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COMPOSTS AND ENRICHED COMPOSTS AS ALTERNATIVES TO CHEMICALS IN PLANT DISEASE CONTROL

M.I. Trillas[a], E. Casanova[a], D. Sant[a], J.C. Tello[b], J. Ordovás[c], C. Borrero[c], M. Avilés[c]

[a]*Facultat Biología. Universidad Barcelona, Avda. Diagonal, 645 E-08028 Barcelona e-mail: mtrillas@ub.edu.*

[b]*Escuela Politécnica Superior Universidad de Almería*

[c]*Escuela Universitaria Ingeniería Técnica Agrícola. Universidad de Sevilla*

Abstract

Composting in well-designed systems is a better way of treating organic wastes than incineration or landfilling [1, 2, 3]. It retrieves waste materials for reuse in greenhouses or on fields in an environmentally acceptable way. The final products can be used as soil improvers or fertilisers and also to suppress plant pathogens [3, 4, 5, 6, 7, 8]. The suppressive effects of compost can be enhanced by inoculation with specific strains of biological control agents [9, 10; 11].

Spain is one of the largest areas in Europe where horticultural crops and flowers are intensively cropped in plastic greenhouses, in fields or in containers [12]. In addition to the municipal solid wastes generated in large cities, several hundred tons of agricultural wastes (wine and oil industries, cork, rice hulls, etc.) are produced every year [13]. Unfortunately, the practice of disinfecting soil with methyl bromide or other chemicals is extremely widespread [14, 15]. However, as a member of the EU, Spain is required to reduce these practices in the short term.

Since 1997, in the context of interregional projects, we have been working on compost production (processes and methodology), and characterization (for horticultural purposes), and evaluation of its natural suppressive capacity. We report on the suppressive action of composts from different wastes against two important plant pathogens (*Fusarium oxysporum*, and *Rhizoctonia solani*). We are also working with other plant pathogens, such as: *Verticillium dahliae*, *Botrytis cinerea* and *Pythium aphanidermatum*. We also describe the benefits of *Trichoderma asperellum*, biocontrol strain T-34 [16] isolated and developed from composts from urban residues [17, 18, 19]. *T. asperellum*, T-34, is able to consistently reduce diseases produced by *F. oxysporum* and *R. solani* when added to the developed composts and peat substrates [11].

Introduction

The agricultural wastes composted by our group using the windrow system are made up of cork bark (*Quercus Ruber*, L.) from the cork industry, grape marc from the alcohol industry and olive marc from the olive oil industry mixed with gin trash from the cotton industry.

These materials are composted annually in piles of between 100 and 40 m^3 - the process lasts between four and six months [17] - and form the basis for our experimental material. Composted urban waste (collected from markets or selected from the waste disposal system and mixed with the leftovers from pruning) provided by Metrocompost, a Barcelona company (Medi Ambient, Generalitat de Catalunya) [11], has also been analyzed for over six years now. These materials are analyzed physically, physical-chemically and chemically with a view to formulation and adaptation for use in the growing of plants in pots [20].

Suppressiveness is analyzed by means of bioassays in controlled atmosphere chambers. The bioassays permit the establishment of interaction at three levels: pathogen (virulent and in sufficient concentrations to produce disease), plant (a highly sensitive variety) and atmosphere (temperature, humidity and nutrients), all optimized to make the disease occur [1, 4, 5 and 6]. In order to elucidate the relationship between the micro-organisms and the chemical/ physical atmosphere, the materials are or are not submitted to microbiological cleaning, by sterilization or in autoclave, stove at 60º C, for six days [5,11, 21]. With regard to the biological control agent *Trichoderma asperulum (T-34)*, its capacity to colonize and reduce the incidence of diseases produced by *F. Oxysporum* and *R. Solani* is assessed.

The biological community of the different composts is evaluated both for genetic diversity (identification and richness, by semi-selective methods, respiration, phospholipids, etc.) and for functional diversity (β-glucosidase activity, hydrolisis of fluorescein diacetate, use of carbon substrates, etc.) [17,19, 22].

Materials and Methods

Bioassay with tomatoes (*Lycopersicon esculentum,* cv. Roma) and *Fusarium oxysporum* (isolated RAF70). The substrates are or are not (controls) inoculated with the pathogen, mixed and shaken vigorously in a bag with 2 litres of substrate / compost and distributed among five pots. Four tomato seeds germinated in vermiculite are transplanted per pot (in stage 2-3 real leaf). The pots are placed randomly in a phytotron (25± 2ºC, photoperiod 16:8 h light: darkness, with a PAR light of 150-210 $\mu E\ m^{-2}\ s^{-1}$). The pots are watered according to the plant's growth needs and fertilized with Peters foliar feed 27-15-12 (Scotts). The disease is evaluated according to the symptoms in a scale of severity, where O = healthy plant, 1 = plant with slight infection (< 50% of leaves chlorotic or wilted), 2 = seriously infected plant (> 50% have wilted but the plant is still alive) and 3 = dead plant. The bioassays also evaluate the incidence of the disease expressed in percentages, where 0 indicates that all the plants are healthy and 100 indicates that they are all diseased, also expressable in terms of the relative length of infected xylem, at the end of the bioassay [11, 17, 19].

Different concentrations of pathogen 5 x 10^4, 10^5, 5 x 10^5 ufc/ ml are used in the different bioassays and the inoculum may be talcum [11] or conidia [19] but can also be prepared with soil [5].

Bioassay with cucumber (*Cucumis sativus,* cv. Negrito) and *Rhizoctonia solani* (AC-4 isolated). The substrates are or are not (controls) inoculated with the pathogen and are mixed in a bag with 2 litres of substrate/compost and distributed in five pots. 15 cucumber seeds are added to each pot. The pots are placed randomly in a phytotron (25 ± 2º C, photoperiod 16:8 light: darkness, with a PAR light of 150 -210$\mu E\ m^{-2}\ s^{-1}$). The pots are watered and fertilized with Peters foliar feed 27-15- 12 (Scotts). The disease is evaluated on day 7 using the following severity scale: 0 = healthy plant, 1 = small lesions in the root/ base of stem, 2 = large lesions in the root/base of stem, 4 = post-emergency wilted plants and 5 = pre-emergency seed rot. Evaluation may also be carried out on the basis of disease incidence, that

is to say the proportion of seedlings affected. In this scale the values are 0 (minimum) and 1 (maximum). The *R. Solani* inoculum was prepared in the soil in accordance with the Ko and Hora method [24].

Inoculum of the biological control agent, *Trichoderma asperellum (T-34)* is inoculated in crop substrates a week before use in the bioassay. Various concentrations of the inoculum, 10^3, 10^4, and 10^5, are evaluated in the bioassays. The behaviour of T-34 in the aforementioned bioassays is studied, this being or not being incorporated into the substrate/compost, which in its turn is or is not inoculated with the pathogen, *F. Oxysporum* or *R.Solani*.

Microbiological activity, the hydrolysis rate of the fluoroscein diacetate (FDA) [23] is evaluated in the composts/substrates, the values being expressed by unit of volume, owing to the differences between the apparent densities of the materials under study [11].

Results and Discussion

The results presented are the mean values of as a minimum three bioassays and they have been analyzed statistically with the different versions of the SPSS programme. These experiments have been carried out in the laboratories and phytotrons of both the University of Seville and the University of Barcelona.

In Table 1 we see the average values for the physical properties of the tested materials, whether pure or formulated, to achieve the appropriate aeration and retention of water available for the plant.

Table 1: Physical properties of peat, composts and other materials used in the plant growth media

	Peat	Cork Compost (CC)	Grape Marc Compost (GMC)	Olive Marc Compost (OMC)[1]	OMC + Rice hulls (1:1 vol)	Compost Peat Vermiculite (2:1:1 vol)[2]
Bulk Density (gr cm^{-3})	0.10	0.25	0.41	0.62	0.43	0.20
Particle Density (gr cm^{-3})	1.60	1.63	1.68	2.09	2.063	1.88
Total Pore Space (%vol)	93.8	84.8	75.5	70.3	79.2	89.4
Easily Available Water (%vol)	21.0	20.0	11.5	15.0	9.9	13.5
Water Buffer Content (%vol)	5.6	5.0	2.8	3.2	2.2	3.5
Not-Easily Avail Water (%vol)	38.1	40.0	35.7	42.4	30.2	38.4
Air Capacity (%vol)	29.3	20.0	24.9	9.7	36.9	33.4
Useful Water (%)	26.4	25.0	14.9	18.2	12.1	17.0

[1]Compost made from olive marc + cotton gin trash (2:1 vol)
[2]Compost made from vegetable and animal wastes + yard wastes and formulated with peat and perlite

As is well-known, peat presents optimal air and water percentages, 29% and 26% respectively, which can be easily used by the plant. These optimal values are also found for

cork compost, although cork has a slightly smaller air content (20%). Both grape and olive marc compost and substrate formulated on the basis of urban waste with peat and perlite have slightly lower useful water levels for the plant (15, 18 and 17% respectively). Olive marc compost has a low air content (10%), which is why mixing it with rice hulls is proposed. Values of about 37% are thus obtained, although this mix reduces water availability (12%). The reduced water availability in the composts obtained can be corrected by watering more frequently, although this should not mean increasing the amount of water used.

Table 2 shows the results in terms of microbiological activity for some of the materials studied, measured as capacity to hydrolyse fluoroscein diacetate. This table does not give levels of significance since results from various experiments have been mixed. The first group is made up of peat, cork compost and grape marc compost and in it each material is significantly different from the others. Cork, for instance, has a high level of microbiological activity. Urban waste compost mixed with peat and vermiculite was analyzed along with another group of materials, like the compost itself without formulation and the peat-vermiculite mix [11]. The most notable result is the finding that the microbiological activity of peat is lower than that of the composts.

Table 2: Microbial activity, measured as hydrolysis of FDA, in plant growth media (controls)

	Peat	CC	GMC	CPV
FDA activity (µFDA cc-1 min-1)	0.51	2.410	0.979	1.47

Table 3 shows the results obtained for the colonizing capacity of T-34 in the materials studied, using the suspension – dilution technique in a semiselective medium for the isolation of *Trichoderma* spp. [25].

Table 3: Colonization capacity of *Trichoderma asperellum* (T-34) using two inoculum concentrations (10^3 and 10^5 cfu / ml).

	Initial concentration T-34 (cfu/ml)	Concentration *Trichoderma* spp. (1 week) (cfu/ml)	Concentration *Trichoderma* spp. (2 weeks) (cfu/ml)	Concentration *Trichoderma* spp. (3 week) (cfu/ml)
Peat	10^3 10^5	8.7×10^3 4.7×10^4	6.1×10^3 2.2×10^4	9.6×10^3 4.0×10^4
Cork compost	10^3 10^5	2.4×10^3 2.2×10^4	2.0×10^3 3.9×10^4	1.6×10^3 2.1×10^4
Grape marc compost	10^3 10^5	1.2×10^3 ---	3.6×10^3 8.0×10^4	1.6×10^3 6.4×10^4
Olive marc compost	10^3 10^5	2.7×10^3 8.5×10^4	3.4×10^3 1.3×10^5	0.5×10^3 2.4×10^4
Compost: peat: vermiculite	10^3 10^5	3.5×10^3 ---	1.4×10^3 3.2×10^4	2.3×10^3 2.6×10^4

With these selective media one cannot differentiate between the native populations of *Trichoderma* spp. which the material may contain from the T-4 populations that we have incorporated. We have seen that a week is sufficient for T-34 to establish itself. With inoculations of 10^3 cfu/ml the populations stabilize at around this magnitude and multiply by ten when inoculated with 10^5 cfu/ml *Trichoderma* spp. populations stabilize at about 10^4 cfu/ml and in almost no material is established at 10^5 cfu/ml. The exceptions are olive marc compost during the first and second weeks and grape marc compost during the second week. But neither does it stabilize at such high concentrations in the material from which it was isolated (urban waste compost, peat, vermiculite).

Table 4 and Figure 1 show the results obtained in the various tomato and *Fusarium oxysporum* bioassays. In the first case they are expressed as the length of xylem infected by the pathogen. It should be emphasized that the significance level is always equivalent, both if the results are expressed in terms of disease levels throughout the bioassay and if they are given as length of infected xylem. The most notable results of Table 4 are the high level of disease control achieved with grape marc compost, oscillating between 0 and 3% according to the dose of pathogen inoculum used. Also worth noting is the fact that the suppressive capacity of this compost is due both to the (natural) action of the micro-organisms and to its chemical properties (microbiological cleaning in a stove) and that it is moderately suppressive, with values similar to those of natural cork compost (17 and 30/ 35% of affected stem). In the case of cork we see that suppressive capacity is due exclusively to the micro-organisms. Natural peat has values like those of cork compost with microbiological cleaning for the two concentrations of pathogen studied and the disease results are significantly higher than they are with natural composts.

Table 4: Evaluation of suppressiveness for some of the compost / substrates studied. The pathogen *Fusarium oxysporum* f.sp. *lycopersici* was used at two concentrations. Disease is expressed as length of xylem showing brownish.

MATERIAL	Length of stem affected (cm)	
	F. oxysporum 10^{-4}cfu ml^{-1}	*F. oxysporum* 10^{-5} cfu ml^{-1}
Cork compost, natural	17.74 b	34.94 b
Cork compost, heated[1]	38.92 d	38.84 bc
Grape marc compost, natural	0.0 a	3.25 a
Grape marc compost, heated[*]	17.14 b	30.15 b
Peat	30.49 cd	49.01 cd

[*] This means that the material has been in the oven at 60 ºC for one week.

The data shown in Figure 1 are from studies carried out with urban waste compost: peat: vermiculite, -natural, sterilized or sterilized with added T-34-. The results obtained show that we are talking about a compost with good suppressiveness for the high concentrations of *F.oxysporum* used (10^5 cfu/ml). One should stress that this compost was 50% formulated and that its suppressiveness was lower without peat and vermiculite. In the course of the 30-days bioassay the disease affected 50% of the plants. This suppressiveness was due to the micro-organisms, as is demonstrated by the fact that the disease affected almost 100% of the plants when the compost was sterilized. Also worth emphasizing is the role of T-34 in the reestablishment and improvement of natural compost suppressiveness, which affected less than 20% of the plants. This compost should be formulated with other materials, principally because of its salt content and also to improve its water retention capacity [11].

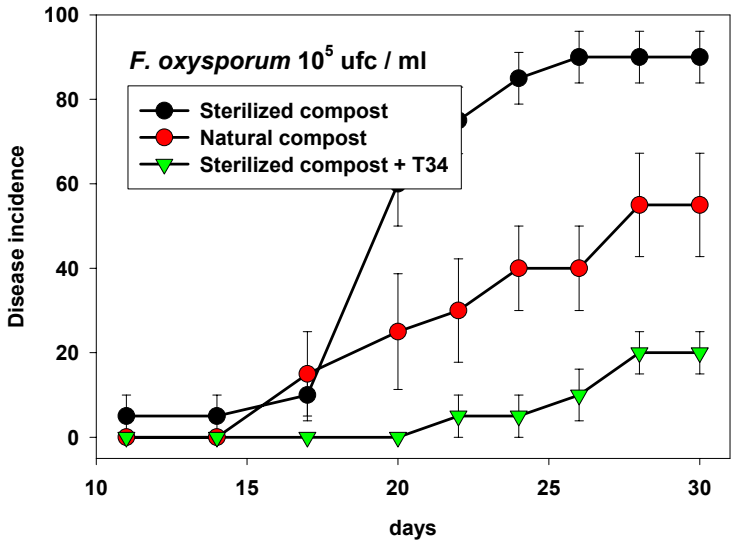

Figure 1. Fusarium wilt disease incidence curve for tomato plants grown in Compost: Peat: Vermiculite. Sterilized compost was autoclaved at 121 ºC, 1h, for 3 consecutive days.

Figure 2 shows the results for the cucumber and *Rhizoctonia solani* bioassay. The materials are those analyzed during the year in which the compost was obtained and after a year's maturation.

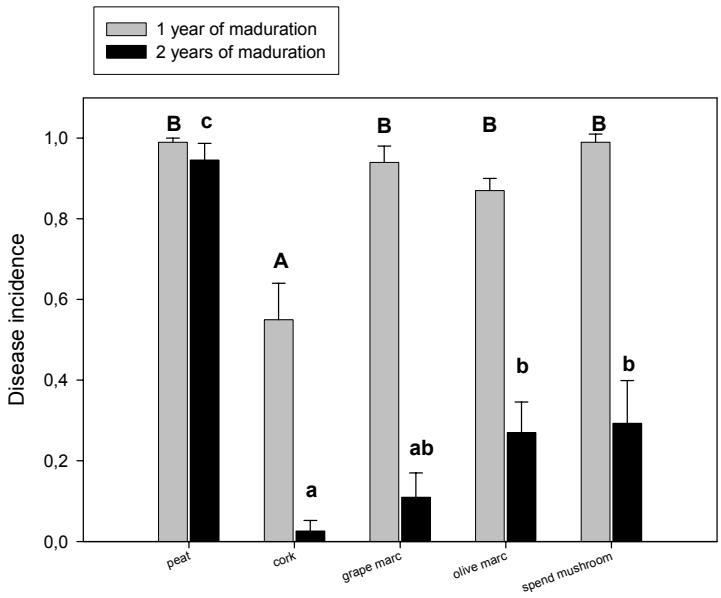

Figure 2. Rhizoctonia damping-off disease incidence for peat and composts, first year of maturation and at the second year of maturation.

As will be readily observed, the best material is first-year cork compost. Nevertheless, the most striking thing is the improvement in the control of this disease with all the composts with longer maturation time. We show here the results with spent mushroom compost, provided by the RECOMSA company in Cuenca. Peat too is a conducive material for this disease.

Figure 3 gives the role of T-34 in the reduction of disease caused by *R.solani* in a moderately suppressive compost as well as T-34 capacity to reduce the incidence of disease in a conducive material like peat and to convert it into a material with a capacity similar to that of natural cork compost. It should be stressed that in these experiments T-34 was inoculated one week before the bioassay at a concentration of 10^3 ufc/ml.

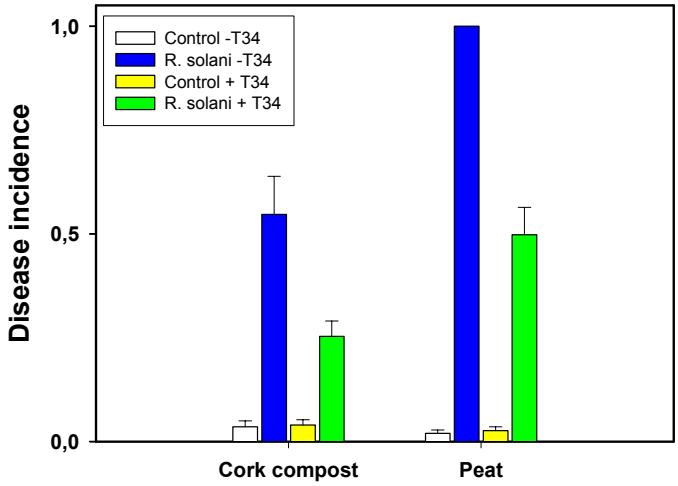

Figure 3: Rhizoctonia damping off disease incidence. Role of *Trichoderma asperellum* T-34 in the reduction of disease. T-34 was inoculated one week previous to the bioassay at a concentration of 10^3 cfu/ml substrate.

Table 5 summarizes the suppressiveness of the composts studied for the two pathogens referred to and also against *Botrytis cinerea* (a foliar pathogen) and *Verticillium dahliae.*

Table 5. List of materials studied and their activity against different plant pathogens.

	Suppressiveness against various pathogens			
Material	*Fusarium oxysporum lycopersici*	*Verticillium dahliae*	*Rhizoctonia solani*	*Botrytis cinerea*
Heated Peat[a]	conducive	conducive	conducive	conducive
Peat	conducive	conducive	conducive	conducive
Peat 2	conducive	conducive	conducive	conducive
Compost:Peat:Vermiculite 2:1:1 (CPV)	highly suppressive	----------	highly suppressive	suppressive
Grape Marc Compost (GMC)	highly suppressive	----------	suppressive	suppressive
Cork Compost (CC)	suppressive	suppressive	highly suppressive	suppressive
Olive Mark Compost (OMC)	suppressive	----------	suppressive	suppressive
Spent Mushroom Compost (SMC)	suppressive	----------	suppressive	suppressive

[a] This material was kept at 60º for 1 wk to decrease microbial activity.

Conclusions

It is possible to compost agricultural and urban wastes and recover materials of great microbiological wealth and a high content of nutritive elements, which can be used alone or formulated as substrates for the cultivation of potted plants. All the materials studied show physical properties in the optimum range for use, requiring less adjustment for irrigation, container size and species.

The mechanisms offered by the micro-organisms present in these composts against different pathogens vary and possibly more than one is involved in each system [1,4,6,11,19]. The chemical composition of these materials is also varied and this affects the micro-organisms which colonize them. In the case of *Fusarium oxysporum*, for example, the populations most involved in cork compost are *Pseudomonas fluorescents,* while grape marc compost are various kinds of actinomycetes [19] and urban waste compost plays an important role *Trichoderma* spp.[11]. The importance of iron and other microelements in the sporulation and virulence of *F.oxysporum* is well established in the bibliography [26] and we have also found pH involvement in composts close to neutrality or slightly basic, which enhances control of vascular fusariosis [11,19]. In a general way, one can talk of the high microbiological activity offered by composts in comparison with other substrates used traditionally in the cultivation of plants. This greater activity hinders the establishment of pathogenic micro-organisms. Composts act as a nutrient source, not only for the micro-organisms that compete with the pathogens, but also for those which parasitize them or produce toxic substances. Apart from all this, we should not forget the role the micro-organisms present in the compost may play at plant level, increasing/ facilitating defence reactions (systemic resistance).

On the basis of our results, we see that different strategies can be applied to increase or strengthen compost suppressiveness. One is to allow the composts to mature for a few months, another to enrich them with specific biological control agents like, for example, *Trichoderma asperellum* T-34.

These composts, either alone or enriched with biological control agents, play a beneficial role in reducing diseases produced in plants by edaphic pathogens. New prospects are also opened up regarding the part composts might play (at plant level) in combatting airborne diseases (like *Botrytis cinerea*). These materials, properly composted and matured, thus offer a singular added value, since the vegetable species cultivated in them are growing in an environment less favourable to the development of diseases. Plants growing in composts will need less pesticides, with all the accompanying advantages, not only for the producer's pocket, but also for the safety of the sprayer, the consumer and the environment.

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