under different P levels on AM root colonization, spore number and

phosphorus							
Treatment (T)	Colonization (%	5)	Spores nun	Spores number (100g dry soil)			
Treatment (1)	P_0	P_{\perp}	P_2	P_0	$\mathbf{P}_{_{1}}$	P_2	
Control	20	23	24	82	85	90	
AM fungi	53	55	57	152	157	160	
Compost	26	28	30	78	80	84	
AM fungi + compost	58	61	66	155	165	173	
T (T)	Recove	ery (%)					
Treatment (T)	P ₀		P,			P,	
Control							
AM fungi	47.5		45.2			28.7	
Compost	30.5		35.3			26.3	
AM fungi + compost	52.2		72.4			36.9	

Spore Number (Per g of Soil): Table VII shows the number of spores/g soil. Also, the obtained data revealed that the number of spores/g soil increased with inoculation by AM fungi and with the same trend as data show in AM root colonization. In this connection, data also showed that the number of spores/g soil were about similar in the soil at 15 (P_1) and 30 kg P_2O_3 /fed (P_2) added as compared to inert phosphorus content (P_0).

Phosphorus Recovery: P recoveries were higher effect by inoculation of AM + organic compost as compared to AM or compost alone (Table VII). The lowest recovery of 26.3% was recorded with the highest P rate (30 kg P₂O₅/fed, P₂). Recoveries of P, however, were decreased with the subsequent increase in p application rate. The lowest value of P recovery was observed with organic manure application.

Comparing the performance of all the bio-fertilizer and AM + organic compost under 15 kg P₂O₅/fed (P₁), the obtained data showed that such treatments enhanced the grain yield, P-uptake and P-recovery, with a significantly effect over the AM or organic compost. This may be explained that a long time interaction (aging) of soluble P with soil leads to a positive effect on its reaction with solid phase of soil[2]. Also, such condition may be due to the presence of calcium carbonate and the formation of relatively insoluble reaction products with Ca, Fe and Al leading to P fixation. All these processes leading to the fixation process, which is delayed when applying fertilizer in the form of bio-fertilizer as grown plant absorbs this nutrient quickly and directly from the soil solution. In addition, the positive effect of bio-fertilizer may also be due to optimum soil pH which facilities maximum utilization of applied Pas well as micronutrients to crops^[43].

Conclusion: From results previously presented, it can be concluded that inoculation of AM + organic compost in the calcareous soil increased productivity of maize plants, which is more attributed to nutrients availability in soil, their easily mobility and uptake by plant roots. On the other hand, results showed that the phosphorus solubility tended to increase with increasing the levels of phosphorus added. Also, solubility of micronutrients increased in the soil, may be due to lowering pH and /or airwater balance.

In general, pronounced response had been obtained in the solubility of Fe, Mn, Zn and Cu when macorrhizal was accompanied with organic compost addition than AM inoculation or organic compost added alone. This may be due to the addition of organic compost improved the physical properties of the soil, and increased the supplying power of available nutrients to plants.

Increase in yield was obtained as a result of the combined treatment of (AM inoculation + organic compost) in case of applied 15 kg P_2O_5 /fed. In general, application of AM inoculation + organic compost helps plants to attain more nutrients through the extended absorption surface, thus can help to overcome problem of P sorption in soil particularly in calcareous ones. Mycorrhizal inoculation percent and number of spores/g of soil gradually increased with inoculation by AM and AM + organic compost, but without increased in treatment of organic compost alone as compared to the control one.

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