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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

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Maize (*Zea mays* L)

Abstract Thirty isolates of *Thiobacillus* sp. were successfully isolated on Thiobacillus enrichment medium from different plants rhizosphere. *Thiobacillus* A1, *Thiobacillus* A2 gave the lowest pH and the highest total sulphate in liquid medium after one week. These two isolates were used as bioinoculants. The effect of inoculation with the two selected isolates and/or arbuscular mycorrhizal fungi (AMF) on onion and maize plants was studied in pot experiment under green house conditions. Mineral fertilizers were applied at the recommended dose of N, P, K and sulphur(S) besides rock phosphate (RP) as P fertilizer. Results showed that, inoculation gave significant increases in N, P, K and S concentrations in plants rhizosphere soil at the sampling periods of 60 and 90 days from planting. Highest values of nutrient concentrations were found in soils fertilised with NK + S + rock phosphate (RP) and inoculated with AMF + *Thiobacillus* A1. Total sulphur-oxidising bacterial counts, dehydrogenase activity (μg TPF/100 g dry soil Day^{-1}) and CO_2 evolution (mg CO_2/100 g soil) were determined in rhizosphere of the tested plants. Soil biology was affected by either mineral or biofertilizer treatments. Significant differences were found for samples collected after 60 days for onion and 90 days for maize fertilised with NK + S + RP and inoculated with AMF + *Thiobacillus* A1 which gave the highest significant differences over control. Mycorrhizal spores number and AMF infection percentages in plants roots greatly affected by AMF inoculation especially when combined with *Thiobacillus* A1 after 60 days from planting. Dry weight of onion bulbs and maize shoots as well as NPK contents significantly affected by AMF and *Thiobacillus* inoculations than the control plants.

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Introduction

During the 19th and early 20th centuries inorganic compounds containing nitrogen, potassium and phosphorus were synthesized and used as mineral fertilizers (loynachan et al., 1993). Due to increasing human population, fertilizers were used to increase crop production and meet the rising demands for food. Increases in the production cost and the hazardous nature of chemical fertilizers for the environment led to resurgence of the interest in the use of biofertilizers for enhanced environmental sustainability, lower cost production and good crop yield (Egamberdiyera, 2007). Phosphorus (P) and sulphur (S) are two necessary nutrients that increase the growth and yield of plants. Deficit of these nutrients in soil, as usually compensated by using of chemical fertilizers, but these fertilizers have harmful effects on the environment and decrease the quality of agricultural products. Therefore, biological fertilizers are more interested for using in agricultural ecosystems. Endomycorrhizal fungi are an integral part of most plant species in nature. It is well documented that application of mycorrhizal fungi caused increases in water and nutrient absorption and transmission special phosphorus to host plants cells and improve growth as well as photosynthesis which produce more assimilation. Also mycorrhizae has synergistic effects with most of other microorganisms that have increasing effect on yield and yield components of most crops (Lukiwatid and Simanungkalit, 2002; Marulanda et al., 2003; Heggo and Barakah, 2003). Mycorrhizal fungi can increase drought resistance of plants (Barakah and Heggo, 1998; Abdel-Fattah and Shabana, 2002; Davies et al., 2002) to some extent through enhanced water uptake at low soil moisture levels (Davies et al., 1996). Increased stomata sensitivity to leaf air vapour pressure deficient and increased root hydraulic conductivity by lowered leaf osmotic potential for greater turgor maintenance (Auge, 2001). Accumulation of some solutes in mycorrhizal plants may serve as osmoregulators (Meddich et al., 2002) and improved nutrition particularly P (El-Tohamy et al., 1999). Sulphur is one of the essential plant nutrients classified as secondary nutrient. It is essential for all plants and is indispensable for the growth and metabolism. Thiobacilli play an important role in sulphur oxidation in soil. Sulphur oxidation is the most important step of sulphur cycle, which improves soil fertility. It results in the formation of sulphate, which can be used by plants, while the acidity produced by oxidation helps to solubilise plant nutrients and improves alkaline soils. These bacteria can solubilise the soil minerals through the production of H_2SO_4 that reacts with these non-soluble minerals and oxidised them to be available nutrients (i.e., Mg, Al, Mn, K and P) to the cultivated plants. Mycorrhizal fungi interact with a wide assortment of organisms in the rhizosphere. However, due to the effect of the interaction between *Thiobacillus* bacteria and mycorrhizae, the previous studies revealed that the result of this interaction can be in three aspects, i.e., positive, neutral and negative on either the Mycorrhizal root association or on a particular component of the rhizosphere (Mostafavin et al., 2008).

Onion (*Allium cepa* L.) is one of the most important commercial crops among vegetables and spices. Onion is only vegetable in which India figures prominently in the world for production and export (Singh and Joshi, 1978). Onion has immense medicinal values and is useful in fever, dropsy, catarrh and chronic bronchitis. Maize (*Zea mays* L.), a crop species of tropical/subtropical origin, was cultivated at higher geographical latitudes. Therefore, it was important to optimise the yield potential of maize under marginal climatic conditions, in particular the combination of suboptimal temperate and moderately high light intensity.

The aim of this work, uses to study the interaction between arbuscular mycorrhizal fungi (AMF) and *Thiobacillus* bacterial inoculants on onion and maize plants grown in sandy soil under green house conditions to evaluate the effect of their combination on plant growth parameters.

Materials and methods

*Isolation of **Thiobacillus** sp.*

Thiobacillus sp. was isolated from rhizosphere of four plants (maize, onion, eggplant and cabbage) grown in four fields at Ismailia Experimental Station of ARC and Mainshiat Al-Kanater (Kalubia). Ten gm of rhizosphere sample was inoculated into 250 ml of *Thiobacillus* enrichment medium (Vishniac and Santer, 1957). The medium was incubated at 30° for 7 days for activation without shaking. Thereafter, the medium was replaced by adding 10 ml of the growth to 1L of fresh medium; the process was repeated for five transformations in order to ensure the suppression of growth of any anaerobic bacteria in the soil and activate sulphide oxidising bacteria. The development of turbidity in the medium was assumed to be due to microbial growth. Turbid samples were streaked onto solid medium, incubated under the same conditions and the single colonies were further purified using two or three successive streak plate dilutions. The Gram staining and microscopic examinations were used to monitor the purification process and the collected isolates were further assessed for their morphological characteristics.

Sulphide oxidation activity test

For this purpose, each of the collected isolates was inoculated separately into 100 ml of *Thiobacillus* enrichment medium, uninoculated medium was used as a control. Flasks were incubated at 30 °C/for 2 days. Determination of total sulphate and pH were carried out at the end of incubation period according to Issam and Antoin (2007).

Preparation of inocula

Bacterial inoculum

Active cultures of the efficient isolates of *Thiobacillus* were grown separately in *Thiobacillus* enrichment medium (Vishniac and Santer, 1957). Cell suspensions containing about 6×10^8 cfu/ml of eachisolate were used as a standard inoculum. Inoculated treatments were inoculated with 20 ml of cell suspension for each pot at planting.

Extraction and characterisation of AMF

Spores of arbuscular mycorrhizal fungi (AMF) were extracted from the rhizosphere of maize plants grown in the Ismailia Experimental Station of ARC by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The extracted spores were kept moist by storing at 4 °C until used. The collected AM fungi were identified according to the key of Schenck and Perez (1990) using morphological characteristics of hyphae, attached hyphae, chlamydospores, azygospores and sporocarp. The extracted fungal spores were a mixture of different genera as follow: *Glomus* sp. and *Gigaspora* sp.

Mycorrhizal inoculation was performed with 10 ml of spore suspension (28 spores/ ml) in each AMF inoculated treatments at planting.

Pot experiment

Host plant and soil used

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.

Sandy soil was collected from newly reclaimed soil at Ismailia. Soil was subjected to physical and chemical analysis (Table 1) along with microbiological analysis. The microbiological analysis of the used soil showed that the total microbial count was 3×10^6 (cfu/g dry soil) whereas it did not contain any spores of AMF and sulphur-oxidising bacteria.

The mechanical and chemical characteristics of the used soil were determined at Soil Department Soils, Water & Environment Research Institute, Agricultural Research Centre, ARC, Giza, Egypt.

Experimental design

Two separate pot experiments were carried out in the green house of Soils, Water & Environment Research Institute, Agricultural Research Centre, ARC, Giza, Egypt to study the effect of the two efficient isolates of *Thiobacillus* individually or in combination with mycorrhizae, on growth and NPK contents of onion and maize plants in presence of the recommend dose of nitrogen, potassium and phosphorus which added as rock phosphate or super phosphate, with or without sulphur. The trials were planted in pots with 35 cm diameter and 15 cm depth which filled with 7 kg newly reclaimed sandy soil. Rock phosphate (RP) contain (10% P) or super phosphate (P) contain (15.5% P_2O_5), were added in rate of 107 kg and 300 kg/feddan for onion while the rates of maize were 86 kg and 200 kg/feddan for maize plants, respectively. The recommended dose of potassium nitrate, as a source of nitrogen and potassium, is 75 unite N/feddan for onion plant and 100 unite N/feddan for maize plant. The sulphur powder was added at recommended dose (1.7 ton/feddan), as a source of S for tested plants. The previous mineral fertilizers were mixed with soil before sowing. Each pot was planted with 3 bulbs of onion or 4 seeds of maize. Pots of each plant species were divided into 5 groups for mineral fertilizer (control, NPK, NPK + S, NK + R.P, NK + S + RP) and each group were further subdivided into 6 biofertilizers groups (control, SOB isolate (A1), SOB isolate (A2), AMF, SOB (A1) + AMF, SOB (A2) + AMF). Pots were planted in winter and summer seasons for onion and maize, respectively. Three replicates were prepared for each treatment. Plants were left to grow under green house conditions and watered with tap water to keep moisture content at 60% water holding capacity. Pots were arranged in green house in completely randomized design.

Parameters measured

Rhizosphere soil samples were taken after 60 and 90 days of sowing and subjected to some chemical and microbiological analyses. Plants were harvested after 90 days from planting. Dry matter of bulbs and maize plant were measured after drying in oven at 70 °C for 3 days, Bulbs and shoot of maize were ground to analyse P, K and N contents. The percentage of maize and onion root infection with AM Fungi was estimated after 60 days of planting as described by Trouvelot et al. (1986).

Chemical analysis

Onion bulbs and maize plants after harvesting as well as soil samples were analysed for P, K and N according to Cottenie et al. (1982) and available sulphur in soil was determined as described by Issam and Antoin (2007).

Biological analysis

Some biological activities were determined in collected soil samples from onion and maize rhizospheres at periods of 60 and 90 days from planting. Dehydrogenase activity (DHA) (µg TPF/100 g dry soil day^{-1}) and CO_2 evolution (mg CO_2/ 100 g soil) were determined as described by Casida et al. (1964) and Pramer and Schmidt (1964), respectively as well as total counts of bacteria and total sulphur-oxidising bacterial counts were done. Numbers of AMF spores per 100 g of rhizosphere samples were estimated after 60 days of planting (Gerdemann and Nicolson, 1963).

Statistical analysis

Data were statistically analysed according to Gomez and Gomez (1984). L.S.D test at 5% level of significance was used for comparison between the means of different treatments.

Table 1 Physiochemical analysis of the used soil.

Total $CaCo_3$	Organic matter (%)	Particle size distribution (%)				Texture
		Corse sand	Fine sand	Silt	Clay	
(a) Mechanical analysis						
1.4	0.40	4.4	80.5	10.6	4.5	Sandy

pH 1:2.5	EC dS m^{-1}	Soluble ion (meq/L)							Available nutrients		
		Cations				Anions			µg g^{-1}		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}	N	P	K
(b) Chemical analysis											
7.92	3.1	11.7	3.47	13.06	1.77	0.69	11.4	17.91	85	25	125

Table 2 Concentrations of N, P, K (ppm) and S (mg SO_4/100 g soil)in rhizosphere of onion and maize plants influenced by endomycorrhizal fungi (AMF) and/ or *Thiobacillus* isolates (A1, A2) inoculation under different treatments of mineral fertilizers after 60 days of planting.

Treatments		Plant species							
Mineral fertilizer	Biofertilizers	Onion				Maize			
		N	P	K	S	N	P	K	S
Un amended (Control)	Un inoculated	107.3	5.3	183.2	8.4	102.7	3.6	126.1	4.1
	Thiobacillus A1	168.0	6.5	253.5	14.1	140.0	4.3	157.3	7.5
	Thiobacillus A2	121.3	6.9	206.6	11.3	116.7	4.0	117.0	4.2
	AMF	135.3	9.2	291.2	9.4	107.3	4.3	164.5	6.0
	AMF + A1	219.3	9.4	339.2	16.8	172.7	6.1	182.0	8.0
	AMF + A2	154.0	6.9	271.7	9.9	107.3	4.3	137.8	4.3
N P K	Un inoculated	163.3	6.4	251.1	10.4	116.7	4.2	137.8	6.3
	Thiobacillus A1	224.0	10.8	291.2	18.9	149.3	5.2	153.4	8.5
	Thiobacillus A2	168.0	8.2	305.5	10.8	121.7	5.1	148.0	5.6
	AMF	228.6	9.6	360.0	11.7	154.0	5.8	220.0	7.0
	AMF + A1	247.3	10.9	496.5	22.5	182.0	6.5	198.0	8.6
	AMF + A2	144.6	7.5	415.3	12.5	135.3	4.7	166.4	6.7
N P K + Sulphur (S)	Un inoculated	130.6	8.0	174.1	11.4	107.3	5.5	128.0	5.4
	Thiobacillus A1	205.3	10.6	383.5	18.0	177.3	6.9	175.5	7.3
	Thiobacillus A2	172.3	8.1	251.1	11.1	112.0	5.1	167.0	7.0
	AMF	182.0	10.5	549.1	12.1	163.3	5.8	163.8	7.5
	AMF + A1	242.6	11.2	563.5	21.0	186.7	6.8	218.4	8.5
	AMF + A2	172.6	8.4	375.7	11.6	126.0	5.7	173.5	6.7
N K + Rock Phosphate (RP)	Un inoculated	156.0	6.2	262.5	11.2	107.3	3.7	159.9	6.8
	Thiobacillus A1	233.3	9.9	388.0	19.9	177.3	5.5	197.6	9.8
	Thiobacillus A2	214.6	8.7	270.3	12.5	135.3	4.6	157.9	7.2
	AMF	210.0	10.7	384.6	12.8	163.3	6.2	194.8	8.5
	AMF + A1	266.0	11.2	563.5	21.0	210.0	7.9	220.5	8.1
	AMF + A2	178.6	8.4	237.6	10.9	149.3	5.8	172.9	7.4
N K + S + R.P	Un inoculated	172.6	7.3	206.6	9.5	144.7	4.0	130.7	7.0
	Thiobacillus A1	233.3	10.9	515.1	17.3	200.7	6.8	195.6	7.9
	Thiobacillus A2	187.0	8.8	265.8	10.4	149.3	4.9	148.2	6.3
	AMF	228.6	11.0	343.8	12.5	186.7	6.4	227.5	7.5
	AMF + A1	273.0	11.1	648.0	23.6	210.0	6.6	242.5	8.7
	AMF + A2	224.0	9.1	314.6	14.7	163.3	5.7	207.4	7.2
LSD (0.05)	Mineral	22.48	0.83	55.39	2.22	20.28	0.79	25.15	1.01
	Biofertilizers	29.02	1.08	71.51	2.86	26.18	1.03	32.47	1.30
	Min * Bio	31.79	1.18	78.33	3.13	28.68	1.13	35.57	1.43

N: Nitrogen P: Phosphorus K: Potassium S: Sulphur & Biofertilizers: AMF, *Thiobacillus* A1, *Thiobacillus* A2 and Mixture of A1 or A2 with AMF.

Results and discussion

Isolation and purification of ***Thiobacillus***

Thirty isolates with distinct colony morphology were obtained on *Thiobacillus* enrichment medium, each consisting of small rod-shaped cells, Gram-negative and motile. The isolates were recognised as *Thiobacillus* sp. because of their ability to aerobic growth and use thiosulphate as a sole source of energy. In addition, all isolates could use carbon dioxide as a sole source of carbon. Two isolates of *Thiobacillus* sp. (*Thiobacillus* A1, *Thiobacillus* A2) were chosen from 30 isolates from Ismailia and Mainshiat Al-Kanater, which gave the lowest pH and highest total sulphate in *Thiobacillus* enrichment liquid medium after one week. The result were confirmed by Buisman et al. (1991) who reported that certain strains of sulphur-oxidising bacteria belonging to genus *Thiobacillus* can oxidise free sulphide to elemental sulphur. During the process, they derive energy for growth from the oxidation of reduced sulphur compounds. These isolates were further used for onion and maize inoculation.

Effect of inoculation with arbuscular mycorrhizal fungi and Thiobacillus isolates on different parameters of onion and maize plants

Available nutrients in rhizosphere soil

Data present in (Tables 2 and 3) clearly show that the N, P, K, S concentrations in rhizosphere of onion and maize plants positively responded to arbuscular mycorrhizal fungi (AMF) + Sulphur-oxidising bacteria (SOB) *Thiobacillus* isolates (A1, A2) inoculation with each mineral fertilizers

Table 3 Concentrations of N, P, K (ppm), S (mg SO_4/100 g soil) in rhizosphere of onion and maize plants influenced by endomycorrhizal fungi (AMF) and/or *Thiobacillus* isolates (A1, A2) inoculation under different treatments of mineral fertilizers after 90 days of planting.

Treatments		Plant species							
Mineral fertilizer	Biofertilizers	Onion				Maize			
		N	P	K	S	N	P	K	S
Unamended (Control)	Un inoculated	74.6	2.6	111.1	4.2	116.7	6.3	156.7	9.9
	Thiobacillus A1	121.3	4.3	146.8	6.6	172.7	7.1	273.6	14.0
	Thiobacillus A2	79.3	4.0	115.0	5.0	168.0	6.9	205.4	8.2
	AMF	116.0	5.5	114.9	7.3	214.7	7.5	261.3	9.1
	AMF + A1	140.0	8.7	157.2	8.0	294.0	7.8	324.3	17.3
	AMF + A2	98.0	6.4	112.4	7.3	168.0	7.3	263.6	11.7
N P K	Un inoculated	93.3	4.2	100.0	5.3	200.0	7.4	275.6	12.8
	Thiobacillus A1	116.6	6.5	134.5	9.5	214.7	12.6	421.2	16.0
	Thiobacillus A2	112.0	5.4	135.3	6.0	200.0	8.3	291.2	13.0
	AMF	126.0	6.4	215.7	8.7	308.0	9.4	330.2	17.6
	AMF + A1	182.0	8.4	258.6	9.5	336.0	10.0	448.7	22.8
	AMF + A2	119.0	6.4	256.0	6.4	214.7	8.7	293.8	14.9
N P K + Sulphur (S)	Un inoculated	98.0	5.0	113.0	5.6	207.0	8.0	294.4	13.0
	Thiobacillus A1	130.6	6.8	185.2	9.4	233.3	8.5	301.5	20.8
	Thiobacillus A2	107.3	5.1	156.6	8.7	191.3	7.9	294.4	17.9
	AMF	144.6	7.6	152.7	8.6	298.7	9.9	305.5	19.5
	AMF + A1	149.3	9.2	239.1	9.4	354.7	11.2	328.8	24.7
	AMF + A2	112.0	6.7	224.9	7.0	214.7	9.7	299.5	17.3
N K + Rock Phosphate (RP)	Un inoculated	88.6	4.8	118.9	6.5	210.0	7.0	265.9	12.0
	Thiobacillus A1	102.6	6.5	209.6	9.9	238.0	8.7	367.2	21.4
	Thiobacillus A2	98.0	5.8	171.6	6.8	214.7	8.4	291.2	14.1
	AMF	130.6	7.0	222.2	8.7	298.7	8.6	374.4	13.5
	AMF + A1	149.3	8.1	279.4	12.1	350.0	9.3	410.2	23.6
	AMF + A2	116.6	6.7	127.4	7.3	233.3	8.6	310.7	14.7
N K + S + R.P	Un inoculated	98.0	5.1	102.6	6.3	200.7	7.3	225.5	15.3
	Thiobacillus A1	126.0	7.7	146.9	10.5	270.7	8.9	400.2	21.6
	Thiobacillus A2	102.6	6.8	129.3	6.9	235.7	7.9	265.9	16.2
	AMF	149.3	8.1	186.5	9.1	242.7	9.1	423.6	17.4
	AMF + A1	140.0	8.5	190.4	10.0	345.3	9.6	521.3	26.9
	AMF + A2	112.0	6.2	142.9	6.3	270.7	9.1	383.5	17.0
LSD (0.05)	Mineral	20.71	0.84	33.55	0.83	22.75	1.03	39.36	1.66
	Biofertilizers	26.73	1.09	43.32	1.07	29.37	1.33	50.82	2.15
	Min * Bio	29.28	1.19	47.46	1.17	32.17	1.46	55.67	2.36

N: Nitrogen P: Phosphorus K: Potassium S: Sulphur & Biofertilizers: AMF, *Thiobacillus* A1, *Thiobacillus* A2 and Mixture of A1 or A2 with AMF.

treatments, as compared with both or single inoculation with AMF or SOB after 60 and 90 days of planting. Inoculation with *Thiobacillus* A1 was more effective than *Thiobacillus* A2. The AMF + *Thiobacillus* A1 inoculation gave the highest significant effect for nutrient concentrations of rhizosphere soil in presence of the recommended dose of nitrogen, rock phosphate, potassium and sulphur fertilizers at periods 60 and 90 days. This observation was obvious in the tested parameter, e.g. nitrogen (N), phosphorus (P), potassium (K) and sulphur (S) (ppm). These parameters exhibited high increases in the rhizosphere of onion, reached 67.18%, 73.43%, 158%, 127% in respective order, over the uninoculated treatment which amended with NPK as mineral fertilizers only whereas such increases were 80%, 57%, 76% and 38.1% for maize plants, after 60 days of planting. The recorded values of nutrients concentrations gave maximum concentration in onion rhizosphere after 60 day of planting but at 90 days in maize rhizosphere for all treatments (Tables 2 and 3). These results are agreement with Mostafavin et al. (2008) who reported that combined inoculation of biofertilizers (AM Fungi + *Thiobacillus* sp.) caused a significant increases in P and Mn concentrations of soybean plants with mineral fertilizers (NPKS) compared to NP, NPKS and NPK + AMF treatments. The mycorrhizal fungi (AMF) are important component of the microbial, mutualistic symbiotic soil fungi that colonise the roots of most crop plants. They have the ability to enhance host uptake of relatively immobile nutrients (Abdlhafez and Abdel-Monseif, 2006; Azcon et al., 2003). On the other hand, the members of the genus *Thiobacillus* are able to oxidise elemental sulphur to sulphate that results in lower soil pH and increased nutrients availability for plants (Altand, 2006). The highest concentration of S in rhizosphere soil affects the growing plants especially onion crop (El-Shafie and El-Gamaily, 2002). *Thiobacillus* and mycorrhiza could increase all parameters

Table 4 Biological characters of onion and maize rhizosphere soil inoculated with arbuscular mycorrhizal fungi (AMF) and/ or *Thiobacillus* isolates (A1, A2) under different treatments of mineral fertilizers after 60 days of planting.

Treatments		Plant species					
Mineral fertilizer	Biofertilizers	Onion			Maize		
		T.SOB 10^3	DHA	CO_2	T.SOB 10^3	DHA	CO_2
Un amended (Control)	Un inoculated	–	11.1	154.4	–	8.3	54.0
	Thiobacillus A1	81	37.5	247.2	61	10.2	121.9
	Thiobacillus A2	62	20.6	164.8	37	8.9	88.9
	AMF	–	37.1	188.9	–	9.6	152.9
	AMF + A1	106	39.9	286.7	72	12.7	167.9
	AMF + A2	69	26.6	188.5	68	9.0	158.7
N P K	Un inoculated	–	17.2	162.9	–	8.5	112.2
	Thiobacillus A1	106	36.3	290.1	69	12.6	158.5
	Thiobacillus A2	69	18.2	190.1	40	11.9	143.0
	AMF	–	38.4	235.3	–	14.3	165.0
	AMF + A1	108	40.6	361.8	75	16.8	198.4
	AMF + A2	75	29.9	265.6	71	14.4	160.9
N P K + Sulphur (S)	Un inoculated	–	19.9	218.4	–	10.6	136.5
	Thiobacillus A1	106	34.8	242.6	79	12.2	167.0
	Thiobacillus A2	77	29.8	178.9	46	10.9	138.5
	AMF	–	36.3	242.6	–	13.3	144.7
	AMF + A1	107	38.8	356.0	87	15.0	210.1
	AMF + A2	91	33.5	199.3	84	14.2	158.3
N k + Rock Phosphate (RP)	Un inoculated	–	14.9	187.4	–	8.7	141.8
	Thiobacillus A1	108	34.5	291.8	72	14.4	184.0
	Thiobacillus A2	67	24.1	170.9	43	11.0	167.6
	AMF	–	35.4	240.6	–	13.8	188.6
	AMF + A1	111	34.7	377.5	82	16.5	199.0
	AMF + A2	72	31.9	204.2	82	10.9	186.3
N K + S + R.P	Un inoculated	–	20.1	174.7	–	8.6	157.0
	Thiobacillus A1	113	37.5	284.8	82	12.8	197.3
	Thiobacillus A2	81	28.9	201.6	47	12.5	154.5
	AMF	–	33.0	272.5	–	14.1	164.0
	AMF + A1	115	39.0	364.5	104	14.3	235.4
	AMF + A2	91	31.8	212.7	78	12.0	160.0
LSD (0.05)	Mineral	14.71	2.81	34.58	11.90	1.46	26.40
	Biofertilizers	19.00	3.63	44.64	15.37	1.89	34.09
	Min * Bio	20.81	3.98	48.90	16.83	2.07	37.34

T.C.SOB = Total counts of sulphur-oxidising bacteria (cfu/g soil).
DHA = Dehydrogenase activity (μg TPF/100 g soil Day^{-1}).
CO_2 = CO_2 evolution (mg CO_2/100 g soil).

because mycorrhizal fungi increase absorption of water and mineral nutrients Balloei et al. (2009).

Soil biological activity

Data presented in (Tables 4 and 5) revealed that all the tested soil biological activity parameters [total sulphur-oxidising bacterial-counts, dehydrogenase activity (μg TPF/100 g soil Day^{-1}) and CO_2 evolution (mg CO_2/100 g soil)] under different inoculation treatments of *Thiobacillus* A1, A2 and/or AM fungi were higher than those of uninoculated treatments, after 60 and 90 days of planting.

Total sulphur-oxidising bacteria in rhizosphere soil were the highest (104 and 115×10^3 cfu/g soil) when soil fertilised with NK + rock phosphate (RP) + sulphur (S) and inoculated with AMF + *Thiobacillus* A1 while soil inoculated with *Thiobacillus* (A1) alone gave 61 and 81×10^3 cfu/g soil at 60 days, with significant increase equal to about 70% and 42% in the rhizosphere of maize and onion plants, respectively. The DHA gave maximum values with NPK and AMF + *Thiobacillus* A1 inoculants for onion rhizosphere (40.6 μg TPF/100 g soil Day^{-1})and maize rhizosphere (16.8 μg TPF/100 g soil Day^{-1}), after 60 days of planting (Table 4). Moreover, the dual inoculation of AMF + *Thiobacillus* A1 showed the most pronounced effect on CO_2 evolution and DHA with both tested plants after 90 days of planting with each of the mineral fertilizer treatments (Table 5). Generally, all parameters of biological activity under different mineral fertilizers increased significantly after 90 days of planting for maize rhizosphere soil, whereas it decreased significantly in onion rhizosphere soil compared to the recorded values after 60 days of planting. Data in (Tables 4 and 5) cleared that the inoculation with *Thiobacillus* A1 was more effective than isolate A2 and the inoculation with AMF was more effective when combined with isolate A1 for most of biological parameter.

Table 5 Biological characters of onion and maize rhizosphere soil inoculated with arbuscular mycorrhizal fungi (AMF) and/ or *Thiobacillus* isolates (A1, A2) under different treatments of mineral fertilizers after 90 days of planting.

Treatments		Plant species					
Mineral fertilizer	Biofertilizers	Onion			Maize		
		T.SOB 10^3	DHA	CO_2	T.SOB 10^3	DHA	CO_2
Un amended (Control)	Un inoculated	–	7.3	121.5	–	9.0	165.2
	Thiobacillus A1	62	8.6	211.1	66	24.6	230.0
	Thiobacillus A2	43	8.1	170.5	50	13.9	175.2
	AMF	–	8.4	164.8	–	24.6	200.9
	AMF + A1	71	10.4	244.9	84	36.7	233.0
	AMF + A2	61	8.5	166.7	64	26.3	199.3
N P K	Un inoculated	–	8.9	151.0	–	11.2	217.8
	Thiobacillus A1	79	9.3	241.4	71	39.6	275.7
	Thiobacillus A2	64	9.2	147.5	62	20.2	221.0
	AMF	–	10.7	221.1	–	37.5	231.0
	AMF + A1	96	12.3	254.5	102	40.4	292.0
	AMF + A2	70	11.2	187.0	66	28.8	227.0
N P K + Sulphur (S)	Un inoculated	–	8.0	162.1	–	20.9	253.4
	Thiobacillus A1	64	9.4	193.5	81	30.0	339.6
	Thiobacillus A2	62	9.2	132.6	64	25.8	253.4
	AMF	–	10.7	166.7	–	28.4	355.3
	AMF + A1	93	12.0	193.5	96	38.6	375.7
	AMF + A2	68	9.7	186.6	77	28.8	276.7
N K + Rock Phosphate (RP)	Un inoculated	–	7.7	151.0	–	25.1	218.9
	Thiobacillus A1	76	10.3	183.9	83	35.4	304.4
	Thiobacillus A2	71	8.7	162.1	76	29.2	269.8
	AMF	–	9.8	228.4	–	33.0	370.3
	AMF + A1	100	13.7	235.7	97	38.1	375.2
	AMF + A2	76	10.7	178.2	66	36.2	291.3
N K + S + R.P	Un inoculated	–	9.1	137.6	–	21.8	277.1
	Thiobacillus A1	85	14.7	182.0	91	36.8	341.9
	Thiobacillus A2	81	10.4	166.3	72	37.1	319.7
	AMF	–	13.3	166.3	–	33.5	337.7
	AMF + A1	103	14.3	300.8	112	38.9	361.9
	AMF + A2	78	12.8	213.4	105	31.8	280.9
LSD (0.05)	Mineral	10.50	2.02	21.32	13.53	2.57	29.05
	Biofertilizers	13.55	2.61	27.52	17.47	3.32	37.51
	Min * Bio	14.85	2.86	30.15	19.14	3.64	41.09

T.C.SOB = Total counts of sulphur-oxidising bacteria (cfu/g soil).
DHA = Dehydrogenase activity (μg TPF/100 g soil Day^{-1}).
CO_2 = CO_2 evolution (mg CO_2/100 g soil).

NPK contents of plants

Onion and maize plants were harvested after 90 days from planting, nitrogen, phosphorus and potassium uptake (mg/plant) are shown in (Table 6). Generally, the dual inoculants of *Thiobacillus* A1 and AMF gave the highest records of tested parameters with all mineral fertilizer treatments for the tested plants. The aforementioned inoculation treatment gave the highest significant effect with NK + RP treatment, for onion plant and NK + S + RP treatment for maize plants. This observation was clear in the tested parameters, e.g. N, P, K content (mg/plant).

These parameters exhibited high increase in onion plants, reaching 66.60%, 119.5% and 50.2% increases, in respective order above the same inoculation treatment without mineral fertilizer (control) whereas such increases were 212.6%, 297.4% and 96.45% for maize plants at the end of the experiment. Inoculation with *Thiobacillus* A1 was more effective than isolate A2. Inoculation with AMF was more effective with isolate A1. Marschner (1998) reported that application of sulphur to the soil has several effects; such as reducing pH, improving soil–water relation and increasing availability of metals like P, Fe, Mn and Zn. Arbuscular mycorrhizal fungi increased plant uptake of phosphorus, nitrogen and water absorption. AMF developed on extrametrical mycelium which increased the root P absorbing sites (Bolan, 1991; George et al., 1992; Burkert and Robson, 1994).

Dry weight of plants

Data presented in (Fig. 1) show the stimulatory response of dry weight of onion and maize plants to AMF and *Thiobacillus* inoculation at harvest. Total dry weight of onion bulbs gave maximum value when soil fertilised with NPK + S (5.9 g/plant) &NK + RP (5.7 g/plant) inoculated with AMF +

Table 6 Nutrient uptake (mg/plant)of onion and maize plants as influenced by AMF mycorrhizal and/or *Thiobacillus* isolates (A1, A2) inoculation under different treatments of mineral fertilizers after 90 days of planting.

Treatments		Plant species					
Mineral fertilizer	Biofertilizers	Onion bulb			Maize plant		
		N	P	K	N	P	K
Un amended (Control)	Un inoculated	5.9	1.30	26.0	4.8	4.1	29.7
	Thiobacillus A1	13.3	9.50	67.0	18.6	9.6	49.6
	Thiobacillus A2	10.4	6.00	32.2	10.2	5.7	31.3
	AMF	15.0	10.20	85.5	18.6	9.3	49.2
	AMF + A1	26.7	17.40	148.0	21.4	11.5	62.1
	AMF + A2	15.3	8.40	68.4	12.5	6.2	40.7
N P K	Un inoculated	13.9	9.30	58.8	23.6	7.9	36.0
	Thiobacillus A1	25.8	14.40	112.5	26.0	21.3	73.6
	Thiobacillus A2	18.9	10.20	76.8	25.9	10.2	64.3
	AMF	27.3	18.10	110.0	28.2	19.0	71.9
	AMF + A1	36.4	33.00	177.5	29.0	24.0	110.0
	AMF + A2	21.4	14.30	88.1	26.3	10.6	69.1
N P K + Sulphur (S)	Un inoculated	12.0	7.80	54.6	12.3	9.7	45.4
	Thiobacillus A1	34.1	23.50	156.5	30.0	23.4	78.9
	Thiobacillus A2	19.8	10.20	80.0	21.4	10.0	53.3
	AMF	30.5	20.20	153.7	25.1	15.7	64.8
	AMF + A1	44.2	34.80	205.3	36.7	25.5	125.7
	AMF + A2	18.6	13.00	79.3	22.7	10.6	68.6
N K + Rock Phosphate (RP)	Un inoculated	10.0	9.20	58.6	12.2	8.8	46.2
	Thiobacillus A1	27.9	20.10	144.6	29.9	24.4	80.0
	Thiobacillus A2	17.5	12.60	87.5	19.5	12.8	50.2
	AMF	36.7	25.00	141.1	22.5	18.0	57.6
	AMF + A1	44.5	38.20	222.3	35.1	34.2	121.8
	AMF + A2	19.0	16.70	81.6	19.4	8.8	53.9
N K + S + R.P	Un inoculated	13.8	7.60	60.5	16.9	13.9	51.6
	Thiobacillus A1	36.8	27.10	154.1	42.1	39.3	111.3
	Thiobacillus A2	16.7	10.00	75.9	28.1	13.2	64.4
	AMF	35.6	20.60	128.5	37.3	26.7	108.7
	AMF + A1	43.7	34.70	192.8	66.9	45.7	122.0
	AMF + A2	22.0	10.2	89.2	26.7	13.9	89.3
LSD (0.05)	Mineral	0.13	0.06	0.28	0.10	0.05	0.20
	Biofertilizers	0.17	0.08	0.36	0.13	0.07	0.26
	Min*Bio	0.19	0.08	0.39	0.14	0.07	0.28

N: Nitrogen P: Phosphorus K: Potassium & Biofertilizers: AMF, *Thiobacillus* A1, *Thiobacillus*A2 and Mixture of A1 or A2 with AMF.

Thiobacillus A1, whereas the highest value was 5.4 g/plant for maize plant treated with the previous inoculants in soil amended with NK + S + RP. The significant increases were 103 and 95.55% for onion plant and 101% for maize plants, in respective order, over the uninoculated treatment amended with the recommended dose of NPK. Sulphur as a macronutrient has a positive effect on onion, maize and other plants and is ranked equal to nitrogen for optimising crop yield and quality (Tucker, 1999; Shinde et al., 1996 and Bloem et al., 2004).

Number of AMF spores and infection percentage

Results in (Table 7) showed that the colonisation of AMF ranged from 37–91% and 33–99% in the root of onion and maize plants, respectively, after 60 days of planting. The dual inoculation by AMF and *Thiobacillus* A1 gave the highest significant effect on measured parameters with all mineral fertilizer treatments compared to single inoculation with AMF only. The maximum records of AMF infection percentage and numbers of AMF spores were 91% and 350 spore/100 g soil for onion plants, respectively, with dual inoculation (AMF + A1) in presence of the recommended dose of NK + S + RP. Whereas the dual inoculation gave the maximum value of AMF infection percentage and number of AMF spores/100 g soil with recommended dose of NK + S + RP and NK + RP, for maize plants after 60 days of planting.

These results are in line with the findings of many researchers, they stated that AMF promote plant growth and nutrient uptake of low fertility soils (Marschner, 1995) and AMF can increase the effectiveness of P fertilizer added to soils that are P-deficient or having high P-fixing capacity. For example, in an acidic soil, addition of AMF and rock phosphate fertilizer together were more effective in enhancing the growth of corn than when rock phosphate was added alone (Alloush and Clark, 2001). AMF improved onion growth by increasing leaf area, plant height, and leaf chlorophyll content and probably photosynthesis capacity both leading to greater dry mass and larger bulb (Bolandnzar et al., 2007).

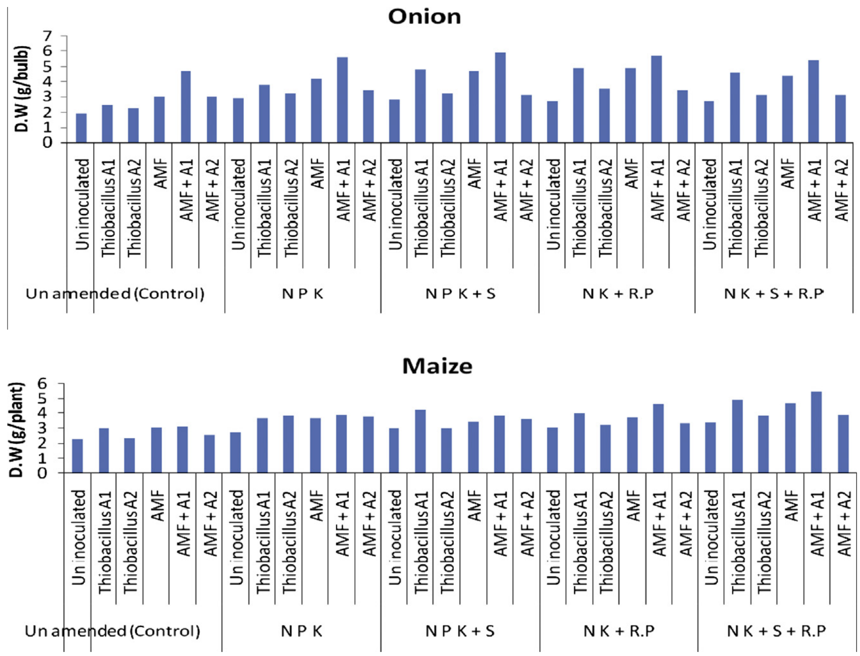

Fig. 1 Dry weight of onion and maize plants as influenced by arbuscular mycorrhizal fungi (AMF) and/or *Thiobacillus* isolates (A1, A2) inoculation under different treatments of mineral fertilizers after 90 days of planting *Onion*: LSD (0.05): Mineral fertilizers (Min) 0.13 Biofertilizers (Bio) 0.17 Min × Bio 0.19 *Maize*: LSD (0.05): Mineral fertilizers (Min) 0.52 Biofertilizers (Bio) 0.67 Min × Bio 0.74.

Table 7 Spore numbers and percentages of AMF mycorrhizal colonisation of onion plants as influenced by AMF mycorrhizal and *Thiobacillus* isolates (A1& A2) inoculation under different treatments of mineral fertilizers after 60 days of planting.

Treatments		Onion plant		Maize plant	
Mineral fertilizer	Biofertilizers	No. Spores/ 100 g Soil	Infection Percentage of AMF (%)	No. Spores/ 100 g Soil	Infection Percentage of AMF (%)
Un amended (Control)	AMF	133.0	37	66.0	33
	AMF + A1	180.0	66	125.0	65
	AMF + A2	133.0	47	108.0	50
N P K	AMF	125.0	67	108.0	45
	AMF + A1	183.0	80	158.0	76
	AMF + A2	158.0	51	116.0	48
N P K + Sulphur (S)	AMF	150.0	59	83.0	54
	AMF + A1	300.0	88	183.0	84
	AMF + A2	250.0	62	141.0	56
N K + Rock Phosphate (RP)	AMF	166.0	65	175.0	53
	AMF + A1	316.0	82	216.0	90
	AMF + A2	175.0	75	125.0	74
N K + S + R.P	AMF	275.0	70	158.0	62
	AMF + A1	350.0	91	183.0	99
	AMF + A2	216.0	74	150.0	86
LSD (0.05)	Mineral	59.81	9.54	50.81	12.91
	Biofertilizers	59.81	12.32	66.50	17.00
	Min * Bio	103.60	16.53	106.0	20.50

Conclusion

In conclusion, dual inoculation with AMF and *Thiobacillus* A1 isolate improved the growth parameters and nutrient contents of onion and maize plants using the recommended doses of N, K, phosphorus as rock phosphate and sulphur fertilizers and enhanced environmental sustainability with good crop yield in sandy soil.

The effect of AMF and the most efficient isolate (*Thiobacillus* A1) individually or mixed inoculants will be tested under field conditions on the tested plants.

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