Suppressive Properties of Composts are Determined by their Raw Materials

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Abstract

Plant diseases occur as a result of infection by microorganisms, primarily fungi. To control these diseases, farmers use chemical pesticides, which can be harmful for environments. Therefore, alternative methods to control phytopathogens are needed. In our study, we focused on the use of comports to control pathogenic Fusarium oxysporum in tomatoes and identify what properties and mechanisms are responsible for the disease suppressive nature of comports. The raw materials used for compost preparation were chicken manure, swine manure, cattle manure, corn and straw. Unsterilized and sterilized variants of comports were used in the experiments. Sterilization of compost had different effects on comports containing different primary substrates indicating primarily abiotic character of suppressiveness of comports prepared on the basis of chicken manure, primarily biotic character of comports prepared on the basis of swine manure, and mixed character of suppressiveness of comports prepared on the basis of cattle manure.

Keywords: Biocontrol Organisms, Composting, Phytopathogen, Suppressiveness

1. Introduction

Plant diseases cause major losses in crop yield annually. Of all groups of microbes the largest percentage of plant diseases are caused by fungi. Fusarium spp., is one of the most widespread fungi causing plant root wilt. To control these diseases, farmers usually use chemical pesticides. There is growing concern over the harmful side effects on the environment as well as human and animal health of using such chemicals. There is now a growing need to develop solutions that do not cause harm to the environment but are also effective. The use of biological control measures is getting more attention as an alternative to chemical control.

Some comports have been reported to be able to control soil-borne plant pests. Use of such comports can solve several environmental problems – waste utilization, soil quality improvement and plant disease control. Comports can be suppressive towards plant pathogens using one of four mechanisms, or their combination. Firstly, beneficial microbes compete for nutrients with plant pathogens in the rhizosphere. Secondly, suppression may be caused by a production of antibiotic compounds by beneficial microorganisms in a process known as antibiotic. These antibiotics are metabolites produced as a result of microbial activity and may be toxic substances or lytic agents. Thirdly, there is the mechanism known as parasitism or predation in which the addition of compost stimulates the growth of populations of micro-arthropods such as beneficial nematodes, springtails and mites which consume pathogen propagules in the soil. The fourth mechanisms is that of activation of disease resistance genes in plants by microorganisms in compost which is known as Induced Systemic Resistance or systemic acquired resistance which is basically a state of enhanced defense which develops when a plant is sufficiently stimulated. In induced systemic resistance certain microorganisms cause plants to turn on or strengthen their natural defense.

Suppressive activity has been divided into two groups, general and specific suppression. General suppression refers to the sum of activities by different microorganisms in compost. Specific suppression refers to a situation where a specific group of organisms suppress a disease which may be due to microorganisms being antagonistic to the pathogen. To strengthen suppressiveness of comports,
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bacterial and fungal strains with biocontrol properties can be added to composts. Thus, *Pseudomonas putida*, *Acetobacter indonesiensis*, *Bacillus pumilus*, *Paecilomyces variotii*, *Streptomyces griseus*, *Acremonium chrysogenum*, *Trichoderma harzianum*, *Gliocladium virens*, *Flavobacterium balustinum* and *Xanthomonas maltophilia* species are known to possess suppressive properties and therefore can be used for this purpose. However, the subsequent fate of these additions is not fully understood because of the complex factors influencing their survival – from biochemical properties of composts, nature of raw materials to competition from the view of microbiota.

Despite the fact that in many cases composts suppressiveness is described in the literature, the exact reasons for occurrence of suppressiveness are not completely understood. Thus, suppressive efficacy of composts may depend on interaction between pathogens, bio-control agents, soil organic matter and plants roots. Maturity of composts may be another important factor that determines the efficiency of using biocontrol strain *P. putida* PCL 1760 for improvement of compost suppressiveness.

2. Materials and Methods

2.1 Preparation of Compost Mixtures

Different compost mixtures were prepared on the basis of agricultural wastes. The raw materials used were chicken manure, corn waste, straw, cow manure and pig manure which were collected from farms in the Republic of Tatarstan (Russia). The compost mixtures contained two or three substrates. The relationship between the substrates in the compost mixtures was chosen in such a way as to ensure the ratio between the content of organic carbon (C$_{org}$) and total nitrogen (N$_{tot}$) was between 9-25 (Table 1.) which reported to be optimal for composting process.

<table>
<thead>
<tr>
<th>Name of mixture</th>
<th>Mass of substrates, %</th>
<th>Sawdust</th>
<th>Corn waste</th>
<th>Chicken manure</th>
<th>Cattle manure</th>
<th>Swine manure</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost 1</td>
<td></td>
<td>44</td>
<td>56</td>
<td>14,25</td>
<td>14,25</td>
<td></td>
<td>14,25</td>
</tr>
<tr>
<td>Compost 2</td>
<td></td>
<td>38</td>
<td>62</td>
<td>9,57</td>
<td></td>
<td></td>
<td>9,57</td>
</tr>
<tr>
<td>Compost 3</td>
<td></td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>13,06</td>
<td></td>
<td>13,06</td>
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<tr>
<td>Compost 4</td>
<td></td>
<td>45</td>
<td>55</td>
<td></td>
<td>14,15</td>
<td></td>
<td>14,15</td>
</tr>
<tr>
<td>Compost 5</td>
<td></td>
<td>40</td>
<td></td>
<td>60</td>
<td>22,53</td>
<td></td>
<td>22,53</td>
</tr>
<tr>
<td>Compost 6</td>
<td></td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>12,96</td>
<td></td>
<td>12,96</td>
</tr>
</tbody>
</table>

2.2 Estimation of Properties of Composts in Dynamics

Compost parameters were measured weekly.

The temperature was measured every day at different levels of the composting mixtures (length and height) with digital thermometer equipped (Scala, GEFU, Germany).

Phytotoxicity of the composts was measured toward oats (*Avena sativa*). The seeds were placed in a 90-mm plastic cups with 5g of compost mixed with 45g of soil. Controls were treated in the same way but with 50g soil rather than compost. The germination index (48 h at 25°C) was calculated from three replicates for 15 seeds per replicate according to the following formula:

$$GI(\%) = \frac{(\text{Seeds germination in treatment x Root length in treatment} \times 100)}{(\text{Seeds germination in control x Root length in control})}$$

To determine the DOC values of each sample, the extraction method outlined by Chefetz was used. A 0,5-ml sample of solution was taken after extraction and immediately analyzed by dichromate oxidation according to the tube digestion procedure described by.

The microbial respiratory activity in the soil was estimated from the amount of C-CO$_2$ released from compost and captured NaOH solution by according to.

2.3 Evaluation of Suppressive Properties of Composts

Suppressive properties of composts were evaluated using “tomato – fusarium” model system. Soil was artificially
contaminated by *Fusarium oxysporum f sp. radicis-lycopersici* (Forl) (10⁵ spores kg⁻¹). The compost samples were placed into the soil in 1:10 ratio, and tomato seeds were sown. After three weeks the number of dead plants was assessed as well as the number of plants with damaged roots. Infected plants were considered those with rotten roots, or with a clearly visible blackening of the root. As a control, there was soil which had not been inoculated with *F. oxysporum*. To eliminate the influence of microbiota on the compost, plant growth and development, sterilized compost was added to the contaminated soil. Compost was sterilized by placing 50g of compost into a 250 ml beaker and placing it in an autoclave overnight. Besides, we performed an additional test where tomato seeds were treated by *P. putida* PCL 1760 biocontrol strain. *P. putida* PCL 1760 was cultured in a solution of 50 ml 1% carboxymethyl cellulose + 50ml L-bullion solution for 24 h. The tomato seeds were then dipped into the culture, after which they were placed on filter paper and then grown in the sterilized compost amended soil.

### 2.4 Statistical Analysis

Each data point represents the average value from triplicate measurements and error bars represent standard deviation. Significant differences among the values of each parameter studied during composting were performed using *STATISTICA Base* (StatSoft). The results were considered significant if the associated P value was below 0.05.

### 3. Results and Discussion

#### 3.1 Monitoring of the Composting Process. Revealing of the Possible Early Time of Supressiveness Appearance

Four parameters (the temperature of the compost mixture, content of soluble organic carbon, respiratory activity of microorganisms and phytotoxicity) were used to monitor the process of composting. All these parameters are widely used to assess the efficiency of the aerobic decomposition of organic matter, as well as for determining the maturity and stability of the composts¹¹,¹⁶,³⁸. Results are presented on Figure 1.

Temperature is an important indicator of the progression of the composting process and it plays a vital role on the succession of microbial communities¹¹. Figure 1(a) shows the temperature dynamics taking place during the composting process which we carried out. In our samples, the temperature sharply increased in all the composts on the second day signifying the start of the thermophilic phase. By day 20 the temperature began to decrease denoting that composts were entering into the cooling phase. There was a general leveling off of temperature after 20 days which suggests that the final curing phase had been reached.

Respiration activity is related to the presence and activity of microbial communities. When there is a high concentration of organic matter microbes respire at higher rates as their populations in the composts are high. Reduced respiratory activity is associated with the depletion of readily available organic compounds mentioned in other studies. Respiration activity is the parameter recommended to measure stability of the composts¹⁴. As shown on the Figure 1(b), the changes in respiration activity of composts were similar. Initial respiration activity values were highest in Compost 2 with a value of about 870 mg C-CO₂ kg⁻¹ and lowest in Compost 1 with a value of 315 mg C-CO₂ kg⁻¹. During the identified thermophilic phase there were general fluctuations in respiration activity for all samples. This occurs as a result of the succession of microbial populations which consume different substrates. After about day 60 respiration activity was generally low and there were very low fluctuations in respiration activity values and values in all samples were below 200 mg C-CO₂ kg⁻¹. These low and stable respiration activity values a characteristic of the curing phase.

One of the most important parameters used to monitor the process of composting, compost maturity and possible onset of suppressive properties, is a measure of the amount of soluble organic carbon (DOC). DOC values indicate the amount of easily degradable compounds in the composting piles (Wang et al.³⁴). Data showing the changes in the content of soluble carbon are presented in Figure 1(c). In all composts samples the initial percentage DOC values ranged from 6% to 14%. During days 0 to 55, we observed a decrease in DOC in all the compost mixtures while significant fluctuations took place in each of the separate samples. This could be explained by break down of the organic compounds in composts by microbes followed by rapid consumption of these compounds. After day 56, less fluctuations of DOC were observed while the absolute values were quite low (between 1 % and 3 %). This is probably due to completion of microbial decomposition
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of easily degradable compounds by r-strategists and beginning of low and stable decomposition of recalcitrant organic compounds by K-strategists. The composts containing chicken manures had high DOC values during the composting process which suggests that chicken manure has a relatively lower bio-stability than the other manures. This agrees with a study carried out by. The composts containing cattle and swine manure generally had low values for DOC owing to high concentrations of recalcitrant carbon.

The purpose of the composting process is to obtain an organic fertilizer and one of the characteristics of the efficiency of the composting process is its maturity and phytotoxicity. We therefore evaluated the effect of composts on oat plants and based on the results obtained we calculated the GI. Results are presented in Figure 1(d). In the beginning all the composts inhibited plants as shown by the low GI index which ranged significantly. From about day 10 to day 70 of composting there was a general increase in the GI values for all composts with fluctuation in values during this period. The fluctuations of GI values supports the dynamics of phytotoxicity reported in literature. In cases where GI increased, this could be attributed to compost behaving like a fertilizer and not necessarily suppressing disease. The highest GI values were observed in Compost 4 containing cattle manure. Cattle manure has low concentration of phytotoxic compounds. Cattle are ruminants and have a high concentration of non-easily degradable organic compounds. Chicken manure containing composts tended to have lower GI values. Chicken manure can therefore be said to have a higher concentration of phytotoxic compounds which can be explained by the fact that poultry animals do not excrete urine and urine is released with feces. This means that poultry excreta is higher in acidic compounds.

Analyzing all four parameters measured in complex, we estimated a time point when the most intensive destruction of organic compounds in the composts had come to completion: according to the temperature dynamics it was day 20-22, and according to the three other parameters – day 55-60. At this time point fluctuations of the parameters became weaker,
respiration activity and content of DOC reached their minimum, and phytotoxicity became stable. According to the literature\textsuperscript{20, 38}, this time point is a earliest possible time for the appearance of composts' suppressiveness. It is economically important, to obtained the composts with required properties as soon as possible. Therefore this approach of determination of earliest expected onset of suppressiveness may be used in practice.

### 3.2 Determination of Suppressive Properties of the Compost towards \textit{F. oxysporum}

We further estimated the influence of \textit{F. oxysporum} contaminated soil treated by the composts on the plants. We measured suppressiveness every two weeks starting from day 56. Composts did not show stable suppressive properties, which suggests that the process of compost maturation had not come to completion yet. The earliest time point at which we observed significant inhibition of \textit{F. oxysporum} and stimulation of the plant growth was on day 70. Results are presented in Figure 2.

![Figure 2. Suppressive properties of the composts.](image)

In the presence of unsterilized composts the percentage of plants that were healthy was higher than those in the soil with no compost amendment - 56%-83% in contrast to 33%. However the number of healthy tomatoes when using compost was lower than that in the control (92-98%). Soil amended with Composts 3-6 had relatively high percentages of healthy tomatoes.

For better understanding of the mechanism of suppressiveness, we additionally analyzed two variants of composts. First, we sterilized the composts in order to reveal microbial role in suppression. Second, we treated tomato seeds with inoculum of \textit{P. putida} PCL 1760 which has previously inhibited \textit{F. oxysporum} by competition mechanisms\textsuperscript{31}. Based on the results obtained the observations could be divided into 3 groups: i) composts where sterilization did not influence suppressiveness and treatment by \textit{P. putida} PCL 1760 caused different effects (Compost 1 and compost 2); ii) composts where sterilization slightly decreased suppressiveness, and treatment of \textit{P. putida} PCL 1760 had no significant effect (Compost 3 and 4), and iii) compost where sterilization highly decreased suppressiveness, and of \textit{P. putida} PCL 1760 let do a significant improved suppressiveness (Compost 5 and 6). In the first group, suppressiveness may be primarily abiotic and chemicals contained in the composts may inhibit growth of the pathogen, while compost components were not inhibiting for biocontrol bacteria. In the second group, suppressiveness may be due to both biotic and abiotic factors, but the biotic elements may not play very significant role, besides, composts are slightly toxic towards \textit{P. putida} PCL 1760. In the last group, inhibition of \textit{F. oxysporum} may be primarily biotic, and compost components are able to stimulate growth of \textit{P. putida} PCL 1760. It is interesting to note that composts inside each group contain the same type of manure: chicken manure for the first, cattle manure for the second, and swine manure for the third group. We can suggest therefore that suppressive and toxic properties of the composts studied are determined by the raw materials which were used for their preparation, even in the later stages of composting process.

### 4. Conclusion

In this work we demonstrated the principle potential to obtain suppressive composts from the agricultural wastes. An approach to reveal the time point of the possible early appearance of suppressiveness was suggested. It was revealed that raw materials play an important role for composts' suppressive and toxic properties.

### 5. Acknowledgement

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### 6. References

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