ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Optimal Doses and Schemes of Suppressive Compost Amendments.

[1]Polina Galitskaya*, [2]Polina Kuryntseva, [2]Liliya Biktasheva, and [2]Svetlana Selivanovskaya.

[1]Department of Landscape Ecology, Institute of environmental sciences, Kazan Federal University, Kazan, Russian Federation
[2]Department of Applied Ecology, Institute of environmental sciences, Kazan Federal University, Kazan, Russian Federation

ABSTRACT

Fungal plant diseases cause dramatic yield losses worldwide. Suppressive composts, which possess both fertilizing properties for plants and inhibiting properties for plant pathogens, represent an effective and environmentally friendly alternative to conventional pesticides. In this work, composts obtained from agricultural wastes using microbial biopreparation were applied to suppress Fusarium wilt in tomato plants in model experiments. We evaluated several doses of compost amendments: 1, 5, 10, 15, 20, and 25%. In our experiments, a dose of 20% was most effective and resulted in disease suppression of 84%. From the three amendment schemes investigated (1 – once before vegetation season, 2 – twice before vegetation season with one month break between amendments, half of the dose each time, 3 – twice, once before winter frost simulation, once before vegetation season, half of the dose each time) with a 20% dose, the first scheme was the most efficient one. Schemes 2 and 3 were 1.6 and 1.5 times less efficient, correspondingly. After a single amendment with 20% of compost, soils were suppressive during two consecutive vegetation periods (21 days each) of tomato plants. During the third vegetation period, suppressiveness decreased by 1.7 times. No significant differences in disease inhibition was found between suppressive compost and conventional fungicide “Maxim” application, based on fludioxonil under laboratory conditions. However, under greenhouse conditions, suppressive compost application was more efficient.

Keywords: Suppressive composts, compost amendments, *Fusarium oxysporum*, plant disease, fungicide

**Corresponding author*

INTRODUCTION

Composting is one of the most effective ways to use organic wastes. The product obtained can be applied as organic fertilizer in agriculture, resulting in improved soil structure and higher nutrient levels [1,2]. Some composts are able to inhibit the outbreak of plant diseases [3,4]. Such a property – suppression – may be due to several mechanisms; however, all of them can be attributed to the presence of specific microorganisms in the composts. These microbes are also called "biocontrol agents" because of their ability to suppress and control the proliferation of plant pathogens [3,5–7]. To improve the suppressive properties of a compost, biopreparation based on biocontrol agents may be performed [8,9].

Both from a scientific and a practical point of view, there is significant interest in data about doses and schemes of suppressive compost application in order to provide maximum efficiency. Numerous studies have evaluated the impacts of compost application on plant yield increases due to biogenic elements introduction or air and water soil regime improvement. Application doses range between 6 and 80 t ha^{-1} for conventional agricultural soils, depending on the soil type, and between 150 and 450 t ha^{-1} for poor and highly degraded soils [10–12]. Doses of suppressive composts that are effective for disease inhibition are comparable or higher than those needed for fertilization. In experiments under laboratory conditions, Malandraki et al. demonstrated that an application of 20% (about 500 t ha^{-1}) of compost led to a 25% decrease of plant disease after 48 days [13]. In a similar study, van der Gaag et al. [12] found that the application of 20% of suppressive compost had different impacts on different plant species; e.g. the number of healthy plants of *Begonia eliator* increased by 43–64%, while the number of healthy plants of *Cyclamen persicum* did not change significantly. The authors of another study found that the application of 20% of compost suppressed the pathogens *Fusarium oxysporum* and *Verticillium dahlia* by three to five times compared to the control [14].

Another crucial factor in decision-making is the duration of soil suppression caused by compost amendments. Since the suppressive properties can be attributed to microbes, microbial survival time and activity in the indigenous soil community are of great importance. Unfavorable abiotic factors, such as low winter temperatures, and biotic factors, such as inter-specific competition and physical withdrawal of plant roots at the end of the vegetation period can contribute to the elimination of microorganisms. Up to date, studies related to the suppressive effects of compost application are scarce. Since authors usually use one vegetation season to estimate soil suppressiveness, it is probably believed that this effect lasts only for one vegetation period, similar to disease inhibition caused by pesticides. However, compared with the effects of potential pesticides, the advantage of suppressive composts, besides the absence of negative environmental effects, is their potential to prevent pathogen proliferation in the soil and not only in the plants. This is especially important for soil-borne diseases such as Fusarium wilt [15–17].

The objective of this study was to reveal the optimal doses and schemes of amendment of suppressive compost obtained from chicken manure and straw and treated with biopreparation in a model experiment with tomato plants and the pathogen *F. oxysporum*. The efficiency of the compost was compared with that of the fungicide "Maxim" (active substance fludioxonil), which is widely used in Russia.

MATERIALS AND METHODS

The compost used in this study was prepared from two agricultural wastes sampled on an "Agroholding" chicken farm situated in the Pestrechinskiy district of Tatarstan Republic, Russia(55°46´ N, 49°43´ E). The wastes (chicken manure and straw) were mixed 2:1 in order to obtain a 10:1 carbon to nitrogen ratio, which is favorable for microbial activity [18–21]. Composting was conducted during 120 days until active processes of organic matter decomposition ceased, as determined by temperature, basal respiration, dissolved organic carbon, and phytotoxicity dynamics (data not shown). Compost was prepared at a temperature of 20-22°C in three replicates in plastic containers with a capacity of 100 liters each. To ensure adequate aeration, compost mixtures were mixed daily. The moisture of the compost mixture was maintained at 55-60%. On the 120^{th} day, compost was treated by biopreparation and incubated for another 60 days under the same conditions.

Biopreparation consisted of four biocontrol agents, using one of four mechanisms of suppressiveness described in the literature: *Trichoderma asperellum* T203 (hyperparasitism) [22], *Pseudomonas putida* PCL1760 (competition) [23], *Pseudomonas fluorescence* WCS365 (induced systemic resistance) [24], and *Streptomyces* *spp.* (antibiosis) [25]. The strains were obtained from the Museum of the Department of Biochemistry and Biotechnology of Kazan Federal University, Russia. *Pseudomonas* were introduced into composts during the active growth phase and *T. asperellum* and *Streptomyces spp.* during active spore formation. On the 120th day of composting, 909 ml kg^{-1} of biopreparation were introduced into the compost. The proportion of each strain was calculated so that the amount of each strain in the biopreparation was between 10^4 and 10^6 CFU g^{-1}.

To estimate compost suppressiveness, tomato plants (*Solanum lycopersicum*) were planted in soil artificially spiked with *F. oxisporum* (10^6 spores kg^{-1}). The soil (Luvisol) was obtained from the Matyushenski forest nursery in Tatarstan, Russia (55°48´07´´ N, 49°16´13´´ E). The compost was introduced into the spiked soil and incubated for seven days; subsequently, tomato seeds were planted and incubated at 24 ± 2°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. Three pots with soil were used as three replicates, and 25 tomato seeds were planted into each pot. Clean soil without *F. oxisporum*, but with the addition of the corresponding compost in the same amount, was used as a control. Spiked soil without compost treatment was used as a negative control.

Compost was added to the soil once in the following amounts: 1, 5, 10, 15, 20, and 25% (w/w). In addition, a dose of 20% was added to the soil as follows: twice before vegetation season with a break of one month between amendments, with half of the dose each time, and twice, once before winter frost simulation, once before vegetation season, with half of the dose each time. Additionally, tomato plants were seeded into 20% amended soil three subsequent times and cultivated for 21 days each time. To compare suppressive properties of the compost with those of a conventional pesticide, we used the fungicide “Maxim” (active substance fludioxonil) according to the manufacturer’s instruction. To equalize the stimulating effects caused by a compost, sterilized compost was added to the fungicide-variant in a dose of 20%.

Comparison between the fungicide “Maxim” and compost was additionally carried out under greenhouse conditions with a dose of compost of 20%. Prior to the experiment, plant seedlings were grown for two months in pots with soil without *F. oxysporum* spores at 24 ± 2°C with daily irrigation and a 16:8 h light: dark regime. We prepared 50 seedlings for experiments in each of the three greenhouses ((1) soil spiked with 10^6 *F. oxysporum* spores and no compost addition; (2) soil spiked with 10^6 *F. oxysporum* spores and treated with sterilized compost and (3) soil spiked with *F. oxysporum* spores and treated with non-sterilized compost). Seeds which were further transferred into the second greenhouse were preliminary treated with “Maxim” according to the manufacturer´s instruction. Other seeds were not treated. Compost was introduced into the third greenhouse one month before plants were transferred. The numbers of dead and injured plants were calculated 15, 30, and 60 days after transfer and compared with the number of plants transferred into the greenhouse. Number of transplanted seedlings was used as a control for further calculations.

Sampling and biological analyses were conducted in triplicate. 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 represent the mean ± S.E.M. of the corresponding replicates.

RESULTS AND DISCUSSION

Figure 1 shows the rates of Fusarium wilt suppression in tomato plants with the use of different compost doses. A dose of 20% was the most efficient one for disease suppression (84%); this dose was also efficient in a previous study [26]. In a similar study, Elsas and Postma [27] showed that a dose of 1% of compost did not result is sufficient suppression, probably because of the low microbial numbers introduced into the soil [27]. However, some authors report that even low doses of suppressive composts may alter disease expression in soils [28]; in their study, doses of 5, 10, and 15% inhibited plant pathogens, but were not as efficient as a dose of 20%. However, they did not observe significant differences in suppression between the three doses. A dose of 25% was less efficient compared with the other applied doses. In contrast, the authors of a different study found a proportional increase in suppressiveness with increasing amounts of compost [29]. In our study, the low number of healthy plants in 25% amended soil may be due to the high concentration of nutrients, e.g. ammonia-containing compounds, which inhibit plant growth [30].

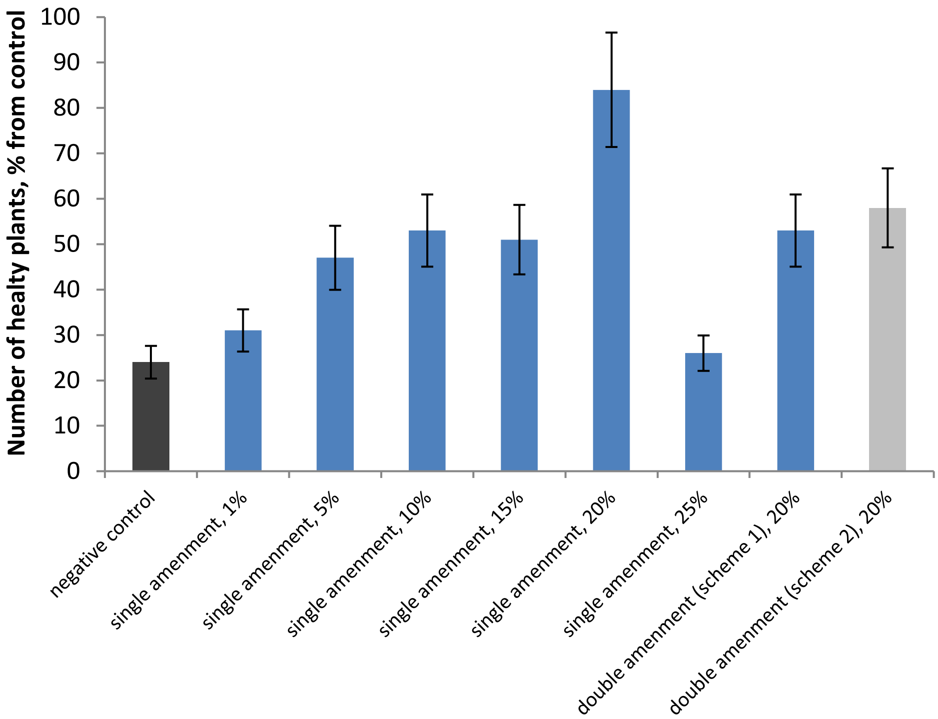

Fig 1. Fusarium wilt severity in soil amended using different doses and schemes of suppressive compost.

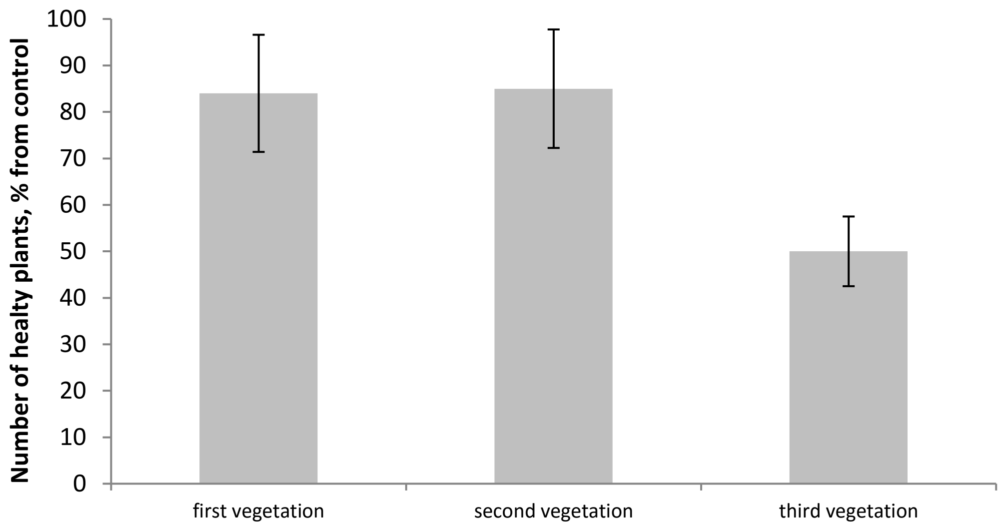

Fig 2. Duration of soil suppressiveness over three vegetation periods of tomato plants.

Further, a dose of 20% was introduced into soil according to the two following schemes: two amendments, 10% each, with a break of one month between amendments. In the first scheme, soil was incubated at 24 ± 2°C between amendments to simulate compost treatment in the early spring time and immediately before planting. We suggested that double amendment may be favorable for survival of the compost microbial community. In the second scheme, soil was incubated at -5 ± 2°C between amendments to simulate winter frost; the purpose of this treatment was to evaluate whether compost microbes are able to tolerate winter temperatures. We also suggested that negative temperatures may change the competition conditions between indigenous and introduced species. As seen in Figure 1, double amendment of soil with compost using half of the doses each was less effective than single amendment using a whole dose. In contrast, the conditions under which the soil was incubated between amendments did have no impact on soil suppressiveness. The ratio of healthy plants after double amendment did not differ significantly from those after single amendment with 5-15% of compost. We therefore conclude that in terms of suppressiveness, single amendments with high doses are preferable over several amendments with low doses. The differences between single amendment with 20% of compost and double amendments are possibly caused by the fact that soils do not maintain suppressive properties for a long time e.g. because of elimination of biocontrol agents from the microbial community. To evaluate this hypothesis, we conducted an experiment in which plants were seeded into compost-amended soil three times consecutively. The results are presented in Figure 2.

The soil containing compost was equally suppressive during three consecutive vegetation periods (21 days each) of tomato plants. In the third vegetation period, the number of healthy plants was estimated to be 1.7 times lower compared with the numbers of the two previous periods. The time of the third incubation corresponds to the time of planting after two amendments; therefore, these results support the hypothesis that suppressive properties of soils obtained from compost amendment decrease over time. However, it should be stressed that disease suppression in the third vegetation period was significantly higher than in the negative control (about 2.5 times). In the literature, different data about the lasting effects of soil suppressiveness after compost application can be found. For example, in the Remade Scotland Organics Fact Sheet, soils are reported to be suppressive up to nine months after compost application [31]. The authors of a similar study demonstrated that suppressiveness lasted for up to 68 days after transferring plants into infected soils treated with suppressive composts [14]. The ability of soils to suppress pathogens for a long time indicates the high potential of suppressive composts for plant disease prevention. This is especially important in regards to soil borne diseases. Suppressive composts inhibit pathogens within the environments they proliferate, whereas conventional pesticides only protect the roots of plants originating from pre-treated seeds [32].

In the next stage of investigation, we compared the efficiencies of a compost dose of 20% and the fungicide “Maxim”. This experiment was conducted in pots under laboratory conditions as well as in conventional agricultural greenhouses. The results are presented in Figure 3.

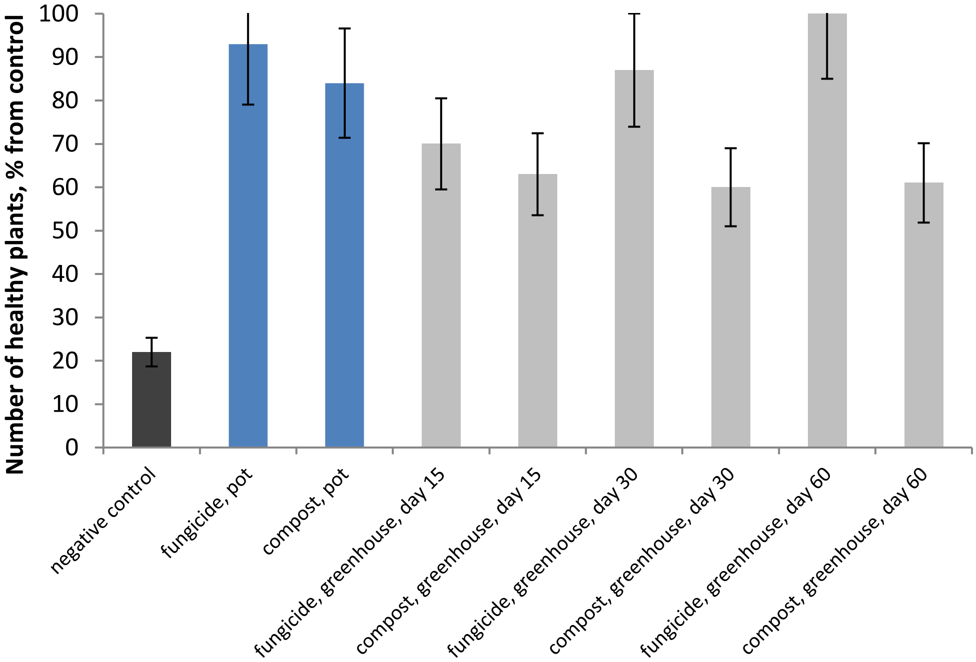

Fig 3. Comparison of efficiency of a 20% dose of suppressive compost and the fungicide "Maxim" in terms of inhibition of Fusarium wilt in tomato plants.

Under laboratory conditions, the number of healthy plants in both experimental set ups did no differ significantly from each other, but as 2.3-2.9-fold higher when compared with the negative control, indicating that both methods may be used for seedling preparation before further transplanting. The choice of an appropriate method should therefore be bases on parameters other than disease prevention, e.g. requirements of organic farming. In the greenhouse experiments, we also did not find significant differences between fungicide and suppressive compost application on the 15th day after transplanting. Interestingly, the number of healthy plans on day 15 in the greenhouse was lower compared to that obtained in the pot experiment. This is may be due to the experimental design. Under greenhouse conditions, plants were transferred from the non-infected into the infected soil after two months of incubation; therefore, plants were inhibited both by the transferring process and by pathogens. In contrast, in the pot experiment, plants were only negatively impacted by one factor (pathogen). In addition, in the greenhouse, wet and hot conditions were prevalent. The authors of a different study showed that increased transpiration under high rainfall and temperature conditions accelerated pathogen infections of plants [33]. On days 30 and 60 after transplanting, we observed significant differences between fungicide and compost treatments, caused by higher disease incidents in the compost treatment and steady disease incidence in the fungicide treatment. Increased suppressiveness over time observed in our study is in agreement with the result of other authors [14,17]. Our results demonstrate the advantage of suppressive compost application over application of fungicides under greenhouse conditions.

CONCLUSION

Based on our results, it can be concluded that single-time compost amendment with a dose of 20% is the optimal scheme for disease suppression in soils spiked with *F. oxysporum*, using a compost prepared on the basis of chicken manure and straw and applied biopreparation. Suppressive compost is equally effective with compared to the fungicide “Maxim” for short-term plantings, but is more efficient for long-term plantings under greenhouse conditions.

ACKNOWLEDGEMENT

This work was supported by the subsidy of the Russian Ministry of Education and Science No RFMEFI57814X0089.

ACKNOWLEDGEMENTS

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

REFERENCES

[1] Epstein E. The Science of Composting - CRC Press Book [Electronic resource]. 1997. URL: https://www.crcpress.com/The-Science-of-Composting/Epstein/9781566764780.

[2] Blaya J., López-Mondéjar R., Lloret E., Pascual J.A., Ros M. Changes induced by Trichoderma harzianum in suppressive compost controlling Fusarium wilt // Pestic. Biochem. Physiol. 2013. Vol. 107. P. 112–119.

[3] Hoitink H., Boehm M. Biocontrol within the context of soil microbial communities: A Substrate-Dependent Phenomenon // Annu Rev Phytopathol. 1999. № 37. P. 427–446.

[4] Mehta C. Palni U., Franke-Whittle I., Sharma A. Compost: Its role, mechanism and impact on reducing soil-borne plant diseases // Waste Manag. 2014. Vol. 34, № 3. P. 607–622.

[5] Galitskaya P., Beru F., Kuryntseva P., Selivanovskaya S. Suppressive Properties of Composts are Determined by their Raw Materials // Indian J. Sci. Technol. 2015. Vol. 8, № 30. P. 1–7.

[6] Noble R., Roberts S.J. Eradication of plant pathogens and nematodes during composting, a review // Plant Pathol.,. 2004. Vol. 53. P. 548–568.

[7] Suarez-Estrella F., Arcos-Nievas M.A., Lypez M.J., Vargas-Garcna M.C., Moreno J Biological control of plant pathogens by microorganisms isolated from agro-industrial composts // Biol. Control. 2013. Vol. 67. P. 509–515.

[8] Postma J., Kok C.J. Effect of compost application on the soil microflora. // Proc. Applying Compost – Benefits and Needs. Brussel, Belgium, 2003. P. 157–161.

[9] Badr El-Din S.M.S., Attia M., Abo-Sedera S.. Field assessment of composts produced by highly effective cellulolytic microorganisms // Biol. Fertil. Soils. 2000. Vol. 32. P. 35–40.

[10] Agegnehu G., Bass A.M., Nelson P.N., Bird M.I. Benefits of biochar, compost and biochar–compost for soil quality,maize yield and greenhouse gas emissions in a tropical agricultural soil // Sci. Total Environ. 2016. Vol. 543. P. 295–306.

[11] Hernandez T., Garcia E., Garcia C. A strategy for marginal semiarid degraded soil restoration: A sole

addition of compost at a high rate. A five-year field experiment // Soil Biol. Biochem. 2015. Vol. 89. P. 61–71.

[12] van der Gaag D.J., van Noort F.R., Stapel-Cuijpers L.H.M., de Kreij C., Termorshuizen A.J., van Rijn E., Zmora-Nahum S., Chen Y. The use of green waste compost in peat-based potting mixtures: Fertilization and suppressiveness against soilborne diseases // Sci. Hortic. (Amsterdam). 2007. Vol. 114, № 4. P. 289–297.

[13] Malandraki I., Tjamos S.E., Pantelides I.S., Paplomatas E.J. Thermal inactivation of compost suppressiveness implicates possible biological factors in disease management // Biol. Control. 2008. Vol. 44, № 2. P. 180–187.

[14] Markakis E., Fountoulakis M.S., Daskalakis G.C., Kokkinis M., Ligoxigakis E The suppressive effect of compost amendments on Fusarium oxysporum f.sp. radicis-cucumerinum in cucumber and Verticillium dahliae in eggplant // Crop Prot. 2016. Vol. 79, № 150. P. 70–79.

[15] Kone S.B., Dionne A., Tweddell R.J., Antoun H., Avis T.J. Suppressive effect of non-aerated compost teas on foliar fungal pathogens of tomato // Biol. Control. 2010. Vol. 52, № 2. P. 167–173.

[16] Siddiqi S., Masud T.I., Nishtar S., Peters D.H., Sabri B., Bile K.M., Jama M.A. Framework for assessing governance of the health system in developing countries: Gateway to good governance // Health Policy (New. York). 2009. Vol. 90, № 1. P. 13–25.

[17] Liu L., Sun C., He X., Liu X., Wu H., Liu M., Tang C., Zhang Y. The secondary compost products enhances soil suppressive capacity against bacterial wilt of tomato caused by Ralstonia solanacearum Lijuan // Eur. J. Soil Biol. 2016. Vol. 75. P. 70–78.

[18] Kumar M., Ou Y.-L., Lin J.-G. Co-composting of green waste and food waste at low C/N ratio. // Waste Manag. 2010. Vol. 30, № 4. P. 602–609.

[19] Sasaki N., Suehara K.-I., Kohda J., Nakano Y., Yang T. Effects of C N ratio and pH of raw materials on oil degradation efficiency in a compost fermentation process. // J. Biosci. Bioeng. 2003. Vol. 96, № 1. P. 47–52.

[20] Iqbal M.K., Shafiq T., Ahmed K. Characterization of bulking agents and its effects on physical properties of compost. // Bioresour. Technol. 2010. Vol. 101, № 6. P. 1913–1919.

[21] Li Z., Lu H., Ren L., He L. Experimental and modeling approaches for food waste composting: a review. // Chemosphere. 2013. Vol. 93, № 7. P. 1247–1257.

[22] Kim T.G., Knudsen G.R. Quantitative real-time PCR effectively detects and quantifies colonization of sclerotia of Sclerotinia sclerotiorum by Trichoderma spp. 2008. Vol. 1171. P. 1–9.

[23] Validov S., Kamilova F., Lugtenberg B. Pseudomonas putida strain PCL1760 controls tomato foot and root rot in stonewool under industrial conditions in a certified greenhouse // Biol Control. 2009. Vol. 48, № 1.

[24] Kamilova F., Lamers G., Lugtenberg B. Biocontrol strain Pseudomonas fluorescens WCS365 inhibits germination of Fusarium oxysporum spores in tomato root exudate as well as subsequent formation of new spores // Environ. Microbiol. 2008. Vol. 10, № 6. P. 2455–2461.

[25] Kinkel L.L., Schlatter D.C., Bakker M.G., Arenz B.E. Streptomyces competition and co-evolution in relation to plant disease suppression // Res. Microbiol. 2012. Vol. 163. P. 490–499.

[26] Tuitert G., Szczech M., Bollen G.J. Suppression of Rhizoctonia solani in Potting Mixtures Amended with Compost Made from Organic Household Waste. // Phytopathology. 1998. Vol. 88, № 8. P. 764–773.

[27] Elsas J.D. van, Postma J. Suppression of Soil-Borne Phytopathogens by Compost // Compost Science and Technology / ed. Diaz L.F., Bertoldi M. de, Bieingmaier W. Waste Management Series, 2007. P. 201–214.

[28] Van Os G.J., van Ginkel J. Suppression of Pythium root rot in bulbous Iris in relation to biomass and activity of the soil microflora // Soil Biol. Bioc. 2001. Vol. 33. P. 1447–1454.

[29] Nelson E.B., Hoitink H.A.J. Factors affecting suppression of Rhizoctonia solani in container media // Phytopathology. 1982. Vol. 72, № 275.

[30] Ivanov A.I., Konashenkov A.A. Agroecological consequences from irregular manure application for vegetable cultivation (in Russian) // Agrochimiya. 2012. Vol. 6. P. 66–72.

[31] Remade Scotland Organics Fact Sheet, Compost Application Techniques. 2006. 4 p.

[32] Zhang Y., Lamm R., Pillonel C., Lam S., Xu J. Osmoregulation and Fungicide Resistance: the Neurospora crassa os-2 Gene Encodesa HOG1 Mitogen- Activated Protein Kinase Homologue // Appl. Environ. Microbiol. 2001. Vol. 68, № 2. P. 532–538.

[33] Yuan S., Wang L., Wu K., Shi J., Wang M., Yang X., Shen Q., Shen B. Evaluation of Bacillus-fortified organic fertilizer for controlling tobacco bacterial wilt in greenhouse and field experiments // Appl. Soil. Ecol. 2014. Vol. 75. P. 86–94.