

# Development of disease-suppressive organic growing media

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## Abstract

**Vigorous seedlings are an important base for vegetable production. Beside the availability of appropriate amounts of nutrients, the health of seedlings is decisive. Soil-borne diseases are a challenging problem in organic seedling production. Here, we present results on the development of disease-suppressive growing media. Three aspects were examined: (i) use of different components of growing media (peat, coconut fiber, wood fiber, compost), (ii) influence of selected organic nitrogen fertilizers and (iii) use of different microorganisms (including commercial biocontrol agents (BCA)). Three plant-pathogen systems were used in this study: cucumber-*Pythium ultimum*, cress-*Pythium ultimum* and basil-*Rhizoctonia solani*. Green waste compost showed a good capability to protect cress against *P. ultimum*. This effect was improved by using a chitin-containing N-fertilizer. However, an inappropriate storage of the compost diminished its efficacy. In contrast to coconut fibers, wood fibers showed a suppressive activity against *P. ultimum* when used as partial substitutes of peat. None of five tested commercial BCAs could improve the suppressiveness of the substrates against *P. ultimum*. However, one of newly tested strains of *Trichoderma* sp. was very suppressive against *P. ultimum*. The tested growing media showed only small differences in suppressiveness against *R. solani* on basil. In contrast, two of the new strains of *Trichoderma* sp., which were intermediately active against *P. ultimum*, could efficiently protect basil against *R. solani*. At the moment, we test combinations of different *Trichoderma* strains, compost, different types of peat and peat substitutes. The aim is to determine whether it is feasible to manufacture growing media which allow the production of healthy and robust seedlings also in the presence of high levels of pathogens.**

**Keywords:** disease suppression, growing media, *Rhizoctonia solani*, *Pythium ultimum*, compost, *Trichoderma*, wood fibers

## INTRODUCTION

Vigorous and healthy seedlings are an important base for vegetable production. Whereby growing media play a decisive role, especially in organic horticulture. Two aspects are crucial: the availability of appropriate amounts of nutrients and the absence of active plant pathogens. In organic growing media, mineral fertilization and pesticides cannot be used, hence these two aspects are mainly influenced by the microbiological activity of the media.

Growing media are complex products and different requirements have to be fulfilled. Beside the aspects of nutrient availability and plant disease suppression, water and air capacities, structure stability, pH-value and salt content are important. All these aspects are influenced by the composition of the growing media. In organic ones, the peat quantity is limited and there are different alternatives to replace it. The nature and characteristics of these substitutes can be very different. Some are minerals, other organic. Some are biologically active, others are not. To obtain an optimal substrate, different materials are mostly used in combination.

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The capacity of a growing media to suppress plant diseases is a very important challenge for the production of growing media for organic horticulture. The used mixture components are normally free from plant pathogens. But some, like peat, are microbiologically inactive and are conducive to pathogens. This means that if a pathogen comes in contact with the media, it can spread very fast in it and might cause important damage to the plant seedlings. Other components, such as quality composts, are microbiologically active and can therefore buffer the media, preventing an invasion with pathogens. Another possibility to prevent the development of plant pathogen in the growing media is the amendment with antagonistic microorganism, for example some *Trichoderma* species.

The aim of this study was to characterize the influence of different kind of amendments of growing media on the development of plant diseases and the potential of an inoculation of *Trichoderma* strains to protect the plant against plant pathogens.

## MATERIALS AND METHODS

### Substrates and fertilizers

The different substrates were used as presented in Table 1.

Table 1. Substrates used for the production of growing media.

Substrate	Specificity	Supplier
Einheitserde Typ 0	White peat + clay	Patzer GmbH & Co. KG, DE-Sinntal
Black peat	not limed	Klasmann-Deilmann, D-Geeste
Wood fibers	Test product	Anonymous
Coco Ter	Coir	Ökohum, CH-Herrendorf
Compost	Green waste compost, 10 mm sieved	Leureko AG, CH-Laufenburg
Digestate	Green waste digestate, 10 mm sieved	Leureko AG, CH-Laufenburg

Except compost and digestates, all the substrates are fertilized with 0.3 g N, 0.1 g P<sub>2</sub>O<sub>5</sub>, and 0.4 g K<sub>2</sub>O L<sup>-1</sup> before use. The fertilizers used are presented in Table 2.

Table 2. Fertilizers used for the production of growing media.

Fertilizer (supplier)	Specificity	N (%)	P <sub>2</sub> O <sub>5</sub> (%)	K <sub>2</sub> O (%)
Horn flour (Hauert)	N-fertilizer, composed of horn	14.0	0.0	0.0
Biosol (Sandoz GmbH)	Contains 40% of chitin, promotes microorganisms decomposing fungi	7.0	0.5	0.5
Chitin Forte (Andermatt Biocontrol)	Contains shrimp shells which are rich in chitin, promotes microorganisms decomposing fungi	2.5	3.0	0.5
Condit 5 (Schmutz)	Hydrolyzed whey, fermented organic plant materials, volcanic minerals	4.0	1.6	1.6
Thomas flour	Phosphate fertilizer	0.0	17.0	0.0
Potassic magnesium (Hauert)	Potassic fertilizer containing magnesium	0.0	0.0	30.0

## Microorganisms

### 1. Plant pathogens.

Two soil-borne plant pathogens were used: *Pythium ultimum* (Strain 67-1) and *Rhizoctonia solani* (Strain F122, from the University of Geisenheim, D-Geisenheim). The different substrates used are presented in Table 1.

The pathogens were grown on malt extract agar medium (MEA). Inoculum was produced on millet (24 g of organic golden millet seeds + 20 mL of demineralized pure ELGA water) in a 100-mL Schott bottle. The medium was autoclaved twice at 121°C for 20 min

each at two days interval before adding four pathogen containing MEA discs ( $\varnothing=0.5$  cm). After 7 days for *P. ultimum* or 3 weeks for *R. solani* when millet seeds were completely colonized by the fungi, the millet was hashed with an onion chopper. The pathogen/millet seed-inoculum was mixed with sand (Vogelsand Vitakraft Sandy, Coop, CH-Basel) in order to ensure a more homogenous distribution of the pathogen within the substrate.

## 2. Biocontrol agents.

Different microbial biocontrol agents were used (Table 3). The commercial products were used as indicated by the supplier. The inoculum of the test strains of *Trichoderma* spp. (Table 3) were produced like the pathogens on golden millet medium (see above). After 7 days, the *Trichoderma* colonized millet was mixed with sterile water and the fungi spores were filtered; the growing media were then amended with  $10^9$  spores  $L^{-1}$ .

Table 3. Microbial biocontrol agents added to substrates.

Microorganism	Product name (supplier)	cfu applied $L^{-1}$
<i>Bacillus subtilis</i>	Serenade Max (Stähler)	15E10
<i>B. amyloliquefaciens</i>	AmyloX (Biogard)	2E10
<i>Pseudomonas fluorescens</i> Pf153	Biofitac Pf1 (Biophyt AG)	2E10
<i>Gliocladium catenulatum</i> J1446	Prestop (Andermatt Biocontrol)	2E9
<i>Trichoderma harzianum</i>	Trianum (Koppert)	6E10
<i>Trichoderma harzianum</i> 720	EMPA, CH-St. Gallen	1E9
<i>Trichoderma harzianum</i> 721	EMPA, CH-St. Gallen	1E9
<i>Trichoderma atroviride</i> 685	EMPA, CH-St. Gallen	1E9
<i>Trichoderma atroviride</i> 722	EMPA, CH-St. Gallen	1E9
<i>Trichoderma koningiopsis</i> 723	EMPA, CH-St. Gallen	1E9

## Bioassays

Disease suppressiveness of substrates was assessed using the model systems *Pythium ultimum*-cress (*Lepidium sativum*), *Pythium ultimum*-cucumber (*Cucumis sativus*), and *Rhizoctonia solani*-basil (*Ocimum basilicum*).

Pots were filled with growth substrate and then the seeds were sown (1 g, 5 seeds and 20 seeds, for cress, cucumber and basil, respectively). All the seeds were organically produced and untreated. After sowing, pots were watered by placing them in a basin with tap water as long as the absorbed water moistened the surface of the substrate. Pots were covered with a plastic foil until plant emergence to ensure optimal humidity conditions for germination. Plants were grown in a grow bank with a day/night lengths of 16 h at 23°C and of 8 h at 18°C, respectively. For cress, the shoot biomass was determined after 6 days. For cucumber, the number of living plants, shoot and root biomass was recorded after 14 days. For basil, the number of living plants was recorded after 2, 3 and 4 weeks, and the mortality of the plants was calculated; after 4 weeks, the shoot biomass was additionally measured.

## Analysis of rhizoplane microorganisms

Bacteria from the rhizoplane were isolated on King's media B (King et al., 1954) and the most abundant bacteria of treatments differing in suppressivity were identified by Maldi-Tof analysis (Benagli et al., 2012; Sauer and Kliem, 2010).

## RESULTS

### Influence of fertilizers and peat substituents in substrate on disease suppressiveness

The development of the symptoms of damping-off disease caused by *Pythium ultimum* on cress and on cucumber was less important in the growing media containing compost as peat substitute than in the growing media containing coir (Figure 1). The tested organic fertilizers did not influence the development of the disease on cress, independently of

whether the peat substitute was coir or compost (Figure 1). Shrimp meal was an exception as it reduced the disease incidence when used in compost media. For cucumber, Biosol and ricin shot increased the disease symptoms in the growing media with coir, but did not influence it in the media with compost (Figure 1).

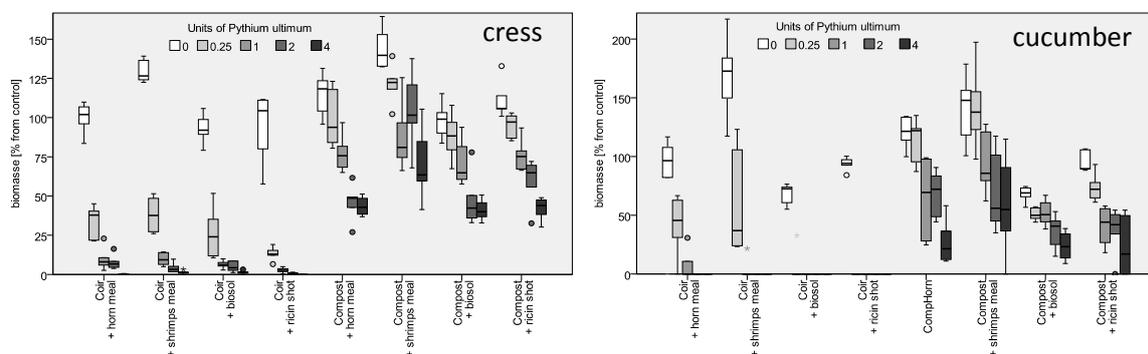


Figure 1. Influence of different organic fertilizers on the disease development of damping-off caused by *Pythium ultimum* by cress and cucumber in growing media. The growing media is composed of 70% “Einheitserde Typ 0” (Pater GmbH) and 30% of coir or green waste compost. 1 unit of *P. ultimum* corresponds to 1 g of a 7 days old *P. ultimum*-millet seed culture L<sup>-1</sup>. The control weight is the average of the cress biomass in the growing media with coir and horn meal without pathogen. Each box plot represents the results of six pots.

The positive effect of the used green waste compost was much less evident if the compost was stored for more than 4 weeks in big bags exposed to the sun (Figure 2). The characterisation of the bacteria populations of the rhizoplane showed a shift in the composition of most abundant bacterial species. Whereby the relative number of *Aeromonas media* isolates was much more important in the rhizoplane of plants growing in *Pythium* suppressive media (Figure 2). In a following experiment, addition of *Aeromonas media* into conducive growing media improved suppressiveness against *P. ultimum* (see Oberhaensli et al., 2017).

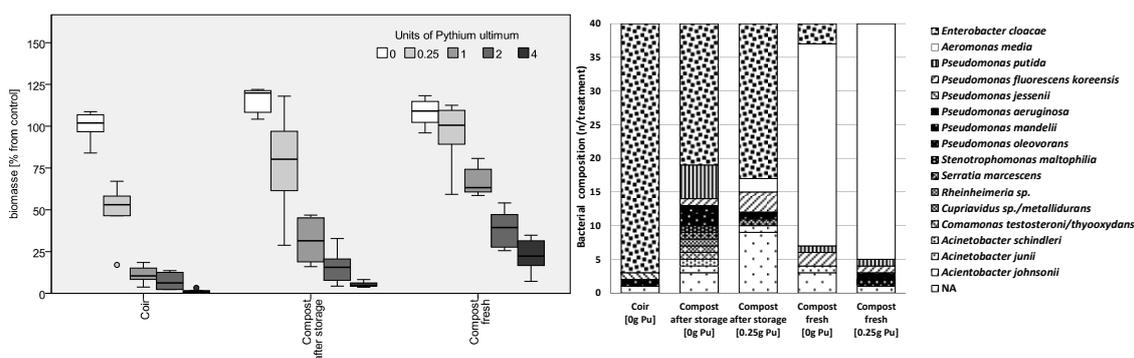


Figure 2. Influence of compost quality on the disease development of damping-off caused by *Pythium ultimum* of cress in growing media (left) and bacterial composition of cress rhizoplane in function of disease suppression (right). Details see Oberhaensli et al. (2017).

The basic component used in the growing mixture influenced also the capacity of compost to protect the plants against disease. The same compost mixed to “Einheitserde Typ0” (composed of white peat and some clay) diminished the incidence of damping off in cress cultures, but showed no effect when mixed with limed black or white peat (Figure 3).

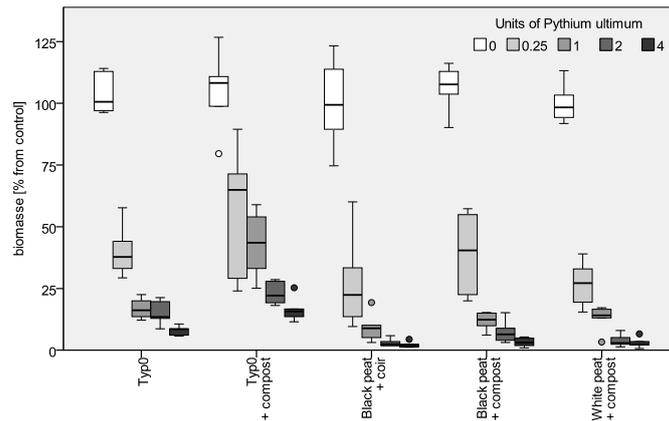


Figure 3. Influence of basic component of growing media on the suppressive capability of compost against damping-off caused by *Pythium ultimum* of cress. 30% of compost or coir was mixed to the basic component. 1 unit of *P. ultimum* corresponds to 1 g of a 7-days-old *P. ultimum*-millet seed culture L<sup>-1</sup>. The control weight is the average of the cress biomass in the growing media with coir and horn meal without pathogen. Each box plot represents the results of six pots.

### Use of biocontrol agents to improve the disease suppressiveness of growing media

Five biocontrol agents commercialized in Switzerland were tested to protect cress against the damping off disease caused by *P. ultimum*: three bacteria (Pf153: *Pseudomonas fluorescens* Biofitac Pf1, Bacsub: *Bacillus subtilis* Serenade Max, Bacamy: *B. amyloliquefaciens* AmyloX) and two fungi (Glio: *Gliocladium catenulatum* Prestop; Tricho: *Trichoderma harzianum* Trianium). Under the conditions of the experiment, none of these products influenced the development of the symptoms of damping off disease caused by *Pythium ultimum* on cress, neither in substrate with coir nor in substrate with compost (Figure 4). Similar results were obtained with cucumber (data not shown).

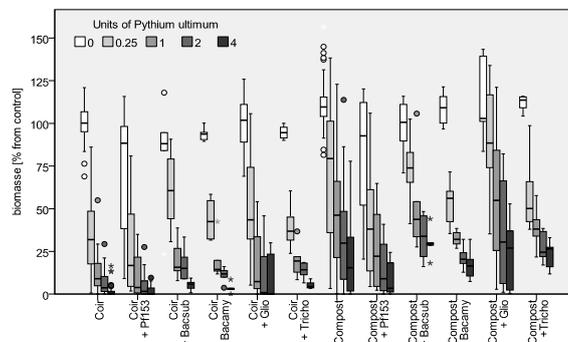


Figure 4. Influence of different commercial biocontrol agents organic fertilizers on the disease development of damping-off caused by *Pythium ultimum* by cress in growing media. The growing media is composed of 70% of "Einheitserde Typ 0" (Patzner GmbH) and 30% of coir or green waste compost. 1 unit of *P. ultimum* corresponds to 1 g of a 7-d-old *P. ultimum*-millet seed culture per litre. Pf153: *Pseudomonas fluorescens* Biofitac Pf1, Bacsub: *Bacillus subtilis* Serenade Max, Bacamy: *B. amyloliquefaciens* AmyloX, Glio: *Gliocladium catenulatum* Prestop; Tricho: *Trichoderma harzianum* Trianium. The control weight is the average of the cress biomass in the growing media with coir and horn meal without pathogen. Each box plot represents the results of six pots.

### Test of new biocontrol *Trichoderma* strains

Five new *Trichoderma* strains (two *T. harzianum*, two *T. atroviride* and one *T. koningiopsis*) were tested for their capability to protect cress against the damping off disease caused by *P. ultimum*. The strain *T. koningiopsis* 723 showed the best protection of the cress plants in limed black peat with 30% of coir (Figure 5). This strain was also able to reduce the disease incidence in growing mixtures with green waste compost and with wood fibers.

These five *Trichoderma* strains were also tested to protect basil against the basal rot disease caused by *Rhizoctonia solani*. In this pathosystem, the two *T. harzianum* strains were the most efficient ones to protect the plants, whereas the strain *T. koningiopsis* 723 showed no efficacy (Figure 6).

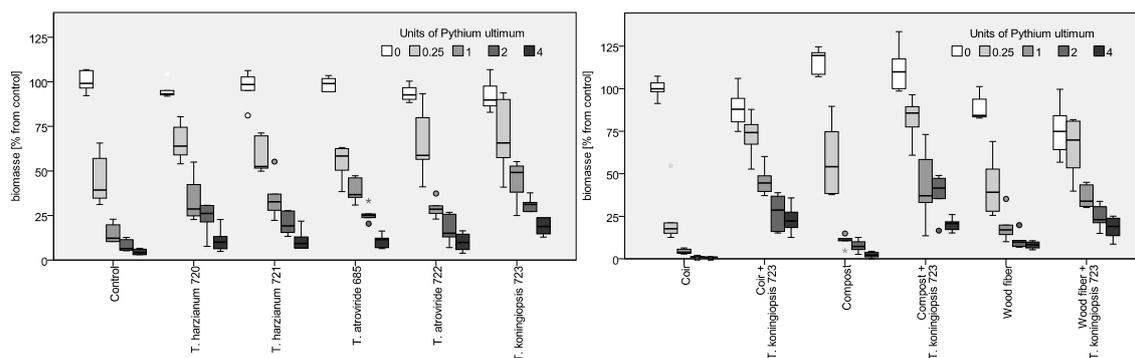


Figure 5. Capability of five *Trichoderma* strains to protect cress plants against damping-off caused by *Pythium ultimum* in cress and cucumber in growing media. The growing media is composed of 70% of limed black peat and 30% of coir (control), of green waste compost or of wood fiber. 1 unit of *P. ultimum* corresponds to 1 g of a 7-d-old *P. ultimum*-millet seed culture L<sup>-1</sup>. The control weight is the average of the cress biomass in the control media without pathogen. Each box plot represents the results of six pots.

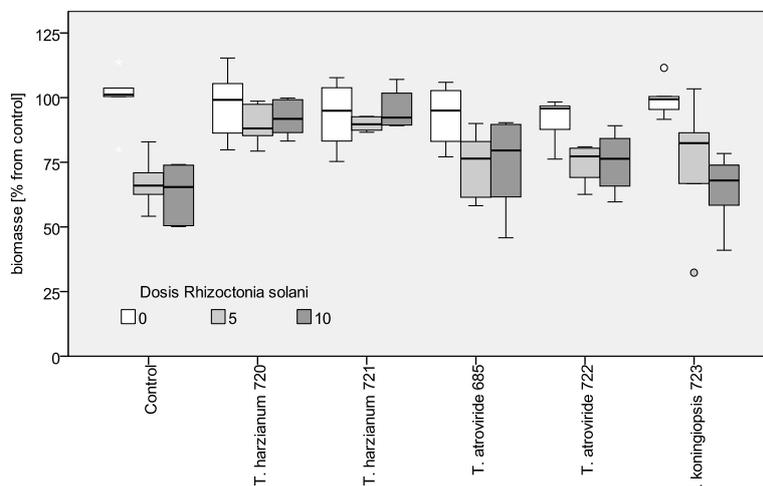


Figure 6. Capability of five *Trichoderma* strains to protect basil plants against basal rot disease caused by *Rhizoctonia solani* in cress and cucumber in growing media. The growing media is composed of 70% of limed black peat and 30% of coir (control). 1 unit of *R. solani* corresponds to 1 g of a 7-d-old *P. ultimum*-millet seed culture L<sup>-1</sup>. The control weight is the average of the cress biomass in the control media without pathogen. Each box plot represents the results of six pots.

## DISCUSSION

Contrary to the other organic fertilisers, shrimp meal reduced the disease incidence in the compost amended growing media. The chitin, in relation to the compost microorganisms, could have been responsible for it, by promoting production of chitin degrading traits of micro-organisms. Suppressiveness of chitin amendments was already observed by different authors (Andres et al., 2007; Giotis et al., 2009). Biosol contains also chitin, but from fungal origin. Because of the easier breakdown of chitin bound in molecular complex of animal origin, the release of chitin from crab shell seems to be easier than from biosol (Gagnon and Berrouard, 1994); thus chitin is easier available to the microorganisms in the soil resulting in a better suppressive effect.

Compost amendment has the highest potential to produce suppressive growing media. However, we observed that this strategy was not always successful. Composts are complex aggregates of organic components with highly diverse microbial communities (Dees and Ghiorse, 2001). These communities varied over time depending on a multitude of factors, and so different was their effect on plant diseases (Fuchs, 2010; Mehta et al., 2014). The difficulty is to be able to predict the suppressive capacity of composts. With our study, *Aeromonas* sp. was the dominant bacterial species in the suppressive compost, whereas *Enterobacter cloacae* was dominant in the conductive compost. *Aeromonas* sp. is typically part of the microbial community of composts in initial stages of the decomposing process (Mehta et al., 2014). It is known, that *A. hydrophila* and *A. cavia*, both closely related to *A. media* (Benagli et al., 2012), can be effective against soil borne pathogens such as *Pythium* spp., *Rhizoctonia solani*, and *Fusarium oxysporum* (Strunz et al., 1978; Inbar and Chet, 1991). So this bacterium could be an indicator for the disease suppressive potential of green waste compost.

The basic component of the growing mixture influences also the capacity of compost to protect the plants. The quality of peat could play a role here, but also the presence and content of clay minerals. It is known that clay minerals can influence the suppressive activity of antagonistic bacteria such as *Pseudomonas fluorescens* (Stutz et al., 1989).

The tested commercial biocontrol agents could not protect the plants against diseases. The conditions in the growing media did not allow the development of their suppressive activity. Some of the new strains of *Trichoderma* spp. showed disease suppressive potential in the tested growing media, either with or without compost. But the efficacy of the strains was specific, and the same strains showed different potentials to suppress either *Rhizoctonia solani* or *Pythium ultimum*. Similar results were also obtained by Pugliese et al. (2011). A combination of *Trichoderma* strains should be tested therefore, in order to broaden their efficacy.

## CONCLUSIONS

The suppressive characteristics of growing media can be influenced by the composition of the basic compounds and the addition of biocontrol agents. Organic fertilizers are less involved in plant protection than compost. However, the suppressive potential of compost varied depending of the compost itself, but also of the other components in the growing media. The quantification of indicator microorganisms such as *Aeromonas* sp. could be helpful to select the most appropriate compost.

The outcome of the next experiments, including the selection of compost, the choice of peat, the use of clays minerals and the application of effective strains of biocontrol agents might lead towards a solution to assure the quality of suppressive growing media. Further research in this direction has to be undertaken.

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