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Impact of Endomycorrhizae and *Acidithiobacillus ferrooxidans* with Sulfur and Phosphorus Nutrition on Onion (*Allium cepa* L.) and Maize (*Zea mays* L.) Plants under Field Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To evaluate the effects of endomycorrhizal fungi (AMF) and sulfur oxidizing bacteria as biofertilizers in presence of phosphors and sulfur as mineral nutrients on onion (*Allium cepa* L.) and maize (*Zea mays* L.) plants under field conitions.
Study Design: Identification of the used bacterial isolate and applied with endomycorrhizae individually or mixed culture as bioinoculum for onion and maize plants grown in sandy soil under field conditions.
Methodology: sequence analysis of 16S rRNA gene was applied to identify the bacterial isolate. Two field experiments were carried out in sandy soil to study the effect of AMF and the sulfur oxidizing bacterium individually or combination with the recommended dose of N, K and P (as rock phosphate or super phosphate) with or without sulphur element, on some growth parameters,

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nutrients content and yield parameter of maize and onion crops .
Results: Results indicated that the bacterial isolate A1 was identified as *Acidithiobacillus ferrooxidans* based upon the 99% of sequence similarity of 16S rRNA gene with the reference strain. Field experiments revealed that after 60 and 90 days, inoculation of onion plants with dual inoculation induced significant response in nutrient content, but this response was found after 90 days in case of maize plant. The maximum data of the sulphur oxidizing bacterial count, dehydrogenase activity and CO_2 evolution in the rhizosphere were obtained after 60 days of cultivation for onion and after 90 days with maize plants. Inoculation with *Acidithiobacillus ferrooxidans* A1+ AMF in amended soil with NK + S + R.P gave the best results in N, P and K content of onion crop, bulbs weight and pyruvic acid content. For maize plant, data indicated that added recommended dose of nitrogen, potassium, sulphur and rock phosphate (NK+S+R.P) with AMF + *Acidithiobacillus ferrooxidans* A1 inoculant led to highest significant increases of nitrogen , phosphours and potassium contents compared to other treatments. Also, there were high significant increases in dry weight of 100 seeds, ear weight and its nutrients content with the abovementioned treatment.
Conclusion: The dual application of a mixture of arbuscular mycorrhizal fungi and *Acidithiobacillus ferrooxidans* A1 seems to have the most pronounced effect on quality and quantity of onion bulbs and yield of maize plants with the recommended dose of sulphur and rock phosphate fertilizers in sandy soil.

Keywords: Onion; Maize plant; biofertilizers; Arbuscular mycorrhiza; Acidithiobacillus; 16S rRNA gene; field conditions.

1. INTRODUCTION

The chemical fertilizers used to enhance crop productivity have often negative effects on the ecosystems [1], including the environmental degradation by leaching of nutrients, especially nitrogen (N) and phosphorus (P) [2]. Except N and P, sulphur (S) is another essential element for plant growth due to its presence in proteins, glutathione, phytochelatins, thioredoxins, chloroplast membrane lipids, and certain coenzymes and vitamins [3]. The S taken up by plant roots is in the form of sulphate (SO_4^{2-}), which undergoes a series of transformation inside the plants and in the environments [4]. Sulfur oxidation accomplished in both chemical and biological processes in the soil, while bacteria belonging to *Thiobacillus* are the most important sulphur oxidizers in the soil [5]. Phosphorus (P) is one of the most important elements for plant growth and metabolism, since it is a constituent of ATP and several vitally important compounds, notably nucleic acids and membranes [6]. Moreover, P plays indispensable role in photosynthetic and respiration reactions. It is also necessary for cell division and development of meristematic tissues. However, the mobility of P is very poor and the P fertilizers are easy to be immobilized in soil, which made the P difficult to be absorbed by plant roots. Mycorrhizae are widespread root-fungus symbioses formed by nearly all of the terrestrial plants. These symbioses are characterized by bi-directional transfer of nutrients between the plants and the mycorrhizal fungi, where the plants provide sugar to the fungi and the latter help the plants on the acquisition of mineral nutrients, especially P, from the soil [7]. Balloei et al. [8] showed that mycorrhiza accompanied by the bacteria of *Thiobacillus* and inoculation of *Thiobacillus* plus sulphur could improve the yield of soybean because of the synergistic effects between the fungus and the bacterium. Numerous studies showed that the phosphours and sulphur nutrition affected the growth, yield, chemical constituents and storability characteristics of onion. In this concern, Bolandnazar et al. [9] found that arbuscular mycorrhizal fungi improved onion growth and development in comparison with the non-mycorrhizal ones. This improvement was resulted from increasing leaf area, plant height and leaf chlorophyll content, which led to greater photosynthesis capacity and subsequently greater dry mass and larger bulb. Based upon the positive effects of mycorrhiza-sulphur oxidizing bacterium association on growth of plants, we conducted this study to investigate the impact of soil amendment with arbuscular mycorrhizal fungi (AMF) and the sulphur oxidizing bacteria as biofertilizers on the growth parameters, nutrient content and productivity of onion and maize plants grown in sandy soil under field conditions.

2. MATERIALS AND METHODS

2.1 Bacterial Isolate and Soil Analysis

A S oxidizing bacterial isolate A1 was used in this study which was isolated and characterized previously [10]. This isolate has high efficient to oxidize sulfide into sulphate as well as gave the best effect on onion and maize plants with or without arbuscular mycorrhizal fungi in pot experiments under net house conditions [10]. The field experiments were conducted at the Agricultural Research Station in Ismailia. The physical and chemical characteristics of the soil were determined according to Baruah and Barthakur [11] and Jackson [12] respectively, and were presented in Table 1. Its microbiological contents, before cultivation, were $4x10^5$ and $6X10^2$ cfu/g dry soil of total bacteria and sulphur oxidizing bacteria respectively, and 83.3 spores / 100 g soil AMF.

2.2 Genetic Analysis

To clarify the taxonomic position of the isolate A1, sequence analysis of 16S rRNA gene was performed. Cellular DNA of the bacterial isolate was isolated as described by Ausubell et al. [13] and amplification of 16S rDNA according to Lane [14] using the universal 16S primers. The PCR-product was purified using QIAquik PCR Purification Kit (Qiagen) and was sequenced in two directions using the previously described primers by Lane [14] in GATC Company (Germany). Sequencing data was analyzed by two different computer alignment programs, DNAStar (DNASTAR, Inc., USA) and Sequence Navigator (Perkin, Corp., USA). The BLAST database [15] of National Center for Biotechnology Information was used to compare the acquired sequence of the isolate A1 with the reported 16S rDNA sequences. The phylogenetic position of the tested isolate was analyzed by the program Phylogenetic Analysis CLC free workbench version 4.5.1.

2.3 Evaluation the Role of The Tested Bacterium with AM Fungi as Biofertilizers for Two Economical Plants Under Field Conditions

2.3.1 Preparation of bacterial inoculums

Active culture of identified isolate was grown in *Thiobacillus* enrichment medium [16] under static condition at 30°C for 5 days. Cell suspension containing about~ 6×10^8 cfu/ml was used as standard inoculum.

2.3.2 AM fungi inoculums

Mycorrhizal spores that contained the mixture of *Glomus* sp. and *Gigaspora* sp. were extracted from rhizosphere of maize plants grown in the Ismailia Experimental Station of Agriculture Research Centre**,** Egypt. Extraction and counting of mycorrhizal spores were carried out according to the method described by Gerdemann and Nicolson [17]. The identification of mycorrhizal spores was carried out according to the key of Schenck and Perez [18] using morphological characteristics of hyphae, attached hyphae, chlamydospores, azygospores and sporocarp.

2.3.3 Field experiment design

Two field experiments were carried out at Ismailia station during July 2012 for maize and September 2013 for onion plants, to study the effects of AMF and the isolate A1 separately or combination with recommended dose of N, K and P (as rock phosphate or super phosphate) with or without sulphur supplement, according to the recommendation of Agronomy Research Institute, on growth parameters, nutrients content and yield parameter of maize and onion crops. Onion (*A. cepa* L., cv. Shandweel) bulbs and seeds of maize (*Z. mays* L.) plants were obtained from Agronomy Research Institute, Agricultural Research Centre (ARC), Giza, Egypt. A spilt plot experiment based on randomized complete block with three replicates for each treatment was carried out. The recommended dose of rock phosphate (R.P) (10% P) and super phosphate (SP) (15.5% P_2O_5), as sources of P were 107 kg and 300 kg/ feddan for onion plants and 86 kg & 200 kg/feddan (=0.42 hectares) for maize plants, respectively. The recommended dose of potassium nitrate as a source of nitrogen and potassium were 75 unites N/feddan for onion plant and 100 unite N/ feddan for maize plant. The recommended dose of elemental sulphur powder was 1.7 ton/feddan, as a source of S for the tested plants. These mineral fertilizers were mixed with soil before sowing. Each plot (3×3.5 m) was planted with 4 grains of maize or 1 bulb of onion / hill. The mineral fertilizers treatments were NPK, NPK +S, NK+ R.P and NK+S+RP and each mineral treatment contained 4 inoculated treatments that were uninoculated (control), bacterial isolate A1, AMF and bacterial isolate A1 + AMF. Inoculated treatments were inoculated with 20 ml of bacterial cell suspension

Table 1. The physico-chemical characteristics of field experiment soil

Particle size (%)				Texture	$CaCO_3$ (%)	Organic matter (%)
Coarse sand	Fine sand	Silt	Clay			
50.4	40.4	3.2	6	sandy	1.40	0.40

Chemical properties		Soluble ions in saturated extract (meq/l)						Available nutrients µg g^{-1}		
ECe dS/m	pH	K^+	Na^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^- HCO_3^-	N	P	K
2.67	8.30	5.33	13.68	8.00	4.00	18.42	9.50 2.70	140	50	132

while mycorrhizal inoculation was performed with 10 ml of spore suspension / bulb or grain (28 spores/ ml) at planting time. The soil samples were taken after 30, 60 and 90 days for onion plants and 60, 90,120 days for maize plants of cultivation for chemical and biological determinations.

2.3.4 Parameters measured

2.3.4.1 Growth parameters

Plants were harvested at the end of experiment. Fresh and dry matter of onion bulbs and maize plants were measured. The yield parameters include dry & fresh weight of bulb / plant (gram) for onion plants and weight of 100 seeds and ear weight of maize plants.

2.3.4.2 Biological analy

Densities of total counts of bacteria and total sulphur-oxidising bacteria were carried out by decimal plate count technique using nutrient agar and *Thiobacillus* enrichment agar media, respectively, at 28-30°C. Some biological activities such as dehydrogenase activity (DHA) (µg TPF/100 g dry soil day^{-1}) and CO_2 evolution (mg CO_2/100 g soil), were determined in collected soil samples from onion and maize rhizosphere according to Casida et al. [19] and Pramer and Schmidt [20], respectively. The spores of arbuscular mycorrhizal fungi in the rhizosphere and percentage of mycorrhizal colonization of root were estimated after 60 days of planting for maize and onion plants by the methods of Gerdemann and Nicolson [17] and Trouvelot et al. [21], respectively.

2.3.5 Chemical analysis

Nitrogen, phosphours and potassium contents were determined in the rhizosphere and plant samples according to the methods of Cottenie, et al. [22]. The sulphur was determined in soil samples according to Issam and Antoin [23]. Pyruvic acid was determined in onion bulbs according to Gordon and Diane [24].

2.4 Statistical Analysis

Data were statistically analyzed according to Gomez and Gomez [25]. Fisher's Least Significant Difference (LSD) at 5 % level of significance was used for comparison between the means of different treatments. In this analysis, data of mineral treatments without biofertilizer inoculums were used for calculate LSD of Mineral (M); biofertilizer treatments without mineral treatments were included for LSD of Biofertilizers (Bio), and the interaction between biofertilizer and mineral treatments were included for LSD of M*Biol.

3. RESULTS AND DISCUSSION

3.1 The Identification of Used Bacterium

Previously, nearly complete sequences of the 16S rRNA genes have been determined for different S oxidizing bacteria [26]. In the present study, nearly complete 16S rRNA gene sequence was obtained for the bacterial isolate A1, which shared 97-98% identity with the closest *Acidithiobacillus* strains. In the phylogenetic tree constructed in this study isolate A1 is closely related to *Acidithiobacillus ferrooxidans* ATCC23270 with similarity level of 99%, therefore, the isolate A1 should be identified as *Acidithiobacillus ferrooxidans* (Fig. 1).

3.2 Performance of onion and maize plants in different treatments under field conditions

3.2.1 Onion Plant

3.2.1.1 Biological activity in the rhizosphere

Data in Table 2 showed that high values of total sulphur oxidizing bacteria were obtained from treatments of dual inoculation supplied with

different mineral fertilizers, particularly NK + S+ R.P. The highest values of CO2 evolution and dehydrogenase activity were 300.8 mg CO_2/100g soil and 27.9 µg triphenylformazan (TPF) / 100g soil day-1, in the same treatment. The maximum values of total sulphur oxidizing bacteria, CO_2 evolution and dehydrogenase activity were obtained after 60 days of planting. The inoculated plants by AMF + A. ferrooxidans A1 with NK+ S+ RP supplement gave the highest values of the mentioned parameters: 1.15x105 cfu/g dry soil 344.6 mg CO_2/100g soil Day-1 and 44.0 µg TPF/ 100g soil Day-1 ,in respective order, after 60 days. The lowest values of the measured parameters were obtained after 90 days of cultivation under different treatments. In general in all of the 3 sampling times, the treatment with NK +S + RP mineral fertilizers inoculated with both of the AMF and A. ferrooxidans A1 gave the highest values which significantly greater than those in the uninoculated treatments. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as measure of microbial activity in soil. Concentration of soil dehydrogenases depends on conditions and intensity of biological conversion of organic compounds. The evolution of CO_2 under field conditions represents respiration by plant roots and soil biota and is a sensitive indicator of abiotic controls root exudates, crop residues decomposition, soil organic carbon turnover and ecosystem disturbance. Chendrayan et al. [27] reported that the increase in dehydrogenase activity was mainly due to higher microbial population. The highest number of Mycorrhizal spores were obtained from added recommended dose of nitrogen, potassium, sulphur and rock phosphate with AMF + *A. ferrooxidans* A1 (600 spores/100 g soil) followed by the same bioinoculant with recommended dose of NPK with or without sulphur (450 spore/100 g soil)

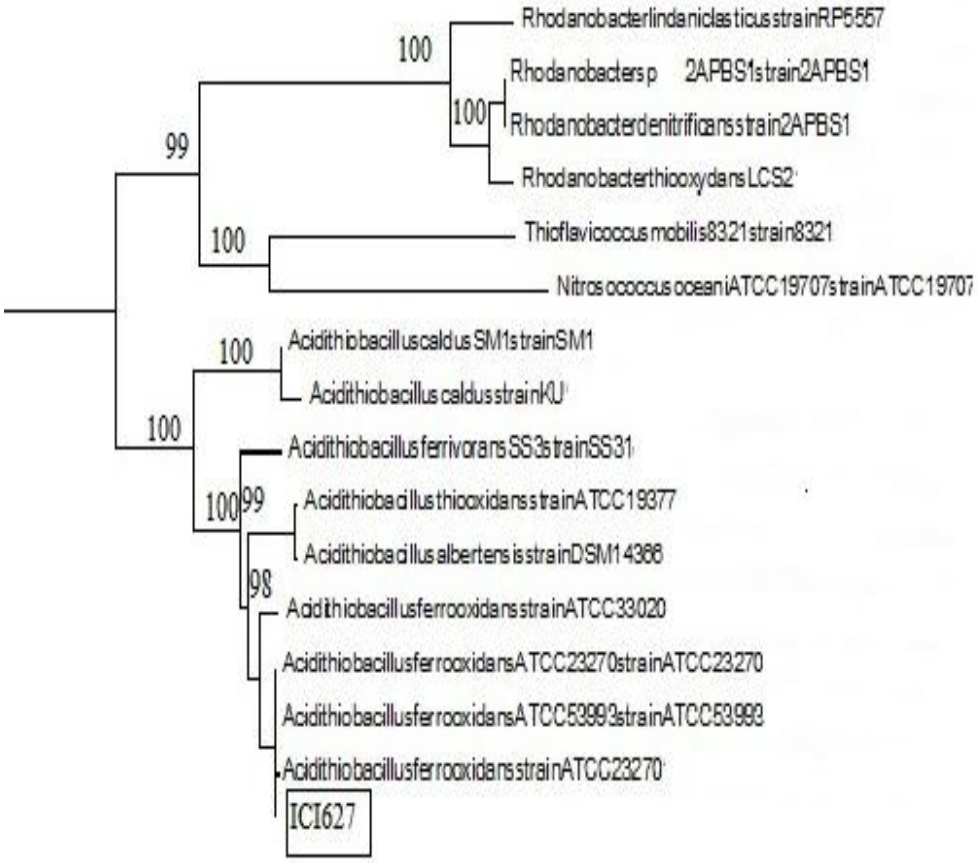

Fig. 1. Neighbor-joining tree showing the estimated phylogenetic relationships of the studied isolate A1 (ICI627) [shown in box] and other closely-related strains based on comparative analysis of 16S rRNA gene sequence

after 60 days of planting. Results also showed that the colonization of AMF ranged from 33-90% in the roots of the tested plants. The highest percentages of colonization were recorded in inoculated plants with AMF + *A. ferrooxidans* A1 in amended soil by recommended dose of NK+R.P with or without sulphur after 60 days of planting (Table 2.). This result is in line with that observed by Alloush and Clark [28] and Bagayoko et al. [29] who stated that in presence of rock phosphate the root colonization by AM fungi increased compared with addition of triple superphosphate which *reduced* mycorrhizal function.

3.2.1.2 NPKS in onion rhizosphere

Data in Table 3 presented that appreciable changes in N, P, K, S content were observed in onion rhizosphere due to inoculation with AMF and / or *Acidithiobacillus ferrooxidans* A1 compared with the uninoculated ones at different ages. After 30 days of cultivation, inoculated plants with AMF + *A. ferrooxidans* A1 gave the highest values of N in presence of NK+RP with or without sulphur. After 60 and 90 days, inoculation of plant with dual inoculants (AMF+ *A. ferrooxidans* A1) induced significant responses in nutrients content in the presence or absence of mineral fertilizers. However, the effects were greater on mineral fertilizers amended plants particularly with NK+S+RP. This observation was reported in nitrogen, phosphorus, potassium and sulphur contents in the rhizosphere being 350, 12.6, 521.3 ppm and 25.6 mg SO_4^{-2}/ 100g soil for plants amended with NK+S+ RP after 60 days of cultivations. The values of NPKS with all treatments decreased after 90 days of cultivation compared to the data after 30 and 60 days due to consume the soil nutrient by onion plants at the end of plant growth period.

3.2.1.3 NPK content of plants

Nitrogen, phosphorus and potassium percentage and uptake (mg/g) in onion bulbs (90 days old) gave maximum concentration when soil fertilized with NK + Rock Phosphate + Sulpher and inoculated with AMF + *A.ferrooxidans*A1, with significant increasing over control treatment (Table 4). The corresponding values of percentages and uptake of nutrients were 3.26% and 324.7 mg/g plant for nitrogen, 2.15% and 214.1 mg/g plant for phosphorus, and 3.46% and 344.6 mg/g plant for potassium. The nutrients especially P and S enhanced quantitative and qualitative characters of onion plants as showed by Nassar et al. [30], in which the foliar application of phosphours and sulphur significantly enhanced their concentration in onion plants. Mahaveer and Alok [31] showed that the inoculation of onion plants with AM Fungi (AMF) can significantly increase bulb diameter, bulb yield and shoot P content, furthermore, AM Fungi improve the uptake of P and other nutrients including N, Ca, S, K, Cu and Zn.

3.2.1.4 Bulbs weight and nutrient components

Pungency in onions is derived from a number of volatile sulphur compounds. Pyruvic acid as indicator for pungency was determined. Data shown in Table 4 revealed that weight of bulbs and pyruvic acid content were significantly enhanced with microbial inoculation. In presence of sulphur with recommended dose of N, P (superphosphate), K, the bulbs fresh weights were significantly greater with dual inoculants compared with the same conditions without sulphur, 64.1 g/bulb with S against 55.0 g/bulb without S. Recommended dose of NK+R.P with dual inoculants gave 50.6 g/ fresh bulb whereas 36.2 g/ fresh bulb with same mineral fertilizer with AMF inoculant only and pyruvate contents were 11.2 and 9.3 µ mole/g bulb respectively. The maximum value of bulbs weight and pyruvate content were obtained from inoculated plants with AMF+ *A. ferrooxidans* in amended soil by NK+S+R.P being 64.7g/bulb and 11.8 µ moles/g fresh weight. Our results were consistent to Mahaveer and Alok [31] that the inoculation of onion plants with AM fungi can significantly increase bulb diameter, bulb yield, shoot dry and fresh weights and shoot phosphours content.

3.2.2 Maize Plant

3.2.2.1 Soil biological activity

The highest values of sulphur oxidizing bacterial counts were obtained in maize plants rhizosphere with dual inoculant in the presence of NK+R.P with or without sulphur after 90 days of planting (Table 5). Generally, the activity of dehydrogenase and CO_2 evolution in the rhizosphere were remarkably stimulated after 90 days of cultivation with all treatments. Biofertilizers treatments enhanced the dehydrogenase activity and CO_2 evolution compared to uninoculated treatments.

Table 2. Biological characters of onion rhizosphere and AMF parameters as influenced by different fertilizer treatments under field conditions

Treatment		Sampling time									AMF	
Mineral Fertilizer	Biofertilizers	30 days			60 days			90 days			after 60 days.	
		T.SOB* ($x10^3$)	CO_2*	DHA*	T.SOB ($x10^3$)	CO_2	DHA	T.SOB ($x10^3$)	Co_2	DHA	Spores /100g Soil	Root colonization (%)
Un-amended	Uninoculated(Control)	38	84.3	10.6	51	163.0	12.6	34	76.7	9.6	166.6	33
	A. ferrooxidans A1	66	185.9	14.2	84	267.2	26.5	56	135.7	12.1	216.6	51
	AMF[#]	57	178.3	15.8	80	216.5	23.0	48	88.2	14.0	250.0	67
	AMF +A1[#]	76	207.8	20.4	92	284.0	35.2	62	137.2	17.8	366.6	68
N P K	Un inoculated	60	105.4	13.4	65	310.5	19.0	50	82.5	11.8	250.0	45
	A. ferrooxidans A1	68	219.6	18.5	87	337.4	34.1	61	143.4	16.9	366.6	52
	AMF	71	248.0	21.1	80	297.8	30.3	61	164.5	16.1	400.0	75
	AMF + A1	81	299.0	23.8	97	347.6	36.7	74	220.1	19.2	450.0	80
N P K + S	Un inoculated	79	126.9	13.8	80	245.3	18.2	57	130.0	12.6	250.0	49
	A. ferrooxidans A1	76	224.2	17.1	100	350.4	34.4	70	143.4	15.1	233.3	57
	AMF	74	260.7	23.6	84	341.1	31.3	64	174.5	15.2	350.0	80
	AMF + A1	83	289.4	23.7	116	359.2	39.8	71	197.5	18.8	533.3	90
N K + R.P[#]	Un inoculated	65	157.2	18.1	77	286.3	27.8	57	100.1	14.5	283.3	57
	A. ferrooxidans A1	71	245.3	20.4	102	371.8	31.8	64	199.8	18.3	300.0	59
	AMF	83	235.7	23.7	87	272.5	31.5	57	189.0	15.5	400.0	81
	AMF + A1	86	264.5	24.7	103	370.7	41.4	81	214.7	19.2	450.0	83
N K + S + R.P	Un inoculated	75	144.9	16.0	104	267.2	26.1	67	118.9	13.5	266.6	50
	A.ferrooxidans A1	89	281.8	20.6	110	328.9	45.0	76	214.3	16.6	350.0	68
	AMF	83	283.3	26.1	107	293.2	35.3	75	142.3	18.3	416.6	72
	AMF +A1	93	300.8	27.9	115	344.6	44.0	81	199.8	22.6	600.0	86
LSD (0.05)	Mineral(Min)	7.76	28.72	4.23	9.95	45.34	4.31	7.48	31.71	2.05	56.18	5.64
	Biofertilizers(Bio)	8.96	33.17	4.89	11.49	52.36	4.98	8.63	36.62	2.36	64.88	6.51
	Min *Bio	10.02	37.08	5.46	12.84	58.54	5.57	9.65	40.94	2.64	72.53	7.28

**. T.SOB=Total count of sulpher oxidizing bacteria (cfu/g dry soil); CO_2 = CO_2 evolution ($mgCO_2$/100g soil); DHA =Dehydrogenase activity (µg TPF/100g soil Day^{-1}).*

[#]. AMF = Arbuscular Mycorrhizal Fungi; A1=A. ferrooxidans A1; R.P= rock phosphate; Each value is average of three replicate

*L.S.D value: Least Significant difference between two values of mineral treatments without bio-inoculum (Min); between biofertilizer treatments without mineral treatments(Bio) and between two values in the same parameters (Min*Bio), according to Duncan's at 5% level*

Table 3. Concentration of nutrients N, P, K (ppm) and S (mg SO_4^{2-}/100 g soil) in rhizosphere of onion plants as influenced by different fertilizer treatments under field conditions

Treatments		Sampling time											
Mineral fertilizer	Biofertilizers	30 days				60 days				90 days			
		N	P	K	S	N	P	K	S	N	P	K	S
Un- amended	Uninoculated(Control)	130.7	5.3	115.7	3.3	140.0	7.2	148.8	4.9	74.7	4.0	98.2	3.2
	A. ferrooxidans A1	205.3	7.4	161.2	8.5	214.7	8.0	266.5	14.8	144.7	6.2	128.7	7.4
	AMF*	210.0	8.3	170.3	6.0	228.7	8.3	187.8	10.3	149.3	6.9	139.1	5.4
	AMF +A1*	219.3	8.7	189.8	7.7	266.0	9.9	267.8	15.7	168.0	7.6	163.8	8.4
N P K	Un inoculated	196.0	6.9	193.9	6.1	247.3	8.7	275.6	9.1	130.7	7.1	104.0	4.2
	A. ferrooxidans A1	233.3	8.6	283.8	11.9	280.0	9.3	304.2	17.1	177.3	7.8	139.1	8.6
	AMF	247.3	9.6	238.9	9.2	289.3	10.1	305.5	11.7	158.7	7.8	150.2	7.1
	AMF +A1	252.0	10.3	292.5	12.4	308.0	10.4	421.2	17.2	191.3	8.2	198.9	11.5
N P K + S	Un inoculated	200.7	7.7	182.0	7.1	219.3	9.4	218.4	7.7	112.0	6.8	159.9	6.5
	A. ferrooxidans A1	238.0	8.9	271.0	13.0	298.7	10.0	287.9	18.5	191.3	8.1	188.2	8.2
	AMF	247.3	9.5	200.2	10.3	308.0	10.0	317.8	12.6	205.3	8.4	184.2	7.9
	AMF + A1	252.0	10.4	308.1	13.3	340.7	11.3	374.4	21.5	205.3	9.8	188.5	12.1
N K + R.P[§]	Un inoculated	210.0	8.8	172.4	6.3	228.7	9.6	221.0	7.9	163.3	7.4	155.3	7.0
	A. ferrooxidans A1	247.3	9.9	251.5	11.9	275.3	9.9	315.2	19.3	186.7	8.4	192.1	9.0
	AMF	266.0	10.1	189.1	10.4	308.0	11.1	225.5	18.7	154.0	9.5	188.5	9.3
	AMF + A1	281.0	10.5	308.7	12.3	345.3	12.6	350.3	21.5	196.0	9.8	195.1	10.0
N K + S + R.P	Un inoculated	228.7	8.0	173.1	9.3	238.0	9.8	269.7	9.3	158.7	7.6	170.5	7.3
	A. ferrooxidans A1	266.0	9.9	269.7	12.1	312.7	10.0	399.7	20.3	196.0	8.8	186.6	10.9
	AMF	256.7	10.4	195.0	10.6	317.3	11.0	343.8	16.6	186.7	9.4	185.9	8.6
	AMF +A1	284.7	10.6	309.4	12.8	350.0	12.6	521.3	25.6	219.3	9.6	199.0	12.1
LSD (0.05)	Mineral(Min)	20.53	0.77	52.04	1.75	26.83	0.93	52.44	2.25	19.47	0.65	21.17	1.65
	Biofertilizers(Bio)	23.71	0.89	60.09	2.02	30.99	1.08	60.55	2.60	22.49	0.75	24.44	1.91
	Min *Bio	26.51	0.99	67.18	2.25	34.64	1.20	67.70	2.91	25.14	0.84	27.33	2.02

** . A1: Acidithiobacillus ferrooxidans A1; AMF= Arbuscular Mycorrhizal Fungi [§]. R.P.: rock phosphate Each value is average of three replicate*

*L.S.D value: Least Significant difference between two values of mineral treatments without bio-inoculum(Min); between biofertilizer treatments without mineral treatments(Bio) and between two values in the same parameters (Min*Bio), according to Duncan's at 5% level*

Table 4. The weight and nutrients content of onion bulbs as influenced by different fertilizer treatments under field conditions after 90 days

Treatments		Weight of onion bulb		Nutrients Content Of Onion Bulbs						
				N		P		K		Pyruvate (µ moles/ g f.w #)
Mineral fertilizer	Biofertilizers	Fresh weight (g/bulb)	Dry weight (g/ bulb)	%	Uptake (mg/plant)	%	Uptake (mg/plant)	%	Uptake (mg/ plant)	
Un amended (control)	Uninoculated (Control)	34.5	5.24	0.92	48.2	0.77	40.3	0.87	45.6	6.9
	A. ferrooxidans A1	48.9	5.86	2.06	120.7	1.29	75.6	1.87	110.0	8.4
	AMF*	39.5	5.68	1.61	91.4	1.52	86.3	1.69	96.0	8.0
	AMF + A1*	52.1	6.15	2.59	160.0	1.85	113.8	2.23	137.1	9.6
N P K	Un inoculated	35.9	5.38	1.75	94.2	1.00	53.8	2.35	126.4	8.4
	A. ferrooxidans A1	48.4	6.40	2.85	182.4	1.52	97.2	2.71	173.4	10.8
	AMF	39.1	5.99	1.99	119.2	1.54	92.2	1.92	115.0	9.8
	AMF + A1	55.0	6.93	2.98	206.5	1.56	108.1	2.82	195.4	11.1
N P K + S	Un inoculated	35.4	5.89	2.12	124.8	0.96	56.5	1.52	90.0	9.5
	A. ferrooxidans A1	52.4	6.55	2.55	167.0	1.48	97.0	2.74	180.4	11.2
	AMF	37.7	6.28	2.43	152.6	1.69	106.1	2.39	150.0	10.7
	AMF + A1	64.1	6.89	2.98	205.3	1.74	120.8	3.20	220.4	11.2
N K + R.P§	Un inoculated	28.1	5.73	2.24	128.3	0.87	50.0	1.72	100.0	8.9
	A. ferrooxidans A1	41.7	6.79	2.36	160.2	1.57	106.6	2.71	184.0	9.2
	AMF	36.2	7.42	2.43	180.3	1.69	125.3	3.20	237.4	9.3
	AMF + A1	50.6	7.43	2.77	205.8	1.87	138.9	2.67	198.3	11.2
N K + S	Un inoculated	53.0	6.66	2.55	169.8	0.97	64.6	1.98	131.8	8.2
+ R.P	*A. ferrooxidans* A1	54.7	7.71	2.82	217.4	1.65	127.2	3.18	245.1	10.6
	AMF	47.8	7.18	3.00	215.4	1.96	140.7	3.22	231.2	9.2
	AMF + A1	64.7	9.96	3.26	324.7	2.15	214.1	3.46	344.6	11.8
LSD (0.05)	Mineral(Min)	0.5	1.00	0.41	0.79	0.15	0.18	0.42	0.57	0.10
	Biofertilizers(Bio)	1.5	1.16	0.47	0.91	0.16	0.21	0.48	0.66	0.12
	Min * Bio	2.9	1.29	0.53	1.02	0.17	0.24	0.54	0.74	0.13

** . A1: Acidithiobacillus ferrooxidans A1; AMF= Arbuscular Mycorrhizal Fungi §. R.P.: rock phosphate Each value is average of three replicate*
*L.S.D value: Least Significant difference between two values of mineral treatments without bio-inoculum(Min); between biofertilizer treatments without mineral treatments(Bio) and between two values in the same parameters (Min*Bio), according to Duncan's at 5% level*

The highest values of these parameters were 331.2 mg CO_2/100 g soil and 30.2 µg TPF /100g soil day^{-1} with dual inoculation amended with recommended dose of nitrogen, potassium and phosphours as superphosphate, followed by the same inoculated treatment with recommended dose of NK+R.P+S being 327.8 mg CO_2/100 g soil and 29.2 µg TPF /100g soil day^{-1} in respective order.The lowest measured

Data in Table 5 shows that the highest numbers of AMF spores and colonization percentage of AMF were obtained from added recommended dose of nitrogen, potassium, sulphur and rock phosphate to inoculated plants with AMF + *A. ferrooxidans* A1 after 60 days of planting. The corresponding values were 400 spore/100 g soil and 98% for number of mycorrhizal spores and colonization percentage, respectively. We observed that *Acidithiobacillus ferrooxidans* inoculant enhanced significantly the colonization percentage and number of spores with all mineral fertilizer treatments compared with AMF inoculation singly. The inoculation with AMF + A1 increased the biological properties the rhizosphere of maize plant more than the single inoculation with either AMF or *Acidithiobacillus*.

3.2.2.2 NPKS in maize rhizosphere

After 60 days, significant differences were observed for N, P, K, S rhizosphere content between the control treatments (uninoculated) and inoculated plants with AMF and *A. ferrooxidans* either singly or in a mixture form (Table 6). The highest recorded figures were obtained by dual inoculation treatment with all mineral fertilizers treatments during the growth period. The highest values of nutrients were obtained after 90 days of cultivation whereas the lowest values were found at the harvest time (120 days). The highest values of nitrogen, phosphours, potassium and sulphur in the rhizosphere were recorded with dual inoculants (AMF+ *A. ferrooxidans*A1) after 90 days of planting. The corresponding values of nitrogen and phosphorus were 396.7 and 14.3 ppm in presence of NK+R.P fertilizer whereas 373.8 ppm and 24.1 mg SO_4^{-2} for potassium and sulphur in soil amended with NK+R.P+S fertilizer, in respective order (Table 6). In this respect, Ochs [33] showed that biological weathering or biochemical is made by microorganisms which produce organic acids, phenolic compound and parameters were recorded after 120 days of cultivation. Similar results were obtained by Chu et al. [32] that the fertilization greatly increased soil microbial biomass CO_2 evolution and dehydrogenase activity after long-term application. The increased biomass and activity by mineral fertilization may be derived from the increased root biomass and exudates because of greater crop yields by fertilization. siderophores. Soluble organic acids affecting rock phosphate weathering in soil could be high molecular weight or low molecular produced by plant roots and soil microorganisms.

3.2.2.3 NPK content of maize seeds

The data in Table 7 showed that nitrogen, phosphours and potassium content of maize seeds were remarkably simulated with biofertilizer treatments. The addition of recommended dose of NK+S+R.P with AMF + *A. ferrooxidans*A1 led to significant increase of nitrogen percentage and uptake of plant compared to other treatments. The highest values of nitrogen percentage and plant uptake were 0.993% and 172.2 mg/g plant respectively with the abovementioned treatment. The dual inoculant gave the highest value of phosphours (0.537%) and potassium (1.86%) content of seeds with NK+ S +R.P. MF inoculant mobilizes P from R.P more efficiently than those indigenous AM fungi which suggest that R.P weathering is highly dependent to the fungal symbiont and the interaction between AMF *Acidithiobacillus* and plant root exudates which increase the the mobilization of nutrient through the metabolic reactions.

3.2.2.4 Yield parameters

Maize plants showed high response to AMF and *Acidithiobacillus ferrooxidans* A1 inoculation either singly or in a mixture forms (Table 7). There were significant increases in dry weight of 100 seeds and ear weight in presence of AMF + *Acidithiobacillus ferrooxidans* A1 with NK+RP+S or NK+RP, compared to uninoculated plants with the same mineral fertilizer. Dry weight of shoot and dry weight of 100 seeds as affected by dual inoculant which gave the best recorded values especially with NK+ S+R.P treatment equal to 266.2% and 41.8% increases respectively over uninoculated plants with recommended dose of NPK (Table7).

Table 5. Biological characters of maize rhizosphere and AMF parameters as influenced by different fertilizer treatments under field conditions

Treatment		Sample time									AMF after 60 days	
Mineral Fertilizer	Biofertilizers	60 days			90 days			120 days			spores /100 g Soil	Root colonization (%)
		T.SOB* ($X10^3$)	CO_2*	DHA*	T.SOB ($X10^3$)	CO_2	DHA	T.SOB ($X10^3$)	CO_2	DHA		
Un -amended (control)	Uninoculated(Control)	38	163.0	10.0	56	182.5	13.3	51	168.7	8.7	83.0	53
	Thiobacillus A1	45	237.3	15.3	83	243.0	19.0	54	224.7	12.5	108.0	71
	AMF #.	49	194.7	13.2	93	200.8	15.9	73	188.6	11.0	225.0	87
	AMF +A1 #	59	240.0	21.6	94	269.5	27.0	101	237.3	14.7	233.0	90
N P K	Un inoculated	59	197.3	15.9	65	247.3	16.1	43	125.6	11.8	116.0	64
	A. ferrooxidans A1	98	221.6	19.5	100	264.5	26.7	71	145.7	12.8	175.0	72
	AMF	74	201.3	24.1	90	254.2	25.9	83	193.6	12.0	258.0	85
	AMF + A1	101	302.1	24.8	107	331.2	30.2	78	251.5	15.7	283.0	95
N P K + S	Un inoculated	63	198.6	15.7	64	224.3	18.2	51	122.7	12.6	133.0	69
	A. ferrooxidans A1	92	219.1	20.6	93	268.5	25.1	63	171.4	14.8	200.0	75
	AMF	79	203.2	22.3	89	265.7	26.0	68	218.0	13.4	250.0	88
	AMF +A1	105	248.1	28.5	114	281.8	29.2	90	333.9	15.0	283.0	95
N K + R.P #	Un inoculated	62	198.6	10.8	69	243.5	17.3	54	143.8	14.2	100.0	75
	A. ferrooxidans A1	84	216.2	11.5	109	291.0	26.1	88	174.7	25.6	158.0	81
	AMF	82	210.5	13.5	89	297.9	25.6	77	191.7	24.3	266.0	85
	AMF +A1	98	235.0	18.4	114	304.8	28.9	102	200.1	26.7	291.0	95
N K + S + R.P	Un inoculated	60	179.1	12.4	83	228.1	14.7	53	158.1	11.7	166.0	68
	A. ferrooxidans A1	99	254.2	21.6	108	271.3	28.3	97	217.1	12.5	233.0	72
	AMF	95	243.5	18.9	115	245.9	25.1	74	180.9	13.5	275.0	86
	AMF + A1	105	235.8	23.7	116	327.8	29.2	103	293.7	19.9	400.0	98
LSD (0.05)	Mineral(Min)	11.36	36.63	2.76	13.99	64.43	3.12	9.02	21.93	2.38	84.67	10.50
	Biofertilizers(Bio)	13.12	42.30	3.18	16.15	74.40	3.60	10.40	25.32	2.75	109.31	14.95
	Min*Bio	14.66	47.30	3.56	18.05	83.19	4.03	11.64	28.31	3.08	146.66	18.00

*Each value is average of three replicate *. T.SOB=Total count of sulpher oxidizing bacteria (cfu/g dry soil); CO_2 = CO_2 evolution ($mgCO_2/100g$ soil); DH A=Dehydrogenase activity (µg TPF/100g soil Day^{-1}). #. AMF = Arbuscular Mycorrhizal Fungi; A1=A. ferrooxidans A1; # R.P= rock phosphate*

*L.S.D value: Least Significant difference between two values of mineral treatments without bio-inoculum(Min); between biofertilizer treatments without mineral treatments(Bio) and between two values in the same parameters (Min*Bio), according to Duncan's at 5% level*

Table 6. Concentration of nutrients N, P, K (ppm) and S (mg SO_4^{2-}/100 g soil) in rhizosphere of maize plants as influenced by different fertilizer treatments under field conditions

Treatment		Sampling time											
Mineral fertilizer	Biofertilizers	60 days				90 days				120 days			
		N	P	K	S	N	P	K	S	N	P	K	S
Un amended	Uninoculated(Control)	130.7	4.5	117.0	5.7	242.7	7.1	168.4	9.6	116.7	2.6	102.0	4.4
	A. ferrooxidans A1	163.3	8.0	157.3	7.6	284.7	10.9	293.8	15.7	186.7	7.3	150.2	8.0
	AMF*	168.0	8.5	164.5	7.5	289.3	12.6	230.8	15.6	149.3	8.1	137.8	5.1
	AMF + A1*	308.0	8.9	167.7	8.7	336.0	13.2	308.8	17.2	210.0	8.2	139.7	8.3
N P K	Un inoculated	205.0	4.7	149.9	6.9	308.0	9.8	261.3	10.7	149.3	4.6	141.7	6.3
	A. ferrooxidans A1	214.7	6.7	153.4	8.1	331.0	10.5	263.3	14.8	191.3	7.1	150.2	8.9
	AMF	233.3	9.2	178.1	8.3	322.0	13.0	326.3	11.5	219.3	6.6	146.3	7.6
	AMF +A1	336.0	9.9	182.0	9.5	345.3	13.4	327.8	18.0	242.7	7.6	154.7	8.3
N P K + S	Un inoculated	214.7	6.2	160.5	7.6	310.0	9.8	258.7	10.1	140.0	4.5	124.1	7.2
	A. ferrooxidans A1	233.3	7.8	175.5	8.4	331.3	9.9	280.2	16.3	200.7	6.9	137.1	7.9
	AMF	214.7	8.2	167.1	8.6	331.3	12.3	284.7	16.3	172.7	8.0	126.8	7.8
	AMF + A1	254.7	8.9	185.4	8.9	374.7	13.0	349.1	20.8	219.3	8.3	161.9	8.4
N K + R.P[§]	Un inoculated	210.0	6.9	159.9	7.8	345.3	9.5	248.3	16.0	154.0	4.0	128.7	7.0
	A. ferrooxidans A1	238.0	7.6	206.1	9.1	373.3	9.7	276.3	16.7	168.0	7.0	130.7	8.6
	AMF	233.3	8.4	172.9	8.5	373.3	11.3	289.3	16.2	205.3	7.2	148.2	7.9
	AMF + A1	350.0	8.5	252.2	9.1	396.7	14.3	313.9	17.8	219.3	7.9	152.8	9.1
N K + S + R.P	Un inoculated	200.0	7.0	141.0	7.4	322.0	9.7	262.6	10.7	177.3	6.9	137.8	6.0
	A. ferrooxidans A1	270.6	8.4	239.2	8.3	331.3	11.4	308.8	17.5	214.7	8.8	148.2	7.4
	AMF	270.7	8.8	236.6	8.3	350.0	13.0	269.1	13.8	214.7	8.5	144.4	6.5
	AMF + A1	354.3	10.2	242.5	9.6	359.3	13.9	373.8	24.1	233.3	9.4	170.3	9.0
.LSD (0.05)	Mineral(Min)	28.66	0.93	45.76	0.96	29.03	0.95	77.24	1.87	24.05	0.83	12.58	0.65
	Biofertilizers(Bio)	33.09	1.08	52.84	1.11	33.52	1.09	89.19	2.16	27.77	0.96	14.52	0.75
	Min* Bio	37.00	1.21	59.08	1.24	37.48	1.22	99.71	2.41	31.05	1.07	16.24	0.84

** A1: Acidithiobacillus ferrooxidans A1 ; AMF = Arbuscular Mycorrhizal Fungi [§]. R.P. : rock phosphate Each value is average of three replicate*

*L.S.D value: Least Significant difference between two values of mineral treatments without bio-inoculum(Min); between biofertilizer treatments without mineral treatments(Bio) and between two values in the same parameters (Min*Bio), according to Duncan's at 5% level*

Table 7. Yield parameters and N, P, K content of seeds of maize plants as influenced by different fertilizer treatments under field conditions after 120 days

Treatment		Yield parameters			NPK contents of seeds					
					N		P		K	
Mineral Fertilizer	Biofertilizers	Dry weight of shoot (g/ plant)	Ear weight (g/ear)	Dry weight of 100 grains (g)	%	Uptake (mg/100 seed)	%	Uptake (mg/100 seed)	%	Uptake (mg/100 seed)
Un amended	Uninoculated (control)	6.77	39.5	10.81	0.519	56.1	0.166	17.9	0.84	9.08
(control)	*A. ferrooxidans* A1	9.54	77.7	13.09	0.712	93.2	0.98	128.3	1.32	17.28
	AMF*	9.70	78.7	12.33	0.578	71.3	0.225	27.7	1.29	15.90
	AMF + A1*	13.10	151.9	13.61	0.742	100.9	0.290	39.5	1.54	20.96
N P K	Un inoculated	7.81	57.6	12.23	0.519	63.5	0.250	30.6	1.37	16.75
	A. ferrooxidans A1	12.48	163.6	13.64	0.890	121.4	0.284	38.7	1.63	22.23
	AMF	14.80	160.9	13.24	0.816	108.0	0.355	47.0	1.63	21.58
	AMF +A1	16.30	181.3	14.41	0.963	138.8	0.500	72.0	1.64	23.63
N P K + S	Un inoculated	14.5	127.5	13.86	0.697	9.6.6	0.200	27.7	1.38	19.13
	A. ferrooxidans A1	18.20	172.6	15.53	0.801	124.4	0.252	39.1	1.40	21.74
	AMF	15.80	154.4	14.52	0.712	103.4	0.360	52.3	1.74	25.26
	AMF + A1	23.60	173.3	17.81	0.860	153.2	0.398	70.8	1.76	31.34
N K + R.P[§]	Un inoculated	11.90	123.8	13.89	0.593	82.4	0.200	27.8	1.38	19.17
	A. ferrooxidans A1	15.17	162.6	16.94	0.756	128.1	0.356	60.3	1.54	26.09
	AMF	16.50	164.3	14.29	0.801	114.5	0.340	48.6	1.56	22.29
	AMF + A1	25.30	166.3	18.20	0.846	153.9	0.398	72.4	1.67	30.39
N K + S + R.P	Un inoculated	10.07	90.3	15.30	0.623	95.3	0.319	48.8	1.31	20.04
	A. ferrooxidans A1	13.52	169.8	16.21	0.917	148.6	0.340	55.1	1.66	26.91
	AMF	17.20	161.5	15.91	0.905	143.9	0.404	64.3	1.67	26.56
	AMF + A1	28.60	171.2	17.34	0.993	172.2	0.537	93.1	1.86	32.25
LSD (0.05)	Mineral(Min)	1.55	2.15	1.28	0.08	0.10	0.06	0.17	0.22	0.56
	Biofertilizers(Bio)	2.8733	5.50	1.48	0.10	0.19	0.07	0.22	0.25	0.65
	Min*Bio	4.98	9.43	1.65	0.11	0.25	0.08	0.24	0.28	0.73

**. A1: Acidithiobacillus ferrooxidans A1; AMF = Arbuscular Mycorrhizal Fungi [§]: R.P. : rock phosphate Each value is average of three replicate*

*L.S.D value: Least Significant difference between two values of mineral treatments without bio-inoculum(Min); between biofertilizer treatments without mineral treatments(Bio) and between two values in the same parameters (Min*Bio), according to Duncan's at 5% level*

These results are in compatible with previous finding which demonstrated the positive effects of inoculation of sulphur oxidizing bacteria like Thiobacillus sp on grain yield and grain protein content in soybean [34]. As a result, the natural oxidation of elemental sulphur caused to increase soil sulphate content. The availability of SO4-2 is important for protein synthesis in plant. Additionally Mostafavian et al. [35] found that applying sulphur, alone or in combination with Thiobacillus had stimulating effect on seed yield.

4. CONCLUSIONS

Based on the our results, it can be concluded that the inoculation of onion and maize plants by arbuscular mycorrhizal fungi and *Acidithiobacillus ferrooxidans* A1 with the application of the recommended dose of sulphur and rock phosphate fertilizers has a remarkable positive impact on the quality and quantity of onion bulbs and yield of maize plants .

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Steinshamn H, Thuen E, Bleken MA, Brenoe UT, Ekerholt G, Yri C. Utilization of nitrogen (N) and phosphorus (P) in an organic dairy farming system in Norway. Agriculture, Ecosystems & Environment. 2004;104:509–522.
2. Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS. Role of soil microorganisms in improving P nutrition of plants. Plant Soil. 2002;245:83–93.
3. Williams JS, Cooper RM. Elemental sulphur is produced by diverse plant families as a component of defence against fungal and bacterial pathogens. Physiological and Molecular Plant Pathology. 2003;63:3–16.
4. Vidyalakshmi R, Paranthaman R, Bhakyaraj R. Sulphur oxidizing bacteria and pulse nutrition - A Review. World Journal of Agricultural Sciences. 2009;5: 270-278.
5. Wainwright M. Sulphur oxidation in soils. Adv. Agron. 1984;37:346-396.
6. Marschner H. Mineral nutrition in higher plants. Academic Press, Harcourt Brace Jovanovich Publisher. 1998;674.
7. Smith SR, Barker SJ. Plant phosphate transporter genes help harness the nutritional benefits of arbuscular mycorrhizal symbiosis. Trends Plant Sciences. 2002;75:189-190.
8. Balloei F, Ardakani MR, Rejali F, Ramzanpoor R, Alizade GR, Mohebbati F. Effect of thiobacillus and mycorrhiza fungi under different levels of sulfur on yield and yield components of soybean. International symposium “Root Research and Application” Root RAP, 2-4, Boku- Vienna, Austria; 2009.
9. Bolandnzar SA, Neyshabouri MR, Aliasgharzad N, Chaparzadeh N. Effect of mycorrhizal colonization on growth parameter of onion under different irrigation and soil conditions. Pakistan Journal of Biological Sciences. 2007;10: 1491-1495.
10. Mohamed Amal A, Eweda Wedad EE, Heggo A M, Hassan Enas A. Effect of dual inoculation with arbuscular mycorrhizal fungi and sulphur-oxidising bacteria on onion (*Allium cepa* L.) and maize (*Zea mays* L.) grown in sandy soil under green house conditions. Annals of Agricultural Science. 2014;59:109–118.
11. Baruah TC, Barthakur HP. A textbook of soil analysis. Vikas Publishing Housing, PVTLTD, New Delhi. 1997;35-67.
12. Jackson ML. Soil chemical analysis. Prentice-Hall of India Private Limited, New Delhi; 1973.
13. Ausubell FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Current protocols in molecular biology. Greene Publishing Associates, Wiley Inter science, New York; 1987.
14. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. Nucleic acid techniques in bacterial systematics. Wiley, New York; 1991.
15. Altschul SF, Boguski MS, Gish W, Wootton JC. Issues in searching molecular sequence databases. Nature Genetics. 1997;6:119-129.
16. Vishniac W, Santer M. The Thiobacilli. Bacteriological. Rev. 1957;21:185-213.
17. Gerdemann JW, Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society. 1963;46:235.
18. Schenck NC, Perez Y. Manual for the indentification of VA Mycorrhizal fungi. 3rd.

ed., INVAM. Univ. of Florida, Gainesville. 1990;268.
19. Casida LE, Klein DA, Santoro T. Soil dehyderogenase activity. Soil Science. 1964;98:371-376.
20. Pramer D, Schmidt EL. Experimental soil microbiology. Burgess Publisher Company. Minnesota, USA. Sons Ltd. Beecles and London. 1964;31-32.
21. Trouvelot A, Kough JL, Gianinazzi-Pearson V. Measure dutaux de mycorrhization VA d' un system radiculaire recherche' de methods d'estimation ayant une signification functionelle In: Gianinazzi- Pearson V, Gianninazzi PS. editors. Physilological and genetical aspects of mycorrhizae. INRA, Paris. 1986;217.
22. Cottenie A, Verloo M, Kiekns L, Velghe G, Comerlynek R. Chemical analysis of plants and soil lab. Anal. And Agroch. St. State Univ., Ghent, Belgium. 1982;63.
23. Issam IB, Antoin HS. Methods of analysis for soils of arid and semi-arid regions. Food and Agricultre organization of the United Nations, Rome Italy. 2007;89-90.
24. Gordon EA, Dian MB. Modified method for the determination of pyruvic acid with dinitrophenylhydrazine in the assessment of onion pungency. Journal of the Science of Food and Agriculture. 2003;83:1210–1213.
25. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. John Wiley and Sons, Inc. New York, USA; 1984.
26. Moore E, Arnscheidt A, Kruger A, Stromple C, Mau M. Simplified protocols for the preparation of genomic DNA from bacterial cultures. In: Akkermans ADL, van Elsas JD, de Bruijn FJ (Eds). Molecular microbial ecology manual, Dordrecht, The Netherlands: Kluwer Academic Press. 1.6.1. 1999;1-15.
27. Chendrayan K, Adhya TK, Sethunathan N. Dehydrogenase and invertase activities of flooded soils. Soil Biology and Biochemistry. 1980;12:271-273.
28. Alloush GA, Clark RB. Maize response to phosphate rock and arbuscular mycorrhizal fungi in acidic soil. Communications in Soil Science and Plant Analysis. 2001;32:231-254.
29. Bagayoko M, George E, Römheld V, Buerkert A. Effects of mycorrhizae and phosphorus on growth and nutrient uptake of millet, cowpea and sorghum on a West African soil. Journal of Agricultural Science. 2000;135:399-407.
30. Nassar KEM, Magda A, Ewais A, Mohmoud Awatef A. Raising of the quantity and quality of onion crop by foliar application of phosphorus and sulphur. Journal of Advance Agricultural Research. 2006;11:410-418.
31. Mahaveer PS, Alok A. Enhanced growth and productivity following inoculation with indigenous AM fungi in four varieties of onion (*Allium cepa* L.) in an alfisol. Biological Agriculture and Horticulture. 2000;18:1-14.
32. Chu H, Lin X, Fujii T, Morimoto S, Yagi K, Hu J, Zhang J. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. Soil Biology & Biochemistry. 2007;39:2971-2976.
33. Ochs M. Influence of humidified and non-humidified natural organic com-pounds on mineral dissolution. Chemical Geology. 1996;132:119–124.
34. Shinde DB, Kadam RM, Jadhav AC. Effect of sulfur oxidizing micro-organisms on growth of soybean. J. Maharashtra Agric. Univ. 2004;29:305-307.
35. Mostafavin SR, Pirdashti H, Ramaznpour MR, Andarkhor AA, Shahsavari A. Effect of Mycorrhizal, Thiobacillus and Sulfur Nutrition on the chemical composition of soybean (*Glycine max* (L.) Merr. seed. Pakistan Journal of Biological Sciences. 2008;11:826-835.

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