EFFECTS OF SEWAGE SLUDGE AND MYCORRHIZATION ON TOMATO SEEDLING GROWTH

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ABSTRACT. Tomato seeds cv INCA 9-1 were inoculated with a *Glomus fasciculatum* fungus strains. Mycorrhizal (M) and non-mycorrhizal (NM) seeds were grown in a Ferralsol soil alone and soil was amended with municipal sewage sludge at 30 $t.ha^{-1}$ rate. Pot experiments were carried out under greenhouse conditions and 30 days after seeding, treatment effects on plant growth were determined by means of shoot and root dry matter production and mycorrhizal infection. Our results show that studge and mycorrhiza effect on plant had different behavior. Studges enhanced shoot growth, whereas mycorrhiza promoted root development. The application of sewage sludge to soil tended to decrease tomato root colonization by *Glomus fasciculatum* after 30 days.

Key words: sewage sludge, *Glomus fasciculatum*, inoculation, plant response, tomato

RESUMEN. En experimentos en condiciones controladas se evaluó el efecto de la aplicación al suelo de lodo de la Estación Depuradora de Aguas Residuales "Quibú" y la inoculación de una cepa de HMA *Glomus fasciculatum* a semillas de tomate (*Lycopersicon esculentum* Mill) sobre la producción de la masa seca de la parte aérea y radical así como la colonización fúngica de las plantas crecidas en un suelo Ferralítico Rojo Lixiviado que recibió 30 $t.ha^{-1}$ de lodo. Las semillas inoculadas y sin inocular se plantaron en macetas plásticas de 1 L de capacidad conteniendo suelo con y sin lodo. Las evaluaciones se realizaron a los 30 días después de la germinación donde se encontró que tanto el lodo como los HMA aumentaron la producción de masa seca de la parte aérea, correspondiéndole al lodo la mayor contribución. En contraste en las raíces solo los tratamientos donde se aplicó la inoculación aumentaron la producción de masa seca. La aplicación de lodo y micorriza de manera conjunta, no modificó la distribución de la masa seca en los órganos en relación con el suelo solo. El establecimiento de la asociación micorrízica se afectó por la disposición de lodo al suelo.

Palabras clave: lodos residuales, *Glomus fasciculatum*, inoculación, respuesta de la planta, tomate

INTRODUCTION

Modern agriculture is designed to find alternatives for reducing the use of chemical fertilizers, such as: making good use of sludge through its availability to soil (1, 2), as well as inoculating plants with different fungi and bacteria (3, 5).

Each element has its own particular features. Sludge, which is made up by solids resulting from waste water processing, when added to soil stops being an environmentally dangerous residue to become a source for improving soil. It also contributes with agronomic values through the supply of organic matter and nutritional elements, such as nitrogen and phosphorous, mainly, in addition to potassium, calcium and microelements, in lower amount (6).

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On the other hand, an efficiently mycorrhized root can increase nutrient absorption and translocation to plants (7), as well as explore a higher soil volume than non-mycorrhized roots (8), incorporating such symbiosis into low input agriculture.

Benefits of associating arbuscular mycorrhizal fungi (AMF) with roots from different crops (9, 11), as well as of using organic matter for amending soil, are well documented. However, there is poor information concerning the interaction between both factors and their impact on plant growth. That is why, the objective of the present research was to evaluate the influence of available sewage sludge to soil on tomato seedling development and mycorrhization capacity.

MATERIALS AND METHODS

Sludge was taken from "Quibú" Municipal Sewing Station, located in Havana City.

Table I. Some features of sludge and soil

	Organic matter (%)	pH	Total N (%)	Available P ($mg.kg^{-1}$)	K
Sludge	38	6.7	2.8	1200	0.01 %
Soil	3.01	7.1	0.51	540	0.61 $cmol.kg^{-1}$

A Lixiviated Red Ferralitic soil was used, taken from the National Institute of Agricultural Sciences (14).

For developing the work, a homogeneous soil and sludge mixture was used at a rate of 25 $g.kg^{-1}$ soil, as well as soil alone for seeding tomato (*Lycopersicon esculentum* Mill.) INCA 9-1 variety, either without inoculation or inoculated with an AMF strain of *Glomus fasciculatum*. The inoculum weight corresponded to 10 % seed weight.

Soil and sludge chemical characterization followed that methodologies collected by Paneque (15).

For each treatment, 10 pots with 1L capacity were used and three plants cultivated in each of them under controlled light conditions (16 hours/light and 8 hours/shade), as well as at temperatures of 25±1 and 20±1^{0}C.

Dry matter of plant top and root was evaluated through the gravimetric method; whereas fungal colonization with AMF by estimating plant infection percentage, visual density and endophyte matter (16), 30 days after germination.

For each substrate, relative mycorrhizal dependence index (RMDI) (17) was estimated by:

$$RMDI = \frac{DWm - DWnm}{DWm} \times 100$$

Where:
DWm: dry matter of mycorrhized plant top
DWnm: dry matter of non-mycorrhized plant top

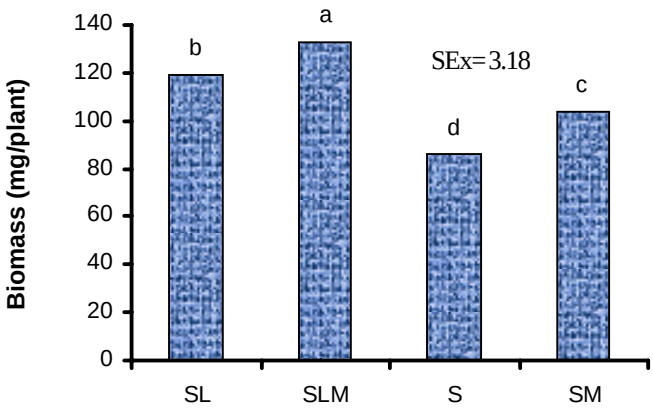
A randomized complete design with factorial arrangements was used and data were processed according to ANOVA. Means were compared following Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Amending Lixiviated Red Ferralitic soil with sewage sludge caused a differential effect on seedlings either from seeds without inoculation or from those inoculated using an AMF *Glomus fasciculatum* strain.

Figure 1 shows that both sewage sludge and AMF improved tomato seedling top as a result of dry matter increment compared to plants growing in soil alone.

According to the results, in relation to soil alone, when sludge was applied there was higher increase (38.5 %) in plant top biomass accumulation than when using AMF (20.27 %). Such behavior could be owing to the higher amount of available nutrients in the soil after applying sludge (18), or to an improvement of soil physical properties (19) due to sludge contribution with organic matter, presenting a value of 38 %. This could increase moisture-holding (20), as well as contribute to improve plant water relations, influencing vegetative growth and development (22).



(SL) soil with sludge, (SLM) soil with sludge and mycorrhizae, (S) soil alone and (SM) soil with mycorrhizae

Figure 1. Dry matter production in the aerial part of tomato seedlings, inoculated with *Glomus fasciculatum* or without inoculation, grown in soil alone and soil with sludge

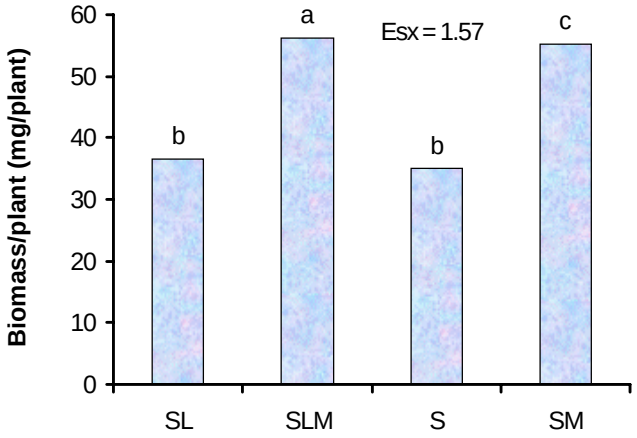
However, when sludge and fungus inoculation were combined, biomass accumulation recorded 53.63 %, it being superior to that achieved by the soil alone, as well as to the one obtained when both treatments were applied separately. It is important to highlight that plants from the medium containing sludge and AMF increased 15 % dry weight production, in relation to plants grown in soil with sludge. A similar increase was recorded in mycorrhized plants when grown in the soil without sludge, showing that mycorrhizal contribution for producing biomass does not depend on sludge.

During the early stages of development, mycorrhized plant growth is delayed, since they go through a parasitic stage caused by symbiosis, where nutrient absorption by plants is limited (23). However, results showed that root growth was only stimulated by AMF application (Figure 2) in all conditions studied, since there were no significant differences concerning dry matter production in roots from mycorrhized plants, regardless of being grown in soil with or without sludge.

On the other hand, previously inoculated plants grown in the soil with sludge, in relation to the soil alone, presented a higher increase in root biomass.

Similarities in root growth response of non-mycorrhized plants, grown with or without sludge, could be caused by heavy metals that make up sludge (24, 25). Such metals could also bring about root toxicity depending on chemical species, concentration and vegetable, inhibiting or delaying growth instead of stimulating it (26).

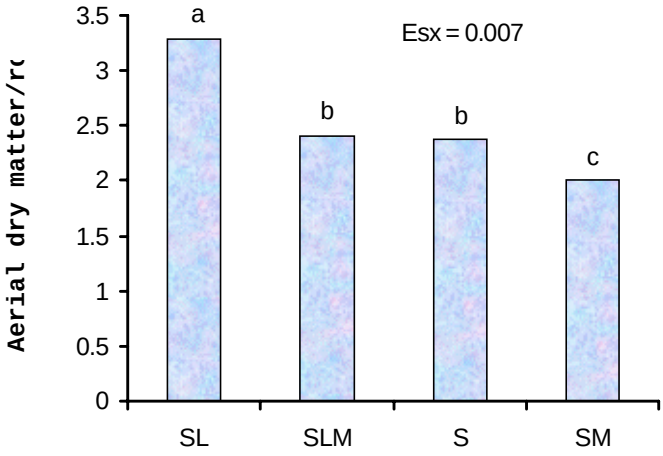
Root growth stimulation, promoted by mycorrizae, followed a similar pattern of behavior to the one found by other authors (27, 28). It was possible due to root physiological functions, which are related to scanning far areas, in order to provide plants with nutrients and water when there is lack of such elements in areas surrounding the roots.



(SL) soil with sludge, (SLM) soil with sludge and mycorrhizae, (S) soil alone and (SM) soil with mycorrhizae

Figure 2. Root dry matter production in tomato plants, inoculated with *Glomus fasciculatum* or without inoculation, grown in soil alone and with sludge

Results concerning the aerial part and root growth, expressed through dry matter accumulation, showed that using seeds coated with AMF in the soil with sludge is a good way of obtaining seedlings with appropriate features for being transplanted.



(SL) soil without sludge, (SLM) soil with sludge and mycorrhizae, (S) soil alone and (SM) soil with mycorrhizae

Figure 3. Relationship between aerial part and root dry matter of tomato plants, inoculated with *Glomus fasciculatum* or without inoculation, grown in soil alone and with sludge

In general, aerial part/root relationship constitutes an important parameter for evaluating responses in mycorrhizal associations and is usually altered by mycorrhizal fungi, due to changes in the absorption capacity of mycorrhized roots.

After evaluating dry weight distribution in both parts of the plant, no change was recorded by the joined application of sludge and AMF in relation to natural conditions (soil alone). On the other hand, an opposite effect was found when both elements were applied separately: sludge increased the relation by promoting plant top growth; whereas inoculation brought about a reduction, causing the same effect on the root, as it is seen in Figures 1 and 2, respectively.

Mycorrhizal dependency of tomato INCA 9.1 cultivar, determined by mycorrhized and non-mycorrhized plant growth, varied depending on the substrate used. According to results, dependence was of 16.7 % when plants where grown in soil alone, whereas such value dropped to 9.2% when sludge was applied. This could be explained, taking into account that adding nutrients to the soil, such as sludge, and having an ppropriate plant humidity level, there is less necessity for plants to be colonized by AMF. Under such conditions, extramatrix hyphae do not play an important role in nutrient absorption directly. However, they bring about other benefits such as soil aggregation (29), achieving better drainage and nutrient mobility.

Results in Table II show that tomato INCA 9.1 variety is sensitive to mycorrhizal colonization, coinciding with other authors (30, 31), who have experimented with different varieties of tomato and *Glomus ssp*. strains.

Table II. Fungal variables of tomato plants, 30 days after germination

	Infection (%)	Visual density (%)	Endophyte weight (mg.g^{-1} root)
Soil	42 a	0.84 a	0.42 a
Soil+ sludge	40 ab	0.60 b	0.38 b
Soil+AM	37 b	0.63 b	0.23 c
Soil+sludge+AM	34 c	0.47 c	0.24 c
	SEx=0.021	SEx=0.005	SEx=0.001

Root colonization of plants grown in soil without sludge reached, values of infection to 42 % as well as endophyte weight of 0.42 mg.L^{-1} root, suggesting the presence of an active propagule community in the soil.

After evaluating the process of fungal colonization, based on infection percentage, visual density and endophyte weight, it was seen that root infection did not vary in non-inoculated plant when sludge was applied to the soil, and decreased when using inoculation. However, visual density was lower when plants grew in soil with sludge than when they did it in soil alone, regardless of whether seeds where inoculated or not. On the other hand, endophyte weight presented a similar behavior when seeds were not inoculated.

Results indicate that mycorrhizal establishment seems to be modified by sludge. As it is seen in Table I, sludge contained phosphorous that increased P levels when released to the soil. This could be the reason why a different plant colonization response was found when using treatments with and without sludge, since high

levels of phosphorus in the soil could reduce or inhibit mycorrhizal development (37).

Even though there is poor information about P release from sludge, in relation to its initial contents in the soil, it is known that such phenomenon depends on some features of sludge, as well as of the crop, the type of soil and climate. Moreover, it has been published that P release is higher during the first 20 days after sludge is available to the soil (33), which is a shorter period of time than the one used in the present experiment. Therefore, a significant increase in P levels could have occurred in the medium during the experimental phase that, together with low crop requirement in such stage of development, could have affected mycorrhization.

Thus, sludge usually contains heavy metals (24), and mycorrhiza tends to protect plants from toxic effects, depending on fungal isolation and metal, exposing themselves to certain damages (34, 35). However, as it is seen in Figures 1 and 2, plant growth was favored by these treatments, which made nutrients more available to plants.

It was also noticed that fungal variables were lower in inoculated plants than in noninoculated ones, regardless of whether sludge was used or not.

Likewise, in most of the evaluated treatments, when sludge was applied to soil, combined with AMF inoculation of tomato seed behavior, was different from the one presented when treatments were used separately. This makes evident the existing interaction between both factors, since their combination stimulates organ growth, not altering biomass relations.

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