CHAPTER VII

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EFFECT OF FERTILIZATION ON THE COMPOSITION OF SOIL FUNGI COMMUNITY

Introduction

Fertility and, simultaneously, the productivity of soil are determined by, among other things, the content of organic matter originating mainly from deficient farm manure and liquid manure. Thus, an increasing interest may be observed in the acquisition of organic matter from other sources, e.g. sewage sludge, municipal solid wastes and municipal green wastes. Owing to a high content of organic matter as well as macro- and microelement, they may be utilized for agricultural purposes as composted organic fertilizers (SPYCHAJ-FABISIAK et al. 2002).

Natural and organic fertilization affects biotic relations in the soil, which is due to an increased content of organic carbon in the soil, especially microbiological carbon and, to a lesser extent, of nitrogen (LARKIN et al. 2006). As observed by HOITINK et al. (1997), fertilization with compost should be adjusted to the contents of macro- and microelements in the soil and to the requirements of plants, as an excess of N, for example, has been found to promote the growth of pathogenic factors, including: Erwinia amylovora and the genus Phytophthora. The high concentration of ammonia N and a low C:N ratio in sewage were reported to stimulate the development of fusarium diseases (KATO et al. 1981). Such elements as: B, Cu, Pb, Mn, Zn, have also been implicated in affecting the structure of the soil fungi community. PRATT (2008) claimed that in soil fertilized with organic wastes of animal origin, as compared to the non-fertilized soil, the concentrations of P, K and Na were significantly higher, whereas those of Mg, Cu and Zn were usually lower and, finally, those of N, Ca, Fe and Mn - were rarely or never higher. That author emphasized, however, that the composition of the soil fungi community remained unchanged under the influence of the above fertilization. A similar opinion was expressed by GÓRSKA and STEPIEN (2007), who claimed that the introduction of organic additives to soil had no effect on the population numbers of hyphae fungi. An opposite claim was made by AWAD and FAWZY (2004), who proved that increasing doses of sewage sludge promoted the growth of bacterial and fungal populations in soil. HOITINK et al. (1997) as well as WEYMAN-KACZMARKOWA et al. (2002) were also convinced that composts and vermicomposts facilitated an increase in the population numbers of soil microorganisms, thus enhancing their

activity and biodiversity. These authors additionally indicated the significance of the type of organic fertilizer used and composting time in determining the structure of the rhizosphere fungi community.

Changes in the counts and functioning of microorganisms in the soil environment affect, among other things, plant resistance to diseases. The inhibiting effect of manure and organic fertilizers on the growth of soil pathogens is relatively well-documented in literature. Fertilization with bovine manure was reported to diminish the population of *Rhizoctonia solani* (TSROR LAKHIM et al. 2001) and that of Streptomyces scabies (LAZAROVITS et al. 2008) in soil, thus reducing infections of potato tubers. The results of other investigations (GORODECKI, HADAR 1990) also confirmed the suppressing effect of that fertilizer on the development of the causative agent of black scurf of potato tubers (Rhizoctonia solani), and additionally on Sclerotinia rolfsii. Organic fertilization in the form of composted plant wastes inhibited the growth of soil pathogens (HADAR, MANDELBAUM 1986), whilst the fresh plant wastes diminished infestation of solanacenous plants with Phytophthora capsici, Alternaria solani and Septoria lycopersicae (KIM et al. 1997, MILLS et al. 2002) and that of pea with Aphanomyces euteiches (WILLIAMS-WOODWARD et al. 1997). LODHA and BURMAN (2000) noted a 20 - 40% reduction in the population of Macrophomina phaseolina – a pathogenic papilionaceous plant – as affected by fertilization with compost of plant waste. In turn, STONE ET AL. (2003) demonstrated that organic additives (paper residues) composted with bark and those not subjected to composting, inhibited the growth of soil pathogens (Pythium spp., Collectotrichum lindemuthianum, Aphanomyces spp.). Numerous authors (SCHUELER et al. 1989, DRAFT, NELSON 1996, HOITINK, BOEHM 1999) reported that composts (e.g. composted household wastes) were likely to suppress the development of some fungi-like organisms (Pythium spp., Phytophthora spp.) and potential pathogens of the genus Fusarium. RINGER et al. (1997) proved that the examined types of composts from household wastes inhibited infections with R. solani to the same extent, but differentiated the intensity of seedlings blight by Pythium ultimum. Composts based on municipal sewage were also found to be significant in plant protection against soil pathogens (SERRA-WITTLING et al. 1996).

Organic fertilization evokes positive changes in the quantitative and qualitative composition of a soil fungi community (SZCZECH 1999). A desirable phenomenon is an increase in the count of beneficial bacteria antagonistic to pathogens. The organic fertilizers applied may, thus, constitute potential biological protection for plants against pathogenic factors.

In the suppression of pathogen development, great significance is ascribed to fungi of the genus *Gliocladium* and *Trichoderma*. BULLOCK et al. (2002) demonstrated a significantly higher count of fungi of the genus *Trichoderma* in the soil subjected to organic than mineral fertilization. The above fungal species are known for their lignolytic and cellulolytic properties. They were shown to intensify biological processes in soil, thus increasing its phytosanitary status (ŁACICOWA, PIĘTA 1989, HOITINK, BOEHM 1999). Other works (BAKER, COOK 1974, NELSON et al. 1983) report that fungi of the genus *Trichoderma* were colonizing sclerotia of *R. solani* and *S. rolfsii*. In turn, CHRISTENSEN (1969) demonstrated that the species *T. harzianum* was producing high quantities of CO₂, ethanol and antibiotics,

which inhibited the growth of some fungal species, including those of the genus *Penicillium* np. *P. jaczewskii*. High numbers of those saprotrophs of the genus *Penicillium* were isolated from soil fertilized with compost from organic wastes by DROZD ET AL. (1996). The high prevalence of those fungi in the natural environment is explained by their high capability to adapt to environmental conditions, e.g. their capability of exploiting various sources of food. SARAIVA et al. (2004) included species of the genus *Penicillium* (apart from fungi of the genera *Aspergillus* and *Fusarium*) in the group of microorganisms most frequently colonizing the organically-fertilized soils.

In the present study, an attempt was made to determine the effect of different organic fertilization compared to non-fertilized plots (control combination) and plots with mineral NPK fertilization and fertilization with manure, on the structure of a community of soil fungi. In addition, *in vitro* tests on PDA culture medium with the addition of aqueous extracts from composts were applied to compute the percentage index of growth inhibition of pathogen mycelium.

Study determinants

An exact field experiment was established in 2004 by the Department of Agricultural Chemistry and Environment Protection at the Agricultural Experimental Station in Bałcyny. Experimental plots with an area of $15m^2$ (at randomized complete block design, in three replications) were located on gley luvisol soil (developed from light silty loam, complex 4 class III, characterized by a high content of P, medium content of K and a low content of Mg, and pH = 5.04). The following crop species were grown in a four-year rotation system: commercial potato, spring fodder barley, winter rape and winter wheat.

The factor analyzed in the study was the type of organic fertilizer. A phytopathological analysis was conducted over the first three years of the experiment on the following plots: I. control (no fertilization), II. mineral NPK fertilization, III. farm manure $10t \cdot ha^{-1}$, IV. farm manure $5t \cdot ha^{-1*}$, V. "Dano" compost $10t \cdot ha^{-1}$ (compost from non-segregated municipal wastes, composted with the "Dano" method), VI. "Dano" compost $5t \cdot ha^{-1*}$, VII. green waste compost $10t \cdot ha^{-1}$, and VIII. green waste compost $5t \cdot ha^{-1}$.

The farm manure and composts from municipal wastes at the dose of 10t x ha⁻¹ were applied in 2004 before potato planting (Jasia cultivar). Doses of mineral fertilizers applied under potato were as follows: 150 kg N (34% ammonium nitrate), 65 kg P (40% superphosphate) and 166 kg K·ha⁻¹ (60% potassium salt). Mineral fertilization on the NPK plot was applied exclusively before sowing. On the plots with farm manure and sewage sludge, the fertilization with N was balanced to 150 kg·ha⁻¹, depending on the content of total nitrogen in the fertilizers, and completed after the main crop with ammonium nitrate. In 2005, only mineral fertilization was applied under spring barley (Justyna cultivar): 90 kg N, 26 kg P and 100 kg K·ha⁻¹ (forms of fertilizers as above). After the harvest of spring barely and before sowing winter rape, mineral fertilization in a dose of 5 t·ha⁻¹ was applied only on plots: IV, VI and VIII. Supplementary fertilization with N up to

120 kg \cdot ha⁻¹ was balanced depending on the total nitrogen content of compost on the above-mentioned plots.

In order to determine species and quantitative composition of fungi in the soil from three sites on particular plots, constituting a given combination, soil samples were collected at a depth of up to 10 cm. In the laboratory, the samples were mixed and their 10-g portions were weighed into 250 ml flasks; 90 ml of sterile water were added to the flasks which were then shaken for 20 minutes to reach a dilution of 10^{-4} . The culture of fungi was run on Martin' medium at a temperature of 22°C, and fungal colonies grown after 5-day incubation were calculated. Results were converted to grams of dry matter, whilst the colonies were inoculated onto agar slants for microscopic identification of species.

A laboratory test was used to determine the effect of aqueous extracts from the composts examined on the growth of potentially-pathogenic fungi: Botrytis cinerea, Colletotrichum coccodes and those of the genus Fusarium (F. culmorum, F. equiseti, F. oxysporum and F. poae). Isolates of the above species, from which single-spore cultures were prepared for the study, originated from the experimental soil. The aqueous extracts were prepared as follows: 2 g portions of dried material were poured over with 100 ml of sterile water for 24 hours. After filtration, the extracts were dosed in 2 ml portions on Petri dishes and poured over with 10 ml of PDA medium with a temperature of 50°C. Next, agar discs 5 mm in diameter overgrown with 7 day mycelium of the pathogens examined were placed on the solidified medium. The dishes with pathogen inoculum on the medium without the aqueous extracts from composts served as a control. After 4 and 8 days, colonies were measured alongside two perpendicular straight lines. The index of mycelium growth inhibition was calculated from the formula: I = $[(\Phi_k - \Phi) : \Phi_k] \ge 100\%$, where Φ_k and Φ denote diameter of fungal culture in the control combination and in the medium with compost extracts, respectively. The results obtained in the study were elaborated statistically with the analysis of variance (STATISTICA® v.8. 2007-08) using the Duncan's test to compare mean values.

Effect of mineral, natural and reduced fertilization on the composition of a soil fungi community

Organic fertilization differentiated the number of colonies of the soil fungi only to a negligible extent. The highest number of fungal colony forming units was noted in the soil fertilized with "Dano" compost applied in doses of 5 tha⁻¹ and was significantly different as compared to the number of CFU in the soil fertilized with a single dose of "Dano" compost (10 tha⁻¹) and with green waste compost in both variants of application (fig. 1).

The species composition of the soil fungi community in particular variants of fertilization appeared to be more diversified. Amongst the isolated fungi, 49 species, yeast-like fungi and asporogenous cultures were identified. The species of potentially-pathogenic fungi identified in the study included: *Botrytis cinerea, Colletotrichum coccodes, Sclerotinia sclerotiorum* and fungi of the genus *Aureobasidium (A. bolleyi* and *A. pullulans)* and *Fusarium (F. culmorum, F. equiseti, F. oxysporum* and *F. poae)*. The highest prevalence of pathogens was noted

in the soil from the control non-fertilized plot (14% of all isolates – fig. 2); only in that fertilization variant was their presence detected in all experimental years. In the second year of the study, a high contribution in the fungal community was reported for species of the genus *Fusarium*.

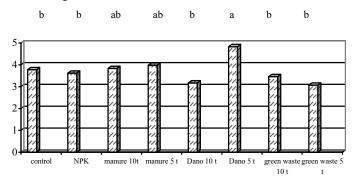
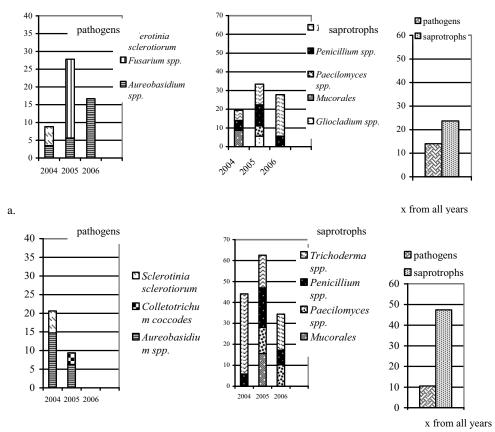


Fig. 1. Number of fungal colony forming units per 1 g of soil (CFU x 10⁵)

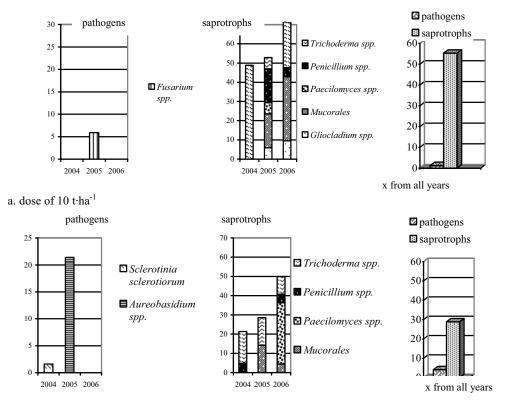


b.

Fig. 2. Fungi isolated from soil: a. without fertilization (control), b. with mineral fertilization (%)

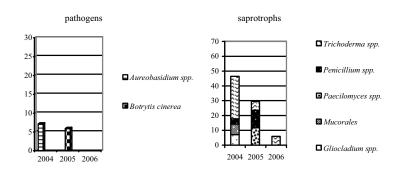
A smaller population of the pathogens was cultured in the soil fertilized with NPK (10.6% - fig. 2b), with *A. pullulans* being the most frequently isolated species.

In soils subjected to organic fertilization, RITZ ET AL. (1997) reported an increase in the count of bacteria, actinomycetes and fungi. They explained the enhanced biological activity of the soil with an elevated content of soluble C in soil and, to a lesser extent, with that of N. Especially favorable seem to be changes affecting an increase in the population of beneficial microflora as specific, biological protection of plants against pathogens (WIDNER ET AL. 1998, SZCZECH 1999). A reduction was observed in the count of pathogens in the soil upon fertilization with farm manure (Fig. 3 a, b) and organic fertilization (compost - Fig. 4 a-d) as compared to the control variant and that with mineral fertilization. The contribution of the pathogens in the fungal community ranged from 1.2% in the soil fertilized with manure at a dose of 10 t ha⁻¹ to 9.1% in the variant with green waste compost applied twice at a dose of 5 t \cdot ha⁻¹ each. In the other experimental variants, the frequency of pathogen occurrence ranged from 5 to 10%. The species of pathogens frequently isolated from the soil analyzed in the study included those mentioned above and those belonging to the generea Aureobasidium and Fusarium, whereas the less frequently isolated species included: B. cinerea, C. coccodes and S. sclerotiorum.



b. dose of 5 $t \cdot ha^{-1}$.

Fig. 3. Fungi isolated from the soil fertilized with farm manure (%)



a. Dano at a dose of 10 t ha-1

b.Dano at a dose of 5 t x ha⁻¹

2004 2005 2006

pathogens

30

25

20

15

10

5

0

30

25

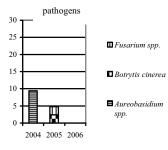
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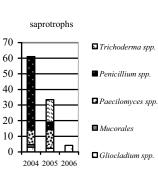
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10

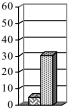
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pathogens

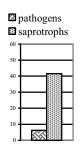




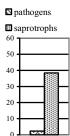
I patogeny I saprotrofy



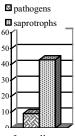
x from all years



x from all years



x from all years



x from all years



d. green waste manure at a dose of 5 t ha⁻¹

Fig. 4. Fungi isolated from the soil fertilized with compost (%)

60 50 Sclerotinia sclerotiorum 40

100

90

80

70

60

50

40

30

Fusarium spp.

saprotrophs 70 60 50 40 30 20 10 2004 2005 2006 Trichoderma spp. Penicillium spp. Paecilomyces spp. Gliocladium spp.

saprotrophs

Trichoderma spp.

Penicillium spp.

Paecilomyces spp.

Gliocladium spp.

Mucorales

c. green waste compost at a dose of 10 t-ha^{-1}

Sclerotinia

sclerotiorum

Fusarium spp.

113

In the fertilization variants analyzed, the latter species occurred sparsely and only in the first year of the study (2004) and did not colonize the soil fertilized with "Dano" compost in either variant of application or that fertilized with farm manure at a dose of 10 t-ha^{-1} .

GORODECKI and HADAR (1990) confirmed the inhibiting effect of fertilization with farm manure on the growth of *S. sclerotiorum*, and that of *R. solani*. In turn, FERRAZ et al. (1999) reported on suppressed sprouting of *S. sclerotiorum* sclerocia in the soil from under a tomato crop fertilized with green wastes.

In the reported study, the perpetrator of grey rot was only isolated in 2005 from the soil fertilized with "Dano" compost in both variants of applications, and its contribution in the fungal community did not exceed 6%. ELAD AND SHTIENBERG (1994) demonstrated that the composts applied were effective in protecting selected plant species against infestation with *B. cinerea*. Single isolates of *C. coccodes* (3.1% of all isolates in this variant) were obtained in this study from the soil fed with mineral NPK fertilizer in the second year of cultivation, i.e. soil from under potato, whereas these isolates were not obtained from the soil fertilized with composts.

In the soil fertilized with minerals, the content of total N is subject to increase and, as reported by ZARZYCKA (1990), better growth of this fungus proceeds under conditions of insufficient supply of this macroelement in the soil, which corresponds to the results of the presented study.

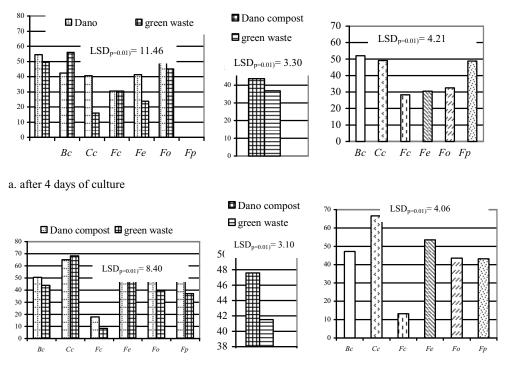
Saprotrophic fungi were most often represented by species of the genera *Gliocladium, Paecilomyces* and *Trichoderma* characterized by the antagonistic action against pathogens, as well as by fungi of the genus *Penicillium* and of the order *Mucorales*. They colonized all communities of soil fungi analyzed in the study. The greatest population of these fungi was noted in the soil environment with farm manure applied at a dose of 10 t·ha⁻¹, with an especially high contribution (33%) of species belonging to the order *Mucorales (Mortierella alpina, M. isabelina, Mucor hiemalis, Rhizopus nigricans* and *Zygorhynchus* spp.).

DOMSCH et al. (1980) included them as permanent components of a fungal community of the soil environment determined as a result of fertilization. In the current study, these were the plots with that fertilizer, i.e. farm manure introduced in a single dose and in separate doses, that were characterized by favorable dynamics of changes in the population of those fungi, i.e. a successive increase in their count in the subsequent experimental years. Species of the genus Trichoderma (T. aureoviride, T. hamatum, T. harzianum, T. koningii, T. viride and T. polysporum) were isolated in consecutive vegetative seasons from the soil in all fertilization variants, except from the soil fertilized twice with 5 t ha⁻¹ of "Dano" compost and from the soil fertilized with green waste compost at both variants of application. Species of the genera Gliocladium: G. catenulatum, G. penicillioides, G. roseum and G. salmonicolor were isolated from the soil less frequently, and their contribution in the fungal community did not exceed 6%, except for the third year of the study in the variants with a single administration of green waste compost and farm manure (16.7 and 9.5%, respectively). Fungi of the genus Paecilomyces most often colonized the soil from under the crop of spring barley in the variant with green waste compost (double administration of the fertilizer) and that of winter rape (3rd

year of the study) in the variant with farm manure applied in a split dose. Ample research studies (HOITINK, BOEHM 1999) have indicated the stimulating effect of various organic fertilizers on the growth of fungi antagonistic to pathogens. In soil fed with organic fertilizers, as compared to that fertilized with minerals, BULLOCK et al. (2002) observed a higher prevalence of fungi of the genus *Trichoderma*.

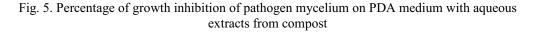
Effect of extracts from compost on the growth of soil fungi

The aqueous extracts prepared from composted municipal wastes, subjected to laboratory analyses, were found to suppress the growth of mycelium of six species of pathogens isolated from soil (Fig. 5.).



b. after 8 days of culture

Explanations: *Bc* –*Botrytis cinerea, Cc-Colletotrichum coccodes, Fc-Fusarium culmorum, Fe-F. equiseti, Fo-F. oxysporum, Fp-F. poae*



The "Dano" compost was characterized by a higher biological activity than the compost from green waste in both analytical periods; i.e. after 4 and 8 days. In the earlier period, the most susceptible to the addition of the extracts in agar medium appeared to be *B. cinerea*, *C. coccodes* and *F. poae* species, whereas in the later period it was the *C. coccodes* species. This confirms the results of the field

experiment, because the *C. coccodes* species did not colonize the soil in any of the plots fertilized with compost, whereas *B. cinerea* occurred in small numbers only in the soil fertilized with "Dano" compost in both variants of its application. In addition, analyses demonstrated the lowest index of growth inhibition of *F. culmorum* mycelium.

KITA ET AL. (1996), in their *in vitro* research on the effect of the addition of aqueous extract to PDA, observed poor growth of colonies and aerial mycelium of such species as: *Rhizoctonia solani* and *F. culmorum*. In turn, STOMPOR-CHRZAN (2001) demonstrated the susceptibility of fungi of the genus *Fusarium* to aqueous extracts from manure-based vermicomposts.

Summary

The results achieved in the study demonstrate that the applied natural fertilization with manure and organic fertilization with composts from municipal wastes modified the qualitative composition of the soil fungi community to a greater extent than its quantitative structure. The positive impact of this type of fertilization was manifested in suppressing the population of pathogenic fungi in respect to the control variant (without fertilization) and the variant with mineral fertilization. The prevailing species isolated in the study were those belonging to Aureobasidium and Fusarium genera. A tendency towards a stronger reduction of pathogen populations was observed in the soil with a single administration of 10 t ha⁻¹ of organic fertilizer as compared to the variant with a double application (5 t ha⁻¹ each). The study also demonstrated an increase in the population of fungi of the genera Gliocladium, Paecilomyces and Trichoderma antagonistic to pathogens. The most positive changes in the population of beneficial fungi were observed in the plots with manure administered both in a single dose and in a split dose. The in vitro test additionally showed that the aqueous extracts from composts added to the culture medium inhibited the growth of mycelium of 6 species of pathogens, though their response was diversified. The least susceptible to the addition of extract into the medium (the lowest index of mycelium growth inhibition) appeared to be Fusarium culmorum species. Finally, a higher fungistatic activity was demonstrated for the extract prepared from the "Dano" compost than for that from green waste.

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