Program Short-term mobility-Anno 2017/18. Soggiorno di ricerca della Dr.ssa Maria Angeles Bustamante Muñoz: Report of the results obtained

Main objective

The main purpose of this project was to study the potential suppressive capacity of different stabilized organic materials against the phytopathogen *Fusarium oxysporum* f.sp. *melonis* (FOM), the causal agent of Fusarium wilt of melon plants. For this, different composts elaborated mainly using sludge from urban and agri-food wastewater treatment plants were evaluated through an *in vitro* assay using as soil-borne pathogen *Fusarium oxysporum* f.sp. *melonis* (FOM).

This research stay is integrated within a research project to evaluate the efficiency of the use of organic amendments, especially composts, as added-value materials that can be used in different agricultural sectors, and in this specific case, as an environmental sound alternative to chemical pest control toward several plant pathogens. The described research project will serve as a preliminary work for an interdisciplinary project involving also melon plants.

Experimental design

During the visit of Dr. Bustamante, within the context of the project previously commented, has been carried out the determination of the potential suppressiveness capacity against *Fusarium oxysporum* f.sp. *melonis* (FOM) in a set of 12 composts previously elaborated using sewage sludge and agri-food sludge mixed with different bulking agents.

The method is an indirect determination derived from the combination of the measurement of the area of inhibition and growth of the fungus versus a control (vermiculite). The determination has been done qualitatively, as the presence of an inhibitory zone around 10 replicates, and quantitatively by measuring the inhibitory zones around the compost samples. The methodology is described in the works of Szczech (1999) and Bustamante et al. (2012). Briefly, 30 mL of sterile water was poured into a Petri plate with a 7-day-old mycelium of FOM on potato dextrose agar (PDA). The fungus was suspended in water by stirring with a sterile swab. Then, 0.1 mL of the suspension was spread on plates with fresh PDA. After 24 h incubation at 28 ºC, heat-treated (sterilized) or not (non-sterilized) compost samples (0.5 g) were placed in the center of each plate. Plates containing samples of vermiculite, heat-treated or not, and prepared as described above, served as non-suppressive controls. There were 10 replicates per treatment. The plates were incubated at 30 ºC for 10 days and the development of fungus was observed.

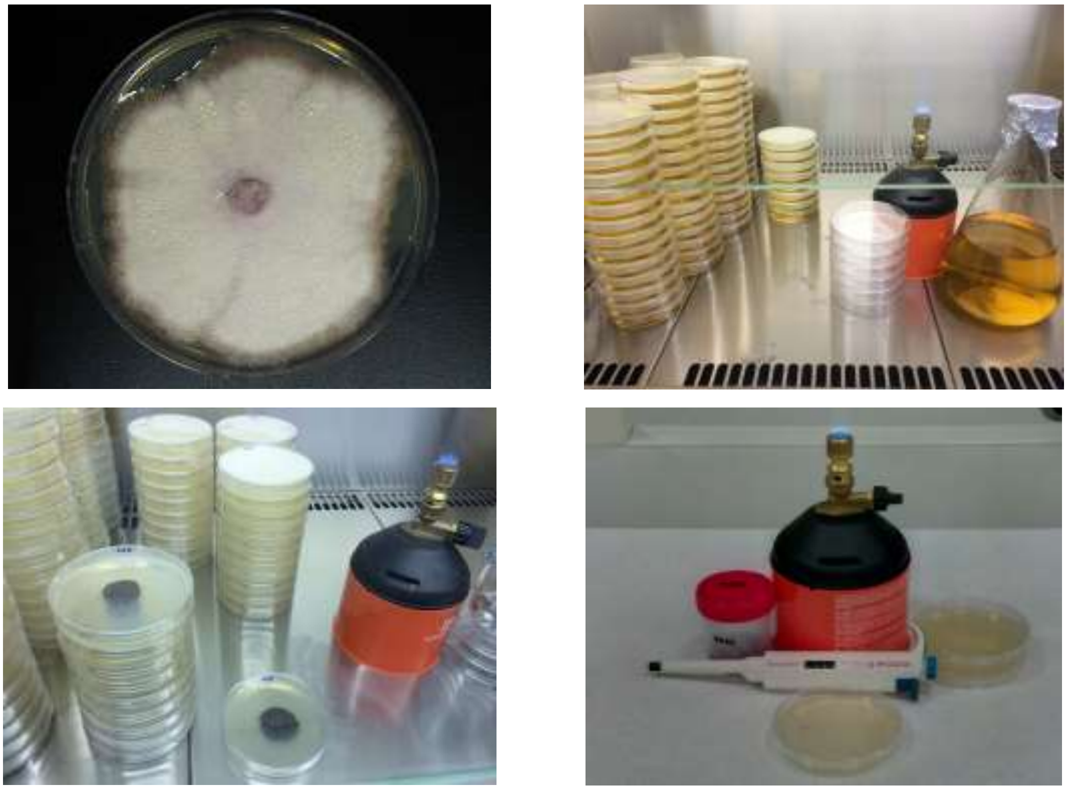

Figure 1. Detail of the experimental steps (in the clockwise direction): a) Plate with FOM; b) Preparation of the infected plates; c) and d) Placement of compost samples.

Results obtained

The results that are shown in this report are the initial results obtained during the research stay of Dr. Bustamante and corresponding to the initial stage of the project previously commented.

In Table 1 are shown the raw materials that were used as ingredients in the elaboration of the compost studied, as well as the proportions, on a dry weight basis, of these components. The compost used were mainly elaborated using sewage sludge with different bulking agents, such as pruning of different plant species usually used in public gardening (mulberry (*Morus alba* L.), palm (*Phoenix dactylifera* L.) trunk and leaves, as well as prunings from vineyard and from giant reed (Arundo donax L.). In addition, composts C10, C11 and C12 were prepared using agri-food sludge, principally obtained after the processing of canned vegetables (pepper and artichoke), and mixed with vineyard pruning or palm trunk waste.

Table 1. Composition (on a dry weigth basis) of the mature compost studied.

Compost type	Proportions of the raw materials in the mixture
C1	29.6 % sewage sludge + 70.4 mulberry pruning
C2	26.3 sewage sludge + 73.7 palm trunk
C3	46.6 sewage sludge + 53.4 palm leaves
C4	29.5% sewage sludge + 36.8 palm leaves +33.7 mulberry pruning
C5	31.4 sewage sludge + 68.6 giant reed waste
C6	42.2% sewage sludge + 57.8 palm leaves
C7	36.2% sewage sludge + 63.8 palm leaves
C8	29.5 sewage sludge + 70.5% giant reed pruning
C9	53.9% sewage sludge + 46.1% garden trimmings
C10	27.5% agri-food sludge (from canned pepper production) + 72.5% palm trunk
C11	38.1% agri-food sludge (from canned artichoke production) + 61.9% vineyard pruning
C12	40.4% agri-food sludge (from canned artichoke production) + 59.6% vineyard pruning

In Figure 2 is shown the *in vitro* potential suppressive effect of the sterilized compost samples, which allows to study the suppressive effect due to abiotic aspects. As it can be seen, the sterilized composts induced a low inhibitory effect, the composts C9 and C10 not showing any inhibition in the growth of *Fusarium oxysporum* f.sp. *melonis*. The raw materials used in the composts preparation seem to produce a different suppressive effect when the material is sterilized, showing the composts C1 and C12 the highest suppressive effect.

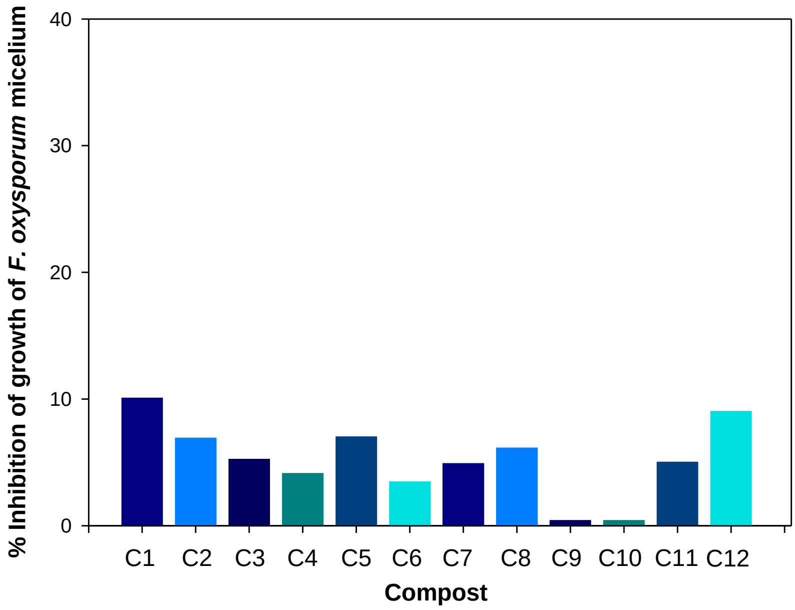

Figure 2. Percentage of inhibition of the growth of *Fusarium oxysporum* f. sp. *melonis* micelium in the sterilized compost samples.

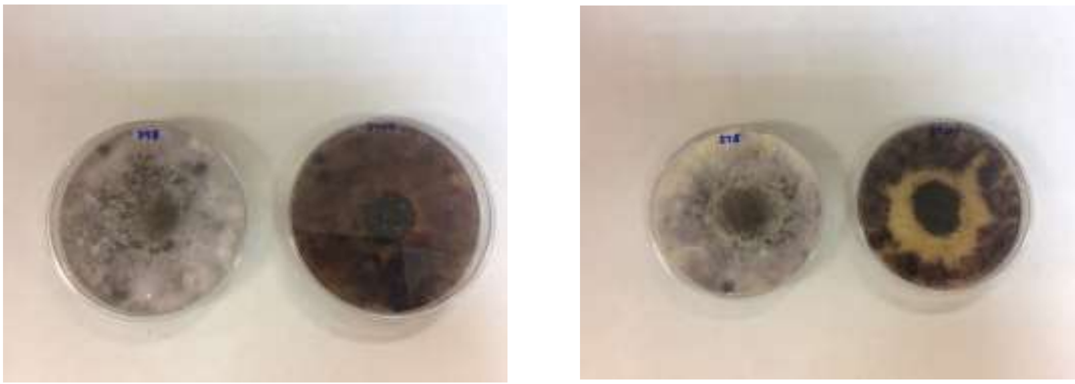

Figure 3. Detail of the suppressive effect on the FOM mycelium in two compost samples. In each photograph, the plate on the left corresponds to sterilized compost; the plate on the right corresponds to non-sterilized compost.

However, in the non-sterilized samples of the mature composts was observed a strong inhibition of growth of *Fusarium oxysporum* f. sp. *melonis* on the agar plates (Fig. 4), showing only C9 and C10 the lowest inhibitory effect in *in vitro* conditions. In the case of the control sample (vermiculite), the inhibitory effect was not observed in the sterilized and non-sterilized samples, which were completely covered with mycelium.

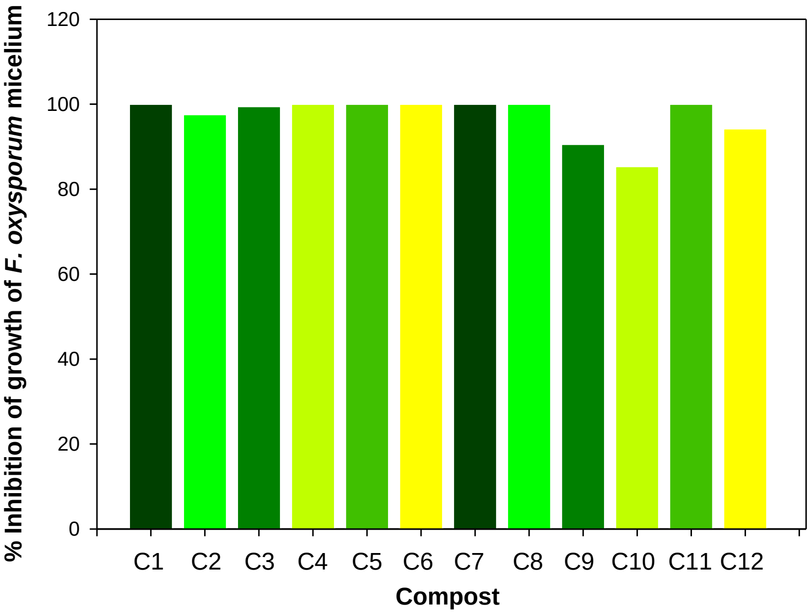

Figure 4. Percentage of inhibition of the growth of *Fusarium oxysporum* f. sp. *melonis* micelium in the non-sterilized compost samples.

Different authors have also reported similar results, observing a higher inhibition in the non-sterilized materials, probably due to the factor or factors that induced the suppression of *Fusarium oxysporum* were eliminated by heating, indicating the biotic nature of the inhibition of fungal growth (Bustamante et al., 2012, Suárez-Estrella et al., 2007, 2012). Szczech (1999) also observed similar results in a study to evaluate the suppressiveness of vermicompost from cattle manure against *F. oxysporum* f. sp. *lycopersici.*

The preliminary results obtained during this short-term stay has shown that practically all the composts elaborated using sewage sludge and agro-industrial wastes (sludge and bulking agents) had a suppressive capacity, in *in vitro* conditions, against *Fusarium oxysporum* f.sp. *melonis*, which opens the way to future investigations related to the potential biopesticide capacity as an added-value property of these specific type of composts.

<u>Future collaborations derived from this research stay</u>

The research stay of Dr. Bustamante has supposed not only the reinforcement of the scientific collaboration with the Italian group, but also the beginning of a new and ambitious collaborative research line associated to the potential added-value of stabilized organic materials, such as composts, as an environmental sound alternative to chemical pest control toward several soil-borne pathogens.

<u>References used</u>

Bustamante, M.A., Alburquerque, J.A., Restrepo, A.P., de la Fuente, C., Paredes, C., Moral, R., Bernal, M.P. (2012). Co-composting of the solid fraction of anaerobic digestates, to obtain added-value materials for use in agriculture. Biomass Bioenergy 43, 26-35.

Szczech, M.M. (1999). Suppressiveness of vermicompost against Fusarium wilt of tomato. J. Phytopathol., 147(3):155-161.

Suárez-Estrella, F., Vargas-García, M.C., López, M.J., Capel, C., Moreno, J. (2007). Antagonistic activity of bacteria and fungi from horticultural compost against Fusarium oxysporum f. sp. melonis. Crop Protection, 26:46-53.

Suárez-Estrella, F., Bustamante, M.A., Moral, R., Vargas-García, M.C., López, M.J., Moreno, J. (2012). In vitro control of Fusarium wilt using agroindustrial subproduct-based composts. J. Plant Pathol., 94 (1):59-70.