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ORIGINAL ARTICLE

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

Amal A. Mohamed [a,*], **Wedad E.E. Eweda** [b], **A.M. Heggo** [a], **Enas A. Hassan** [b]

[a] *Soils Water and Environ. Inst. Agric. Res. Center, Giza, Egypt*
[b] *Microbiology Departments, Faculty of Agriculture, Ain Shams University, Cairo, Egypt*

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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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* Corresponding author.
E-mail address: dr.amalalaa_1986@hotmail.com (A.A. Mohamed).

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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

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