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Suppressive properties of Georgian biowaste composts against *Pythium ultimum*

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Abstract

Goal of the mycological bioassays was the proof of suppressive properties of the Georgian biowastes, which were differentiated in additives (A. straw and other crop residues, B. wooden chips), in seasonal origin, year and maturation stage (A. autumns (compost from 2011, 11 months old), B. winter (compost from 2012, 16 months old). The trials took place in 2012 and 2013. The bioassay, inoculated substrates by *Pythium ultimum*, with peas (*Pisum sativum L.*) as sensitive host is an approved procedure of sterile sand system for the assessment of suppressiveness. The statistical analyses in a 3-way anova procedure (A. year, B. substrate, C. infection level) achieved some noticeable results. The effects of compost charges of the different years were assigned into similar groups. Thus, a year effect could be neglected, in addition the question of seasonal origin and maturation stages. There was a tendency that compost enriched by wooden chips seemed to be more effective in terms of potential regulations against soil-born diseases. The other type of compost was slightly less suppressive, but still significant in most of the cases. Parameters of assessment were fresh matter of above-grown biomass, sprout length, emergence rate, the disease severity and the reduction of disease incidence.

Keywords

Biowaste, Georgia, suppressivenes, *Pythium ultimum*.

Introduction

The use of composts provides many benifical effects for organic and sustainable farming systems. Recycling of organic matter back to soil, providing nutrients for the soil ecosystems, energy sources for soil life, structural material for the improvement of bad physical state of the soil belong to the main useful reasons for their application. Beside that organic matter in general and composts in specific can be highly interesting for the substitution of pesticides in horticultural substrate-based growing-systems and other fields other agricultural practice. The regulatory effect against soil-borne diseases was described in many papers (Hoitink et al., 1986; Hoitink & Boehm, 1999; Bonnanomi et al., 2010; Neher, 2019; Chen et al., 1988; Erhart & Burian, 1997; Boehm & Hoitink, 1992). Schüler et al. (1989) and Bruns (1998) were able to prove the higher suppressiveness of separately collected and composted biowaste compared to composted farmyard manure. Goal of this investigation was the assessment of two Georgian biowaste composts (derived from the compost trial at Marneuli) which differed in their additives (A. wooden chips, B. straw and other crp residues) with regard to their suppressiveness.

Materials and Methods

The bioassay was made with the fungus Pythium ultimum var. ultimum, originated from Dr. Ulber, University of Göttingen. The compost material dated back to trials in **2011 and 2012,** resulted of piles out

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of different seasons, ***autumn and winter***, enriched by different additives, **wooden chips and straw**. Beside that the samples of 2011 had a rotting age of ***11 months***, the ones of 2012 ***16 months***. All in all, four variables wegrowing-t of the bioassays with *P. ultimum* and *Pisum sativum* as sensitive host crop in a sterile sand system. The physico-chemical properties of the composts are described in Table 1.

Table 1. Physico-chemical properties of substrates and composts, used in the bioassays.

Composts Substrates	Year	pH (CaCl2)	DM (%)	Vol. W. (g l^{-1})	Salt (g KCl l^{-1})	N (%)	C/N	Nmin (mg kg^{-1} dm)
Comp_W		7.9	66.2	0812,,0	05.76	1.10	12.9	256,
Comp_S		7.8	66.2	795	10.34	0.90	16.7	497
Sand	2012	6.4	nd	135366	nd	0.001	6,5	nd
Sand+Comp_W		6.7	84.1	1245	nd	nd	nd	nd
Sand+Comp_S		6,7	84.1	1242,7	nd	nd	nd	nd
Comp_W		8.0	57.4	765	6.41	0.74	11.6	nd
Comp_S	2013	8.4	62.2	0696,6	08.02	0.61	12.3	nd
Sand+Comp_W		6.7	83.3	1236	nd	nd	nd	nd
Sand+Comp_S		6.8	82.4	1222,1	nd	nd	nd	nd

nd = not determined, DM = dry matter, mixtures of sand + compost consisted of 20 % (v/v).
Com_W: compost enriched by wooden chips, Com_S: compost enriched by straw.

As could be expected the composts from two years differed in some aspects: most obvious the lower content of salt for the Comp_W variables in both years compared to Comp_S. The N content in 2013 was slightly lower. The same is true for the C/N ratio. Parameters of assessment were pH value, dry matter content, specific weight, salt content, nitrogen, carbon, C/N, and nitrate as fraction of available nitrogen. Less salt for wooden-enriched compost could be a big advantage for the use in growing media and the various salt sensitivities of seedlings.

Mycological methods for the maintenance and the propagation of the pathogen Pythium ultimum followed the instructions of Bruns (1998) and Werren (2011). Substrates and their inoculation were described by Schüler et al. (1989). Pea seeds (1000 Tkw of 280g, undressed) were provided by KWS Lochow GmbH (Var. *'Santana'*) and sterilized by 70% ethanol.

The pot trials (2012 and 2013) were organized in a randomized block design with 5 replicates and placed in a phytotron (day conditions: 19 °C +/- 0,5 °C; 16 h at 10,000 Lux; night conditions: 16 °C +/- 0,5 °C; 8 h in darkness). The substrates (sand, sand + composts) were thoroughly mixed, seeds (8 per 11-pot) were placed in depth of 1.5 cm. Continuous watering was achieved by compensating the estimated water loss. For an undisturbed harvest of the various replicates each pot was surrounded by a small perforated plastic bag 10 days after seeding. The growing days in total were 21 in 2012 and 20 in 2013.

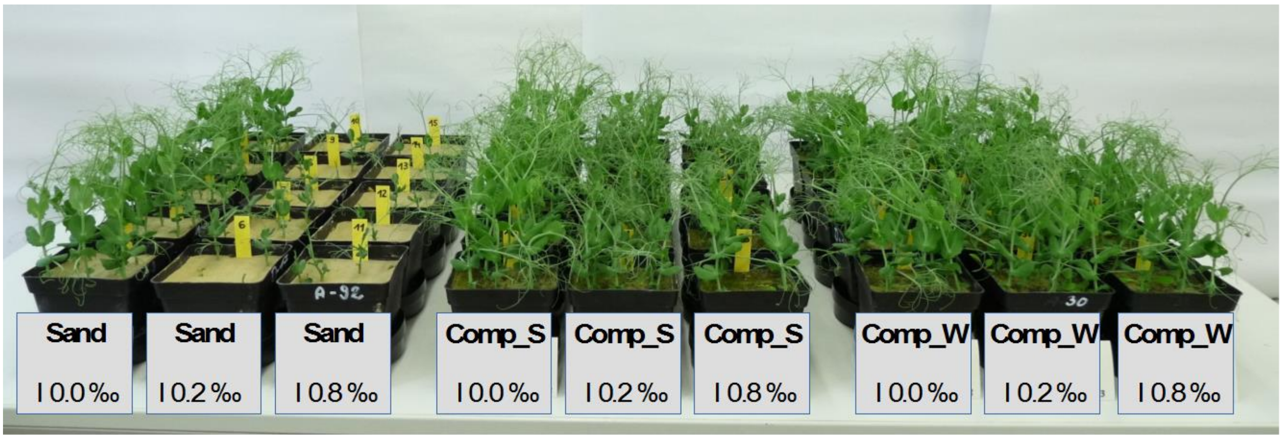


Fig. 1. Cultivation of peas in different substrates in response to different inoculation levels (0.0 ‰, 0.2 ‰, 0.8).

Parameters of assessment

Parameters of assessment were emergence rate, fresh & dry matter of above grown biomass, sprouting length per crop. According to Schüler et al. (1989), Bruns (1998) and Denzel (2008) data of the above grown fresh biomass can be used for the calculation disease severity and the reduction of disease incidence as measurement of the suppressiveness of applied composts. The disease severity or infestation level resulted in the comparison between data of infected and uninfected variables (Bruns, 1998; Werren, 2011).

$$IL\ \% = (FM^{(0)} - FM^{(+)}) / FM^{(0)} \times 100$$

IL= Infestation level

$FM^{(0)}$ = Fresh matter of substrate control

$FM^{(+)}$ = Fresh matter of infected variable

The reduction of infestation (degree of efficiency) compares the difference between infected variable and mineral test variable related to the difference of non-infected mineral control and mineral test variable“ (Werren, 2011).

An important precondition for the estimation of reduction level is the statistical homogenety of uninfected controls (Bruns et al., 2003). The calculation of the reduction of disease incidence followed the formula:

$$RDI\% = (X - FM^{(+)}) / (FM^{(0)} - FM^{(+)}) \times 100$$

RDI = Reduction of disease incidence

X = Fresh matter of infested variable

$FM^{(+)}$ = Fresh matter of infected sand variable in same infection level

$FM^{(0)}$ = Fresh matter of uninfected sand control

Results and Discussion

Fresh matter of above-grown biomass

Suppressive actions could be observed at all tested composts. In both years the achieved biomass responded to the inoculation in similar tendencies, highest growth reductions at highest infestation levels. Except the Comp_W variable in 2013 significant differences towards unfected controls were achieved at all other substrates. High infestation levels also resulted in higher growth reductions compared to low infestation levels, but in no case significantly. Within the evaluation of all data the fresh matter development was found as significantly higher in year 2012 (8.8 g) compared to 2013 (8.0 g), significantly higher in compost variables compared to sand substrate (see Fig 2), and between Comp_W and Comp_S (10.4 g vs. 9.0 g). The results of the Level of infection differed significantly between all levels (11.9 g vs. 7.6 g vs. 5.7 g) (see Fig 3).

Fig. 2. View into the phytotron: After emergence (left); After adding harvest aids in of perforated plastic bags (right).

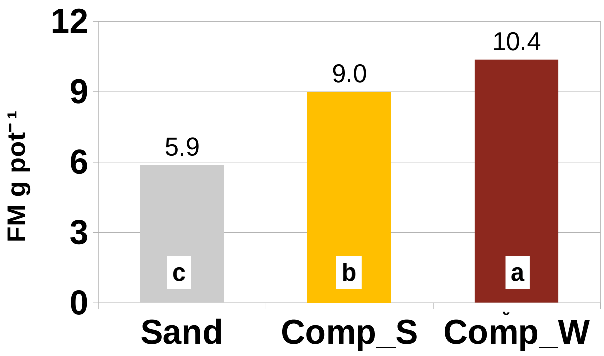


Fig. 3. Fresh matter yield of factor Type of subtrate, including Level of inoculation and Year. Data with unequal letters are significantly different according Tukey ($p \leq 0.05$).
Comp_W: compost plus wooden chips,
Comp_S: compost plus straw.

Table 2. Fresh matter yield in response to type of sustrate, level of inoculation, and carried out in two years. Data with unequal letters are significantly different according Tukey ($p \leq 0.05$). Row and column headed by ALL corresponds to separate terms Year and Substrate. Comp_W: compost plus wooden chips,
Comp_S: compost plus straw.

		Year		
		2012	2013	ALL
Substrate	Infection	g pot⁻¹	g pot⁻¹	g pot⁻¹
Sand	0.0‰	11.9 abc	10.0 b_e	10.9 ab
	0.2‰	6.7 fgh	1.3 i	4.0 de
	0.8‰	4.6 h	0.6 i	2.6 e
Comp_S	0.0‰	12.0 abc	12.8 ab	12.4 a
	0.2‰	8.9 def	8.7 def	8.8 c
	0.8‰	5.1 gh	6.5 fgh	5.8 d
Comp_W	0.0‰	13.4 a	11.3 a_d	12.3 a
	0.2‰	9.2 c_f	10.8 a_d	10.0 bc
	0.8‰	7.6 efg	9.8 b_e	8.7 c
ALL		8.8 a	8.0 b	

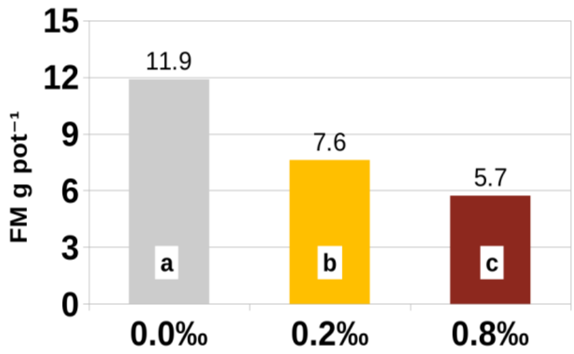


Fig. 4. Fresh matter yield of factor Level of inoculation, including substrate and years Data with unequal letters are significantly different according Tukey ($p \leq 0.05$).

The validity of the bioassay could be assured by the distinct growth reductions of the inoculated sand controls in both study years. The statistical diversities were mentioned before, the partial dimensions of growth reduction were 44 % and 87 % for the low infection, 61 % and 94 % for high infection level in both years.

All (six) compost variables were found homogenous between the years which can be interpreted as valid confirmation of results in 2012. Furthermore, aspects of season (2012: autumn compost, 2013: winter compost) and of degree of maturation (Rotting period of 11 months (2012) and 16 months (2013)) could be neglected.

Table 3. Emergency rate in response to type of substrate, level of inoculation and carried out in two year. Data with unequal letters are significantly different according Tukey (p ≤ 0.05). Row and column headed by ALL corresponds to separat terms Year and Substrate. Comp_W: compost plus wooden chips, Comp_S: compost plus straw.

		Year		
		2012	2013	ALL
Substrate	Infection	N pot⁻¹	N pot⁻¹	N pot⁻¹
Sand	0.0‰	7.8 a	8.0 a	7.9 a
	0.2‰	7.4 ab	3.8 de	5.6 c
	0.8‰	5.4 cd	2.2 e	3.8 d
Comp_S	0.0‰	8.0 a	8.0 a	8.0 a
	0.2‰	7.6 ab	8.0 a	7.8 a
	0.8‰	5.8 bc	6.6 abc	6.2 bc
Comp_W	0.0‰	8.0 a	7.8 a	7.9 a
	0.2‰	7.4 ab	7.8 a	7.6 a
	0.8‰	6.8 abc	7.6 ab	7.2 ab
ALL		7.1 a	6.6 b	

The figures for emergency rates were quite similar to the other parameters assessed. Only the diminishing effect of the inoculation were found more counteracted by the compost variables compared to other parameters. The percentage-wise loss against the particular substrate control was 3 and 23 for Comp_S at 0.2 ‰ and 0.8 ‰ inoculation, 4 and 9 for Comp_W (see Tab. 3).

Sprout length

Table 4. Sprout length in response to type of substrate, level of inoculation and carried out in two years. Data with unequal letters are significantly different according Tukey (p ≤ 0.05). Row and column headed by ALL corresponds to separate terms Year and Substrate. Comp_W: compost plus wooden chips, Comp_S: compost plus straw.

		Year		
		2012	2013	ALL
Substrate	Infection	cm	cm	cm
Sand	0.0‰	10.84 ab	9.38 abc	10.11 ab
	0.2‰	7.86 cd	1.66 e	4.76 de
	0.8‰	7.58 cd	0.80 e	4.19 e
Comp_S	0.0‰	9.94 abc	10.98 ab	10.46 a
	0.2‰	7.80 cd	7.92 cd	7.86 c
	0.8‰	6.08 d	6.02 d	6.05 d
Comp_W	0.0‰	11.35 a	9.82 abc	10.59 a
	0.2‰	8.99 abc	9.84 abc	9.41 abc
	0.8‰	8.48 bcd	9.02 abc	8.75 bc
ALL		8.77 a	7.27 b	

In both years sprout length and fresh matter yields were correlated to a high degree (2012: r = 0.885, p<0.000; 2013: r = 0.992, p<0.000). Crops growing on the sand substrate were mostly afflicted in the year 2013 in which the length was reduced by 82 % and 0.1 % (see Tab.4). The inoculation induced statistically smaller crops in most cases, only crops of the Comp_W substrate were little affected. The statistical homogenity within the annual comparison became obvious again. Similar tendencies could be derived from the means of the term Year*Substrate: the highest (and significant) reductions of infestation level 0.8 ‰ were found in the sequence 59 %, 42 % and 17 % for the substrates Sand, Comp_S and Comp_W. The various subtrate controls were measured by 10.11 cm, 10.46 cm and 10.59 cm and assigned as statistically homogenous.

Disease severity and Reduction of disease incidence

Table 5. Disease severity in response to type of substrate, level of inoculation and carried out in two years. Data with unequal letters are significantly different according Tukey (p ≤ 0.05). Row and column headed by ALL corresponds to separate terms Year and Substrate. Comp_W: compost plus wooden chips, Comp_S: compost plus straw.

		Year		
		2012	2013	ALL
Substrate	Infection	% Subst control	% Subst control	% Subst control
Sand	0.2‰	43.5 bcd	87.1 a	65.3 ab
	0.8‰	61.1 b	93.5 a	77.3 a
Comp_S	0.2‰	25.5 def	32.0 cde	28.8 c
	0.8‰	57.1 bc	49.3 bcd	53.2 b
Comp_W	0.2‰	30.8 de	4.1 f	17.5 c
	0.8‰	42.8 bcd	13.1 ef	28.0 c
ALL		43.4 a	46.5 a	

For the compost substrates the increase of infection degree resulted in higher infection rates of the crops. But only in one case this increase was high enough (>20 %) for a significant difference (Comp_A in 2012 whereas all other substrate groups within the annual perspective turned out to be statistically homogenous.

Table 6. Reduction of disease incidence in response to type of substrate, level of inoculation and carried out in two years. Data with unequal letters are significantly different according Tukey (p ≤ 0.05). Row and column headed by ALL corresponds to separate terms Year and Substrate. Comp_W: compost plus wooden chips, Comp_S: compost plus straw.

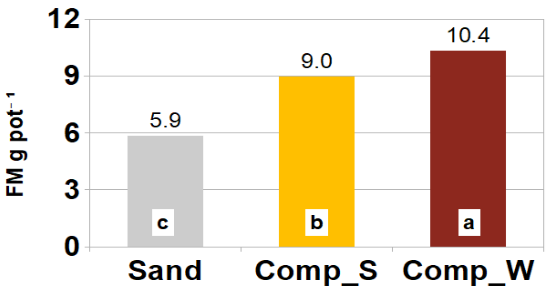
		Year		
		2012	2013	ALL
Substrate	Infection	% Sand control	% Sand control	% Sand control
Comp_S	0.2‰	42.1 cd	84.9 abc	63.5 ab
	0.8‰	6.9 d	62.4 abc	34.6 b
Comp_W	0.2‰	48.3 bcd	109.7 a	79.0 a
	0.8‰	41.2 cd	98.1 ab	69.6 a
ALL		34.6 b	88.8 a	

With regard to the results of the factors year and substrate the significant difference between the growth responses of crops growing on compost-enriched substrate became quite obvious. Compared to the results of the sand substrate group all infection levels of the two compost groups were significantly different.

The higher efficiency of wood-enriched composts explains Bruns (2012) by the fact that composts are highly settled by microbes which are capable for antagonistic reactions. The transformation of organic matter dominated by lignin-rich wooden material results in stable ligno-cellolytic complexes. Microbes adopted to such a system develop high antagonistic capacities, an important tool for the regulation of pathogens.

Root development

Fig. 3. Fresh matter yield of factor Type of subtrate, including Level of inoculation and Year. Data with unequal letters are significantly different according Tukey (p ≤ 0.05). Comp_W: compost plus wooden chips, Comp_S: compost plus straw.



The inoculation by Pythium ultimum affected all roots, visible alterations could be observed (see Fig. 5). By increasing infection level teneous roots tended to disappear. This is mainly true for the highest level, more in peas grown in sand substrate, less in those grown in compost substrate. This was accompanied by disease symptoms at the root crown. The roots of the Comp_W seemed to develop less disturbed than those of Comp_S. A fact which was justified by some of the parameters assessed.

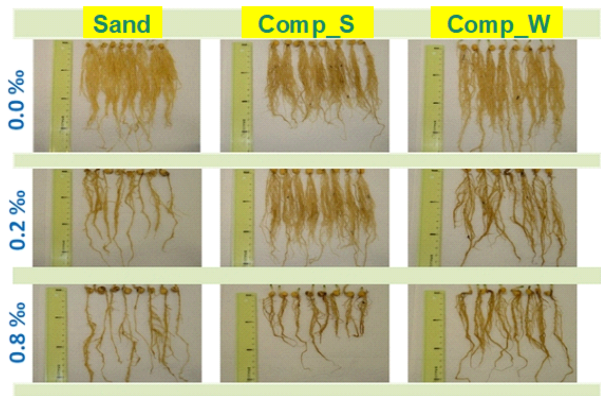


Fig. 5. Root growth in response to Type of substrate and Level of inoculation.

Ebrahimi et al. (2018) could demonstrate very similar results with Iranian house waste compost by using the same test system. Markakis et al. (2016) found similar regulatory actions of Household compost against Verticilium dahliae and emphasized a longer duration of suppressiveness in plant-based composts.

De Corato et al. (2016, 2018) rely that on the existence of higher content of lignin. That corresponds to the found data which indicate the wooden-rich compost as more effective compared to the other type. The potential competition for available nutrients could be another pathway of suppression (Pane *et al.*, 2011; Bonanomi *et al.*, 2007; Suárez-Estrella *et al.*, 2013) derived by different sets of microbiomes (Szczech 1999, Pane *et al.*, 2013).

Conclusion

The separately collected house waste provides a serious source for the production of biowaste compost in Georgia and its beneficial use in agriculture and horticulture (Veeken et al., 2005; Hoitink et al., 1991). Beside the known facts of applied organic matter for the chemical, physical andbiological improvement of soils the specific suppressive properties of such a material let it become a valid source for plant protection measures, in specific against soil borne diseases (Hoitink, 1999; Hoitink et al., 1986; Hoitink & Grebus, 1994). Any system with restricted use of synthetic pestides should intensify the use of such a multipurpose mean.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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