

Research Article**Suppressive properties of composts may be improved
by microbial inoculation**

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ABSTRACT

The use of suppressive composts instead of traditional fertilizers and pesticides is becoming more commonplace in modern agricultural biotechnology. In this study, we investigated the possibility of obtaining composts with suppressive properties from widespread agricultural wastes produced in tatarstan (russia). Straw and corn wastes, as well as chicken, cattle and swine manure were used to prepare 11 two- and three-component mixtures for further composting. It was found that mesophilic phase of composting lasted for 1 to 3 days for all the waste mixtures; the thermophilic phase lasted from 2 to 35 days, and was characterized by significant fluctuations in temperature, i.e. from 27°C to 59°C. In the initial mixture, the dissolved organic carbon (doc) content was between 10 and 62 mg kg⁻¹; it fell significantly on day 13, and then continuously decreased up to day 102, and subsequently remained low. For all the mixtures, maximal respiration activity was observed in the beginning of composting (231.9 mg CO₂-cg⁻¹). After 23 days, this parameter decreased significantly, and fluctuations subsided. The phytotoxicity of the initial compost mixtures varied from 18% (sw+sm) to 100% (cw+chm+sm, cw+chm); however, the trends in the dynamics were similar. After 120 days of composting, 5 of 11 samples were not phytotoxic, and the others were slightly toxic. After 120 days of composting, each mixture was divided into two parts; one was inoculated with a biopreparation consisting of four microbial strains (*trichoderma asperellum* t203, *pseudomonas putida* pcl1760, *pseudomonas fluorescens* wcs365 and *streptomyces* spp.), and the other part was not inoculated. Both parts were composted under equivalent conditions for 60 days. Inoculation led to a slightly shorter period of increasing doc and respiration activity. However, after 3-10 days, no differences were observed between the mixtures. Inoculation did not influence the temperature profile or phytotoxicity of the mixtures. In contrast, the suppressiveness of the composts towards *fusarium oxysporum* increased by 1.2-fold after 60 days, although the inoculated compost mixtures became suppressive 30-58 days earlier as compared with the non-inoculated ones. The influence of inoculation was similar for composts of different types.

Keywords: compost suppressiveness, *fusarium oxysporum*, *trichoderma asperellum*, *pseudomonas putida*, *pseudomonas fluorescens*, *streptomyces* spp

[i] INTRODUCTION

The growing interest in environmental protection and social health has driven investigations in the field of green agricultural management [1]. Traditional strategies of agriculture such as

pathogen-stable plants and synthetic pesticide use do not meet these requirements and lead to decreased soil quality. It should be noted that use of chemical pesticides does not permit the control

of soil-borne plant pathogens, which cause significant losses in crop yields. Therefore, in modern agriculture, new, effective and environmental friendly methods with high efficiency and low costs are needed [2].

The use of suppressive composts is a type of biotechnology because such composts possess both fertilizing and pathogen-controlling properties. As an organic fertilizer, they also allow for the avoidance, fully or partly, of pesticide use [3–6]. When suppressive composts are prepared from organic wastes, another environmental problem, i.e. Waste utilization, may be solved [7]. Despite many publications concerning the suppressiveness of soils and composts, the prediction of suppressiveness or the production of composts with suppressive properties still remains a problem [4,8]. In fact, an analysis of the suppressiveness of different types of commercial composts produced at different times showed that this may range from almost zero to quite high [4,9]. Therefore, inoculation by bio preparation with bio control properties may be a good solution for the creation of suppressive composts [4,10].

Microbial inoculations are often used to intensify the composting process. Inoculations lead an increase in the aromatization level of humic acids, [4,11], an increase in the nitrogen and soluble phosphorous content, the intensification of lignin and cellulose biodegradation, accelerated compost maturation and an improvement in the quality of the final product [4,12–14]. In the literature, publications on the use of inoculations to improve suppressiveness can be found. Often, *trichoderma* micromycetes are used for this purpose [15–19]. Better results may be obtained when two of more biocontrol agents are used in one bio preparation [19,20].

The disease suppressiveness phenomenon may be explained by several mechanisms of interaction between pathogens and bio control agents. These are antibiosis (an antagonistic process mediated by microbes through metabolites, lytic agents and other toxic compounds), the induction of plant systemic resistance (enhanced defensive capacity

developed by a plant when appropriately stimulated), competition (competition between bio control agents and phytopathogens for nutrients and root colonization) and hyperparasitism (direct antagonism where the bio control agent directly attacks a phytopathogen) [21–26]. The aim of this study was to prepare a bio preparation consisting of microbes using all four mechanisms of suppression described above, and to assess its influence on the process of biodegradation as well as on improvements in the suppressiveness of composts prepared from organic agricultural wastes.

[II] MATERIALS AND METHODS

2.1 preparation and inoculation of composts

Compost mixtures were prepared from large-capacity agricultural wastes produced in the pestrichinski agricultural region located in central russia (55°46' n, 49°43' e). The main substrates of the two- and three-component compost mixtures were corn waste (cw) or straw waste, with the addition of chicken (chm), swine (sm) or cow manure (cm). The ratio between the substrates in the compost mixtures was calculated so that the organic carbon to total nitrogen ratio in the final mixture was 10:23. This ratio has been reported to be optimal for microbial aerobic decomposition of organic matter [27–30]. Each composting mixture was prepared in three replicates in plastic containers with a capacity of 100 liters each. Composting was carried out at 20–22°C. Daily compost mixtures were mixed for aeration. The moisture of the compost mixture was maintained at 55–60%.

Inoculation for bio preparation was carried out on day 120 of composting, i.e. In the cooling phase, to enhance the survival of the introduced strains. Before inoculation, each composting mixture was divided into two parts, one of which was inoculated while the other was not. Further parameters of the composts were analyzed in comparison. The composting mixtures used in this study are listed in table 1.

2.2 analysis of composting process parameters

The temperature of the composts was measured daily. For laboratory analyses, composts were sampled every two weeks. The dissolved organic carbon content, (doc), respiration activity of microorganisms as well as phytotoxicity were estimated. The doc content was estimated after extraction of the sample using 0.5 M K₂SO₄ after oxidation by potassium dichromate and concentrated sulfuric acid [31,32]. Respiration activity was estimated on the basis of CO₂ emissions in the process of sample incubation in closed vessels [33]. Phytotoxicity was estimated by a contact method using oat plants (*avena sativa*) as the test object [34,35]. In the phytotoxicity tests, the compost was mixed with soil (luvisol) that was sampled from the matyushenski forest nursery in Tatarstan, Russia (55°48'07''N, 49°16'13''E). The germination index (GI), calculated as $\frac{\text{No. of seeds germinated} \times \text{mean root length in compost}}{\text{No. of seeds germinated} \times \text{mean root length in control}} \times 100$ %, was used as a test function.

Composting without inoculation	Inoculation by bio preparation	Composting with (marked is i) and without inoculation
Analysis of dynamics of temperature, doc content, respiration activity and phytotoxicity	After stabilization of fluctuations of temperature, doc content, respiration activity and phytotoxicity (day 120)	Analysis of dynamics of temperature, doc content, respiration activity and phytotoxicity Estimation of suppressiveness
Sw+chm (c/n=10)		Sw+chm Isw+chm
Sw+chm+cm (c/n=13)		Sw+chm+cm Isw+chm+cm
Sw+cm (c/n=14)		Sw+cm Isw+cm
Sw+sm (c/n=23)		Sw+sm Isw+sm
Sw+chm+sm (c/n=13)		Sw+chm+sm Isw+chm+sm

Cw+cm (c/n=13)		Cw+cm Icw+cm
Cw+chm+cm (c/n=15)		Cw+chm+cm Icw+chm+cm
Cw+chm (c/n=14)		Cw+chm Icw+chm
Cw+chm+sm (c/n=10)		Cw+chm+sm Icw+chm+sm
Cw+sm (c/n=15)		Cw+sm Icw+sm
Cw+sm+cm (c/n=15)		Cw+sm+cm Icw+sm+cm

Table 1. Experiment design

2.3 bio preparation and inoculation

Bio preparation consisted of four bio control strains using one of four mechanisms of suppressiveness described in the literature: *trichoderma asperellum* t203 (hyperparasitism) [36], *pseudomonas putida* pcl1760 (competition) [37], *pseudomonas fluorescence* wcs365 (induced systemic resistance) [38] and *streptomyces* spp. (antibiosis) [39]. The strains were obtained from the museum of the department of biochemistry and biotechnology of Kazan Federal University. *Pseudomonas* were introduced into composts in the active growth phase. *T. Asperellum* and *streptomyces* spp. were introduced in the period of active spore formation. The growth phase of *p. Putida* pcl1760 and *p. Fluorescence* wcs365 before inoculation was estimated on the basis of the optical density curve (600 nm) by cultivation on l-broth at 28°C using a microplate photometer (thermo scientific multiskan™ fc microplate photometer, thermo fisher scientific inc., waltham, usa). The growth phase of *t. Asperellum* t203 and *streptomyces* spp. before inoculation was estimated on the basis of spore number dynamics by cultivation in l-broth at 28°C. Spore numbers were analyzed using a cell counter (scepter™ 2.0 cell counter, merck millipore corporation, darmstadt, germany). 909 ml kg⁻¹ of biopreparation was introduced into compost on the 120th day of composting. The proportion of each strain was calculated so that the

amount of each strain in biopreparation was between to 10^4 cfu g^{-1} to 10^6 cfu g^{-1} (table 2).

Name of the strain	Duration of cultivation before inoculation	Counts in the culture medium	Volume proportion of the culture medium in bio preparation, %
<i>T. Asperellum</i> t203	7 days	0.9×10^4 spores ml^{-1}	99.01
<i>Streptomyces</i> spp.	7 days	9.1×10^5 spores ml^{-1}	0.99
<i>P. Putida</i> pc11760	8 h	9.3×10^{10} cfu MI^{-1}	1.19×10^{-6}
<i>P. Fluorescens</i> wcs365	12 h	2.4×10^{11} cfu ml^{-1}	3.87×10^{-6}

Table 2. Strains in bio preparation

2.4 estimation of the suppressiveness of the composts

Tomato plants (*solanum lycopersicum*) were planted in soil artificially spiked with *f. Oxisporum* (10^6 spores kg^{-1}). The soil (luvisol) was sampled from the matyushenski forest nursery in tatarstan, russia ($55^{\circ}48'07''$ n, $49^{\circ}16'13''$). The composts were introduced into spiked soil (1:4 w:w) and incubated for 7 days, then tomato seeds were planted in the soils and incubated at $24 \pm 2^{\circ}C$ with daily irrigation and a 16:8 h light:dark regime. After 21 days, the number of dead and injured plants was calculated and summed. Clean soil without *f. Oxisporum* with the addition of the corresponding compost in the same amount was used as a control.

2.5 statistical analysis

The sampling and the chemical and biological analyses were conducted in triplicate. The results are expressed on an air-dry soil basis. The data from the experiments were processed using the origin 8.5 statistics package (originlab, northampton, usa). The means were compared using fisher's protected least significant difference at $\alpha = 0.05$. The values in the figures are expressed as the mean \pm s.e.m. Of the corresponding replicates.

[iii] RESULTS

In the first stage of this investigation, we compared the composting dynamics of the organic waste mixtures and then determined if the inoculation of a bio preparation influences this process. We estimated the dynamics of the temperature, doc content, respiration activity and phytotoxicity of the composts. These parameters are recommended to estimate the intensity of aerobic organic matter decomposition as well as to assess compost maturity and stability [40–42].

3.1 compost parameters before introduction of bio preparation

Data about temperature profiles of the composting mixtures during the first 60 days are presented on fig. 1a. Mesophilic stage of composting when bacteria and fungi decompose easily degradable organic compounds such as sugars, amino acids and proteins [43] lasted between 1 to 3 days for the compost mixtures. Thermophilic stage for all the mixtures lasted for 35 days and was characterized by significant fluctuations of temperature – from $27^{\circ}C$ to $59^{\circ}C$. The highest temperatures were observed on the 2-7 days of composting.

according to the literature [44–46], the self-warming of composting mixtures, caused by the intensive biochemical processes of organic matter decomposition, is accompanied by the death of several mesophilic strains, including many pathogens. High temperatures may be a reason for the death of microbes present in the bio preparation, so inoculation should be done in the cooling or maturation stage of composting. The cooling stage began on day 35-44, depending on the compost mixture. Subsequently, the temperature of the compost and the environment did not differ (data after day 60 not shown). One of the important parameters that allows us to estimate the maturity of composts and the possibility of suppressiveness is the doc content [28,42]. The data on doc dynamics in the compost mixtures are presented in fig. 1b. The highest levels of doc were observed in the initial mixtures.

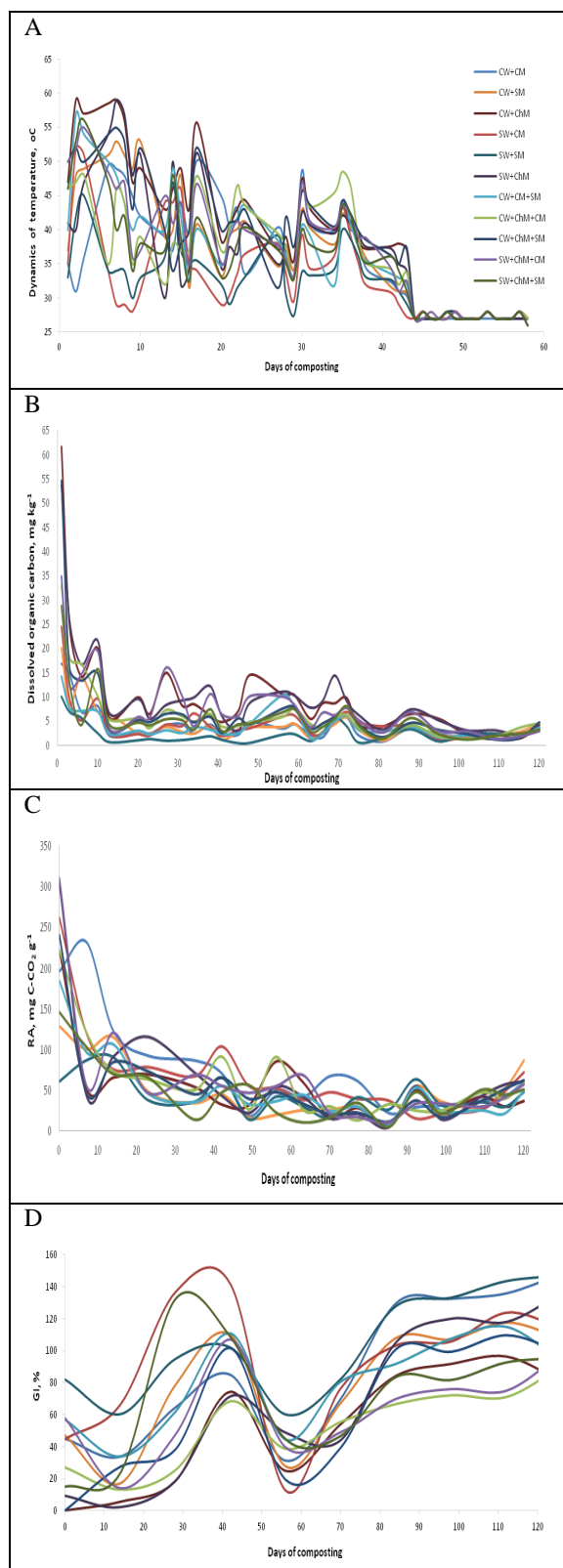
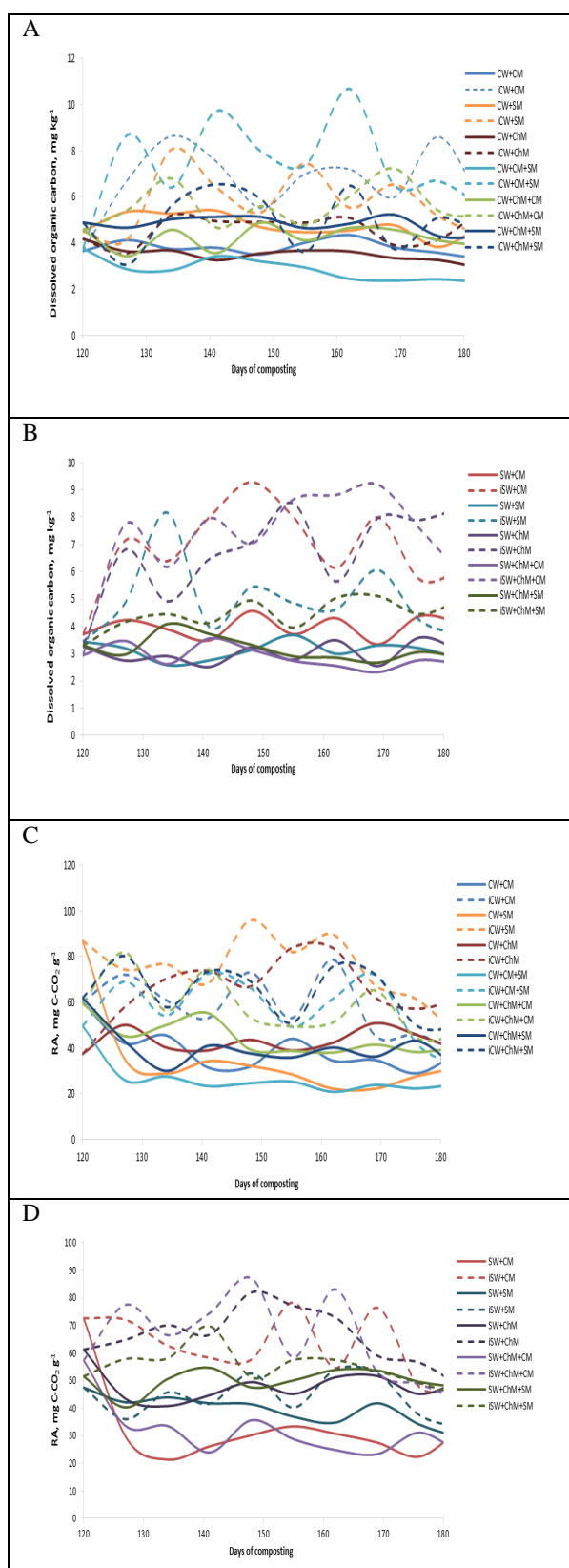


Fig. 1. Temperature (a), doc content (b), respiration activity (c) and gi (d) in the process of composting

organic waste mixtures (before inoculation of bio preparation)

it was estimated to be 62 mg kg⁻¹ in the cw+chm mixture (maximum) and 10 mg kg⁻¹ in the sw+sm mixture (minimum). On day 13, the doc content decreased in all mixtures from 1 (sw+sm) mg kg⁻¹ to 7 (cw+chm) mg kg⁻¹. Furthermore, until 102 days, the doc content was quite low, although it fluctuated in all the mixtures. It is possible that increases in doc occurred as the result of cellulolytic and ligninolytic microbial activity, and decreases in doc occurred with the rapid consumption of easily degradable organic carbon by other members of the microbial pool. After 102 days, the fluctuations in doc subsided, which may reflect the depletion of available organic compounds and the termination of active microbial processes.

The respiration activity of microbes was used as one of the parameters of composting because microbes play a key role in the aerobic transformation of organic compounds. A decrease in respiration activity fluctuations in composts indicates stability [47–50]. As presented in fig. 1b, maximal respiration activity was observed at the beginning of composting for all the mixtures. On day 1 of the investigation, respiration activity ranged between 61 mg co₂-c kg⁻¹ (sw+sm) to 310 mg co₂-c kg⁻¹ (sw+chm+cm). On day 23, the respiration activity of all the composting mixtures decreased to 37-116 mg co₂-c kg⁻¹. The decrease in respiration activity may have been caused by the depletion of available organic compounds, as shown previously by other authors [51–54]. Subsequently, fluctuations in respiration activity were observed. As with the doc content fluctuations described above, this could be connected with the slow transformation of poorly degradable substances to easily degradable ones, followed by rapid consumption of the latter. These fluctuations decreased by day 102, and on day 120 (before inoculation of the biopreparation), respiration activity ranged between 20 mg co₂-c kg⁻¹ (sw+sm, c+chm) and 56 mg co₂-c kg⁻¹ (cw+sm).



Since the goal of composting is to obtain an organic fertilizer, it was very important to assess

how the composts influence plants. Phytotoxicity is often used as an index of compost maturity. In our study, we estimated the gi for oat (*avena sativa*) seeds planted into soil mixed with composts.

As shown in fig. 1d, the initial compost mixtures differed significantly regarding their phytotoxicity, as the gi ranged from 0% to 82%; higher levels of toxicity were observed for compost mixtures that included chm. The phytotoxicity of chicken manure has been demonstrated previously [44,55,56].

interestingly, despite the big differences in initial toxicity, the compost mixtures demonstrated similar trends regarding changes in toxicity over 120 days: in the first 14 days, gi slightly decreased, then increased until day 42. In 7 of 11 compost mixtures, the gi was higher than 100%, but then it went down again until the gi was 13% (sw+cm) to 60% (sw+sm) and then gradually increased. On day 120 of composting, the gi was greater than 100% in 5 of 11 mixtures; in other mixtures, it ranged between 81% and 99%. The changes in phytotoxicity during the process of composting may be caused by the decomposition of organic matter into toxic compounds such as ammonia and low molecular weight organic acids and their subsequent transformation into less toxic compounds [57–59].

3.2 influence of bio preparation inoculation on the composting process

After 120 days of composting, the mixtures were divided into two parts; one part was inoculated with a bio preparation. To simplify the visual form of the results, they are separately presented in fig. 2 for composts containing straw (2a-c) and corn (2d-f).

The introduction of the bio preparation did not change the temperature profiles of the compost mixtures (data not shown). Immediately after inoculation, we observed a brief and slight increase in the doc content in the inoculated mixtures as compared with the non-inoculated mixtures. This may be explained by the fact that microbes in the bio preparation were introduced

with the culture medium they were grown in; this medium contained glucose and yeast extract. After 7 days, no differences were observed between the inoculated and non-inoculated compost mixtures, which may be explained by full consumption of the easily degradable organic compounds introduced with the culture medium. We also observed a brief and slight effect of inoculation on the respiration activity of the composts. Thus, after 60 days, the respiration activity of the inoculated composts was 0.9-4 times higher than that of the non-inoculated mixtures. The phytotoxicity of all the composting mixtures (except for two) was not influenced by inoculation with a bio preparation. Phytotoxicity slowly increased in all the mixtures in the period of 120-180 days.

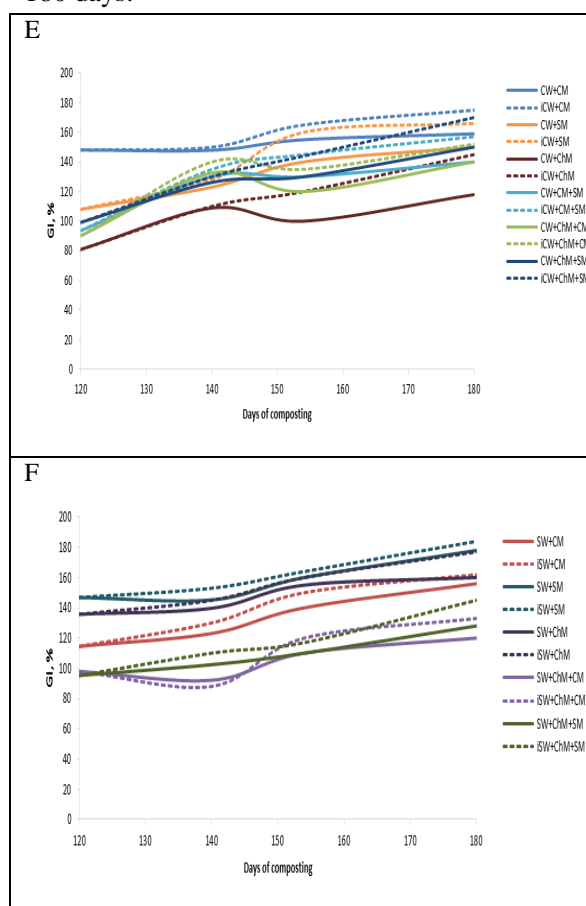


Fig. 2. Doc content (a, b), respiration activity (c,d) and gi (e,f) in the process of composting organic waste mixtures after inoculation of bio preparation

Only two mixtures, i.e. Cw+chm and cw+sm, showed differences in gi between the inoculated and non-inoculated mixtures on day 154.

3.3 influence of bio preparation inoculation on the suppressiveness of the compost mixtures

Results of estimation of composts' suppressiveness are presented in table 3.

Name of the compost mixture	Suppressiveness of the composts of different ages (days), %				
	122	136	150	164	180
Sw+chm	42	65	76	89	97
Isw+chm	66	62	82	91	99
Sw+chm+cm	58	56	45	75	66
Isw+chm+cm	78	78	68	89	75
Sw+cm	50	59	75	78	68
Isw+cm	78	73	88	93	88
Sw+sm	79	75	77	78	72
Isw+sm	85	91	94	95	96
Sw+chm+sm	42	56	58	75	68
Isw+chm+sm	59	71	64	93	96
Cw+cm	33	46	58	62	68
Icw+cm	42	66	90	87	83
Cw+chm+cm	58	65	67	70	72
Icw+chm+cm	77	83	81	90	89
Cw+chm	13	23	27	42	45
Icw+chm	18	31	35	50	53
Cw+chm+sm	17	28	37	43	48
Icw+chm+sm	24	36	54	60	61
Cw+sm	42	46	49	52	63
Icw+sm	62	68	75	67	87
Cw+sm+cm	42	56	59	66	74
Icw+sm+cm	55	77	68	81	83

Composts with high suppressiveness (higher than 80%)

Table 3. Suppressiveness of the composts as revealed by tomato-fuzarium model system

The suppressiveness of the composts was assessed on day 122 after inoculation with the bio preparation. In the non-inoculated composts, suppressiveness at this time point ranged from 13% (cw+chm) to 79% (sw+sm), while in the inoculated composts, suppressiveness was higher and ranged from 18% (cw+chm) to 85% (sw+sm). Over time, suppressiveness tended to increase in both inoculated and non-inoculated composts, and inoculated composts became highly suppressive

(over 80%) earlier than non-inoculated ones. On day 180, the suppressiveness of the non-inoculated composts ranged between 48% (cw+chm+sm) and 97% (sw+chm). Notably, only one non-inoculated compost possessed high suppressiveness (over 80 %) on day 180, while eight inoculated composts demonstrated high suppressiveness.

[v] CONCLUSION

On the basis of temperature, doc content, respiration activity and gi dynamics, it can be concluded that all the composts, independent of their initial substrates, went through significant changes in the beginning of composting and stabilized at the end of the process. Inoculation of the composts with a biopreparation consisting of four biocontrol microbes (*t. Asperellum* t203, *streptomyces* spp., *p. Putida* pcl1760 and *p. Fluorescence* wcs365) on day 120 did not significantly change the composting parameters, although it changed the suppressiveness of the composts. All inoculated composts became more suppressive over time, but the inoculated composts became highly suppressive earlier than the non-inoculated ones.

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