J. Elem. s. 365–375 DOI: 10.5601/jelem.2011.16.3.02

SOIL FUNGAL COMMUNITIES SHAPED UNDER THE INFLUENCE OF ORGANIC FERTILIZATION

Bożena Cwalina-Ambroziak[1], Jadwiga Wierzbowska[2]

[1]Chair of Phytopathology and Entomology
[2]Chair of Agricultural Chemistry and Environmental Protection
University of Warmia and Mazury in Olsztyn

Abstract

The results presented in this paper have been obtained during a three-year experiment (from the second cycle of four-year trials set up in 2004) established in Bałcyny. The following crops were grown in succession: potato, fodder barley, winter oilseed rape and winter wheat. The fertilization treatments included: mineral NPK, FYM and organic fertilization with composted and dried sewage sludge and composted municipal waste. Treatments without soil enrichment or with mineral NPK fertilization were taken for comparison. Farmyard manure and organic fertilizers were applied in two different ways: as a single dose of 10 t ha^{-1} before planting the potato or in two doses, each 5 t d.m. ha^{-1}, before planting the potato and before sowing the oilseed rape. The effect of the applied fertilizers on assemblages of soil fungi was tested. The fungi were cultured on Martin medium.

The fertilizers were determined to have affected the populations of soil fungi. Yeast-like fungi prevailed among the isolated colonies (60-85% of the total isolates). The richest fungal community was obtained from the soil amended with dried and pelleted sewage sludge or municipal green waste compost. However, suppressed growth of pathogenic fungi represented by the species *Aureobasidium pullulans, Botrytis cinerea*, of the genus *Fusarium* and *Sclerotinia sclerotiorum* was observed in the soil amended with natural fertilizers and composts. The growth of pathogens was most strongly inhibited in the soil enriched with a double dose of FYM, composted urban green waste or with dried and pelleted sewage sludge in either of the two application variants. The pathogen-antagonistic fungi, represented by three species of *Gliocladium* and four species of the genera *Paecilomyces* and *Trichoderma*, were more often isolated from the soil fertilized with FYM or organic fertilizers than the unfertilized or NPK-nourished soil. The soil enriched with Dano compost applied in a single rate of 10 t d.m. ha^{-1} was most abundantly populated by these fungi. Among the remaining saprotrophs, fungi of the genus *Penicillium* and the orders *Mucorales* and *Sporotrichum olivaceum* were isolated.

Key words: organic fertility, NPK fertility, soil pathogens, antagonists.

dr hab. Bożena Cwalina-Ambroziak prof. UWM, Chair of Phytopathology and Entomology, University of Warmia and Mazury, Prawocheńskiego 17, 10-720 Olsztyn, Poland, e-mail: bambr@uwm.edu.pl

ZBIOROWISKO GRZYBÓW GLEBOWYCH UKSZTAŁTOWANE POD WPŁYWEM NAWOŻENIA ORGANICZNEGO

Abstrakt

Pezentowane w pracy wyniki pochodzą z 3-letniego doświadczenia (z 2. cyklu 4-letnich doświadczeń założonych w 2004 r.) założonego w Bałcynach. Uprawiano po sobie następujące gatunki roślin: ziemniak, jęczmień paszowy, rzepak ozimy i pszenicę ozimą. Zastosowano nawożenie mineralne NPK, naturalne obornikiem i organiczne: kompostowanymi i suszonymi osadami ściekowymi oraz kompostowanymi odpadami komunalnymi. Kombinacje bez nawożenia i z nawożeniem mineralnym NPK uwzględniono jako porównawcze. Obornik i nawozy organiczne aplikowano w dwojaki sposób: jednorazowo w dawce 10 t ha^{-1} przed sadzeniem ziemniaka lub dwukrotnie w dawce po 5 t s.m. ha^{-1} przed sadzeniem ziemniaka i przed siewem rzepaku. W badaniach określono wpływ aplikowanych nawozów na skład zbiorowiska grzybów glebowych. Hodowlę grzybów prowadzono na podłożu Martina.

Stwierdzono wpływ zastosowanych nawozów na populację grzybów glebowych. Najczęściej izolowano z gleby grzyby drożdżopodobne (60-85% ogółu izolatów). Najbogatsze zbiorowisko grzybów otrzymano z gleby użyźnionej osadem suszonym i granulowanym oraz kompostem z odpadów zieleni miejskiej. Jednak w glebie z zastosowanym nawożeniem naturalnym i kompostami stwierdzono ograniczenie liczebności grzybów patogenicznych reprezentowanych przez gatunki: *Aureobasidium pullulans, Botrytis cinerea*, rodzaju *Fusarium*, *Sclerotinia sclerotiorum*. Najbardziej zahamowany rozwój patogenów zanotowano w glebie z wniesionym dwukrotnie obornikiem, kompostem z odpadów zieleni miejskiej oraz osadem suszonym i granulowanym zastosowanymi w obydwu wariantach. Grzyby antagonistyczne względem patogenów, reprezentowane przez 3 gatunki *Gliocladium*, 4 gatunki rodzaju *Paecilomyces* i *Trichoderma*, częściej niż z gleby bez nawożenia i z nawożeniem NPK izolowano z gleby nawożonej obornikiem i organicznie, z najliczniej zasiedloną glebą użyźnioną Dano w dawce 10 t s.m. ha^{-1}. Wśród pozostałych saprotrofów wyodrębniono grzyby rodzaju *Penicillium*, rzędu *Mucorales* i *Sporotrichum olivaceum*.

Słowa kluczowe: nawożenie organiczne, nawożenie NPK, patogeny glebowe, antagoniści.

INTRODUCTION

Organic substances are added to soil in order to improve its quality by changing the physical (aggregation of soil particles) and microbiological properties (increased biomass content, microbial diversity and enzymatic activity) (DISSANAYAKE, HOY 1999, PUGET et al. 2000, CESPEDES LEON et al. 2006). Soil improvement is achieved, *inter alies*, by adding more carbon to soil (SIKORA, STOTT 1996, van BRUGGEN, SEMENOV 2000). In a study reported by KUNDU and NANDI (1985), a broader C:N ratio stimulated the development of bacteria while inhibiting that of fungi. This effect depended on the concentration of chemical and biological components in organic matter. Natural fertilizers applied in adequate doses, apart from large amounts of organic matter, enrich soil with some macro- and micronutrients. Certain characteristic microbial communities develop in soil under the influence of these fertilizers. For the phytosanitary safety, the share of fungi demonstrating antibiotic and parasitic activity is important. The fungi whose presence in soil is particularly desirable are the ones with enzymatic properties belonging to the genera *Gliocldium* and *Trichoderma*, which can inhibit the development of pathogens (not only the soil ones) by activating the following mechanisms: antibiosis, competition and hyperparasitism (CHERNIN, CHET 2002). Organic fertilizers (SARAIVA et al. 2004), composted plant waste (MAZZOLA, MULLINIX 2005), municipal waste and sewage sludge (LEWIS et al. 1992, TRANKNER 1992) as well as paper-mill residue (COOPERBAND 2001, STONE et al. 2003) provide effective plant protection against such pathogens as *Botrytis cinerea, Pythium* spp.*, Phytophthora* spp., *Rhizoctonia solani, Sclerotinia sclerotiorum.*

This paper contains an analysis of the structure of soil fungal assemblages shaped under the influence of mineral, FYM or organic fertilization.

MATERIAL AND METHODS

The experiment was carried out in 2008-2010 (the second cycle of a four-year experiment started in 2004) on experimental plots in Bałcyny, on grey-brown podzolic soil originating from light silty loam, complex 4 class III (the Polish Norms, BN-78/9180-11). Prior to the experiment, the concentration of P (38.2 mg kg $^{-1}$), K (105.8 mg kg $^{-1}$) and Mg (48.3 mg kg $^{-1}$) in the soil as well as the soil pH = 5.04 were determined. The following crops were grown in succession: potato, fodder barley, winter oilseed rape and winter wheat. The applied fertilization consisted of natural fertilizer: mixed farmyard manure or organic fertilizers: sewage sludge composted with straw (municipal sewage sludge from the WTP in Działdowo, mixed with cereal straw in a 1 : 0.5 ratio and composted in heaps), dried and pelleted sludge (sludge from the WTP in Iława), sludge composted with no added substances (sludge from the WTP in Ostróda), municipal waste compost (Dano compost from unsorted municipal waste in Suwałki), and urban green waste compost from Suwałki. The chemical composition of the organic fertilizers is presented in Table 1. The treatments without fertilization or with the NPK mineral fertilization were taken for comparison. The farmyard manure and organic fertilizers were applied in two ways: in a single dose of 10 t d.m. ha^{-1} before planting the potato or as two doses, each of 5 t d.m. ha^{-1} before planting the potato and before sowing the oilseed rape. For the treatments with the organic fertilizers, depending on their N-total content, nitrogen was balanced to 150 kg ha^{-1} (under potato) and to 120 kg ha^{-1} (under oilseed rape). Spring barley and winter wheat received only mineral fertilization.

For determination of the quantitative and qualitative composition of soil fungal assemblages, in the early August each year, soil samples were collected from the depth of 10 cm from all the plots (at three sites) which made up a given experimental treatment. In the laboratory, the soil samples were shaken and portions of 10 g each were weighed out to place in 250 ml flasks, in which they were shaken for 20 minutes in 90 ml of sterile water, thus obtaining 10^{-1} dilution. The suspension was further diluted (from 10^{-2} to 10^{-4}). Fungi were cultured on Martin medium at 22-23°C. Fungal colonies grown after 5-day incubation were re-calculated per 1 gram of dry matter of soil (DHINGRA, SINCLAIR 1995). Next, the fungi were inoculated onto agar slabs for further species identification. The results of the *in vitro* tests, carried out in a completely randomized design, were processed statistically using analysis of variance (Statistica® 9.0 2010). The means were compared with Duncan's test at the significance level of 0.01.

Table 1

Chemical composition of organic fert

Fertilizer	C org.	N	P	K	Mg	C : N
	(g kg^{-1} d. m.)					
FYM	364.00	20.00	3.72	20.80	4.80	18.2
Sludge composted with straw	276.00	20.56	23.15	9.81	6.11	13.4
Dried and pelleted sludge	386.00	35.15	24.48	3.56	5.65	11.0
Sludge composted with no added substances	410.00	50.45	31.82	3.18	7.27	8.1
Municipal waste compost (Dano)	151.00	12.57	6.92	7.83	5.46	12.0
Urban green waste compost	59.40	5.00	1.88	3.25	4.25	11.9

RESULTS AND DISCUSSION

The applied natural and organic fertilization differentiated counts of the soil fungal populations; in total, 6,192 colonies of fungi were obtained, in which yeast-like fungi made up 76.3%. Filamentous fungi were represented by 50 species. The phytopathological tests have demonstrated that most of the colony-forming units were obtained from the soil amended with urban waste compost and dried and pelleted sludge (Figure 1). The literature contains contradictory reports on the influence of soil organic amendments on assemblages of soil fungi. GÓRSKA and STĘPIEŃ (2007) as well as PRATT (2008) claim that organic fertilization does not differentiate the abundance of filamentous fungal populations. AWAD and FAWN (2004) formulate contradictory conclusions as they suggest that increasing rates of sewage sludge stimulate the development of soil microorganisms and increase their diversity (WEYMAN-KACZMARKOWA et al. 2002). As researchers claim, the amount of organic carbon added to soil plays an important role; to a lesser degree, added nitrogen affects microbial communities.

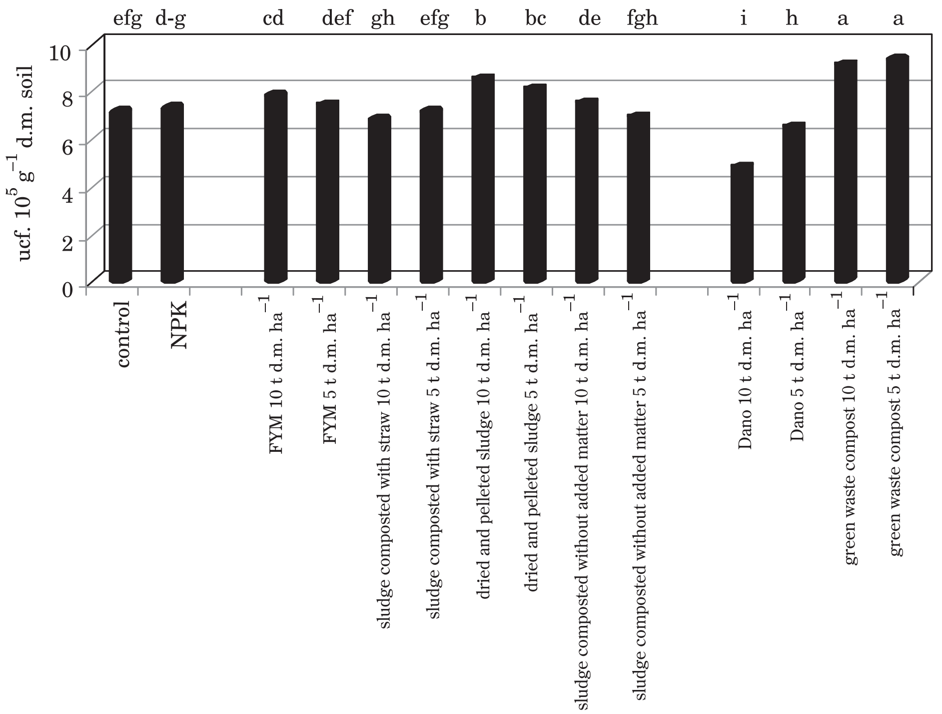

Fig. 1 Number of colony-forming units of fungi in the soil

In the present study, the dominant organisms in the fungal assemblages were yeast-like fungi, which made up from 63 to 85% of total communities (in the treatment with municipal waste compost 10 t d. m. ha^{-1} and urban waste compost in both application variants, respectively) – Figures 2a,c,d. Fifty species and non-sporulating colonies were identified among the filamentous fungi The pathogenic fungi, important for the phytosanitary soil condition, were represented by the following species: *Aureobasidium pullulans, Botrytis cinerea*, four species of the genus *Fusarium* and *Sclerotinia sclerotiorum,* most frequently colonizing the unfertilized and NPK-fertilized soil: 4.6 and 3.6%, respectively, of the total number of isolates (Figures 3a, 2b). The smallest population of the above fungi was cultured from the soil fertilized twice with FYM and urban green waste compost, under spring barley (Figures 3d, 2d). A single application of these fertilizers stimulated the development of potentially pathogenic fungi. However, this effect was accompanied by a growing number of antagonistic fungi, whose presence in soil is desirable (3 species of the genus *Gliocladium*, 5 – *Paecilomyces* and 4 – *Trichoderma*). Based on their experiments, SERRA-WITTLING et al. (1996) concluded that natural fertilizers and composts inhibited the development of *Fusarium* spp. fungi as well as those of *Pythium* and *Phytophthora*, all wide-

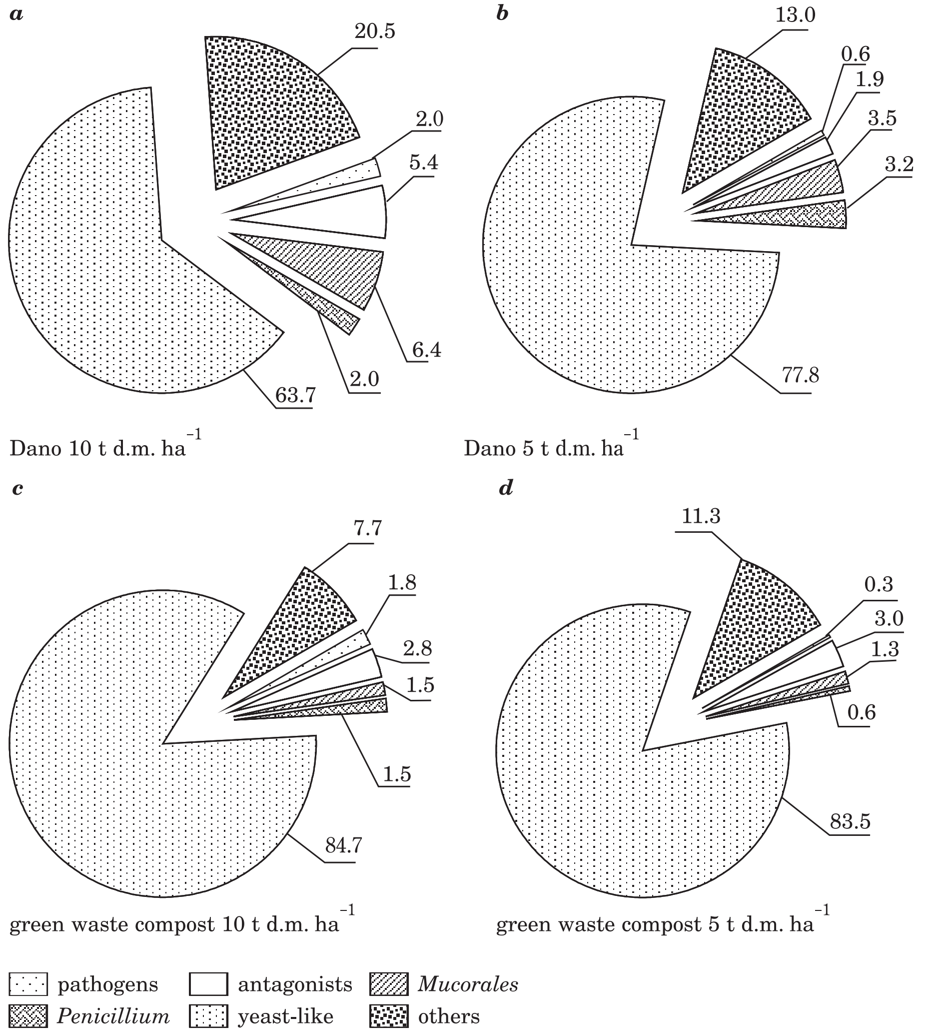

Fig. 2. Fungi isolated from soil fertilized with composted industrial waste (%)

spread in soil. TSROR LAKHIM et al. (2001) as well as LAZAROVITZ et al. (2008) report that the infection of potato by *Rhizoctonia solani* and *Streptomyces scabies* is less severe in soil fertilized with cattle manure. Other researchers (MILLS et al. 2002) point to less severe infection of solanaceous plants by *P. capsici, Alternaria solani* and *Septoria lycopersici* in soil amended with composted plant waste. CATXARRERA et al. (2002) observed weaker symptoms of *Fusarium* wilt of tomato plants when the soil had been enriched with the fungus *Trichoderma asperellum* and compost. BARAKAT and AL.-MASRI (2009) found out a similar effect for the soil enriched with *T. harzianum* and sheep manure.

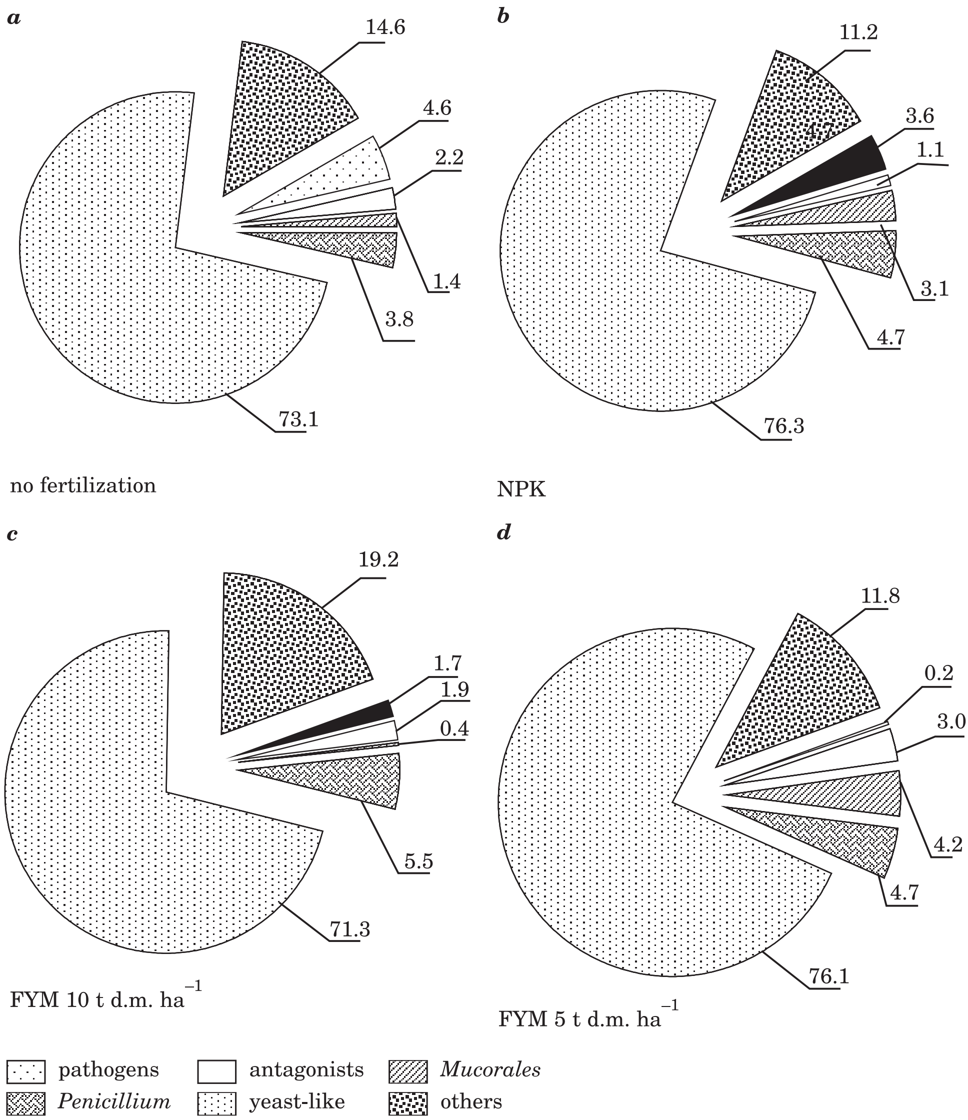

Fig. 3. Fungi isolated from unfertilized, NPK and FYM fertilized soil (%)

Our comparison of the analysed composted sewage sludge has demonstrated that the most favourable soil conditions, in respect of the plants' health, prevailed in the soil amended with dried and pelleted sludge, applied in either of the two systems: 0.6% of the pathogens and 3% of the antagonists (Figure 4c,d). However, the highest number of antagonists colonized the soil enriched with the compost made from unsorted municipal waste,

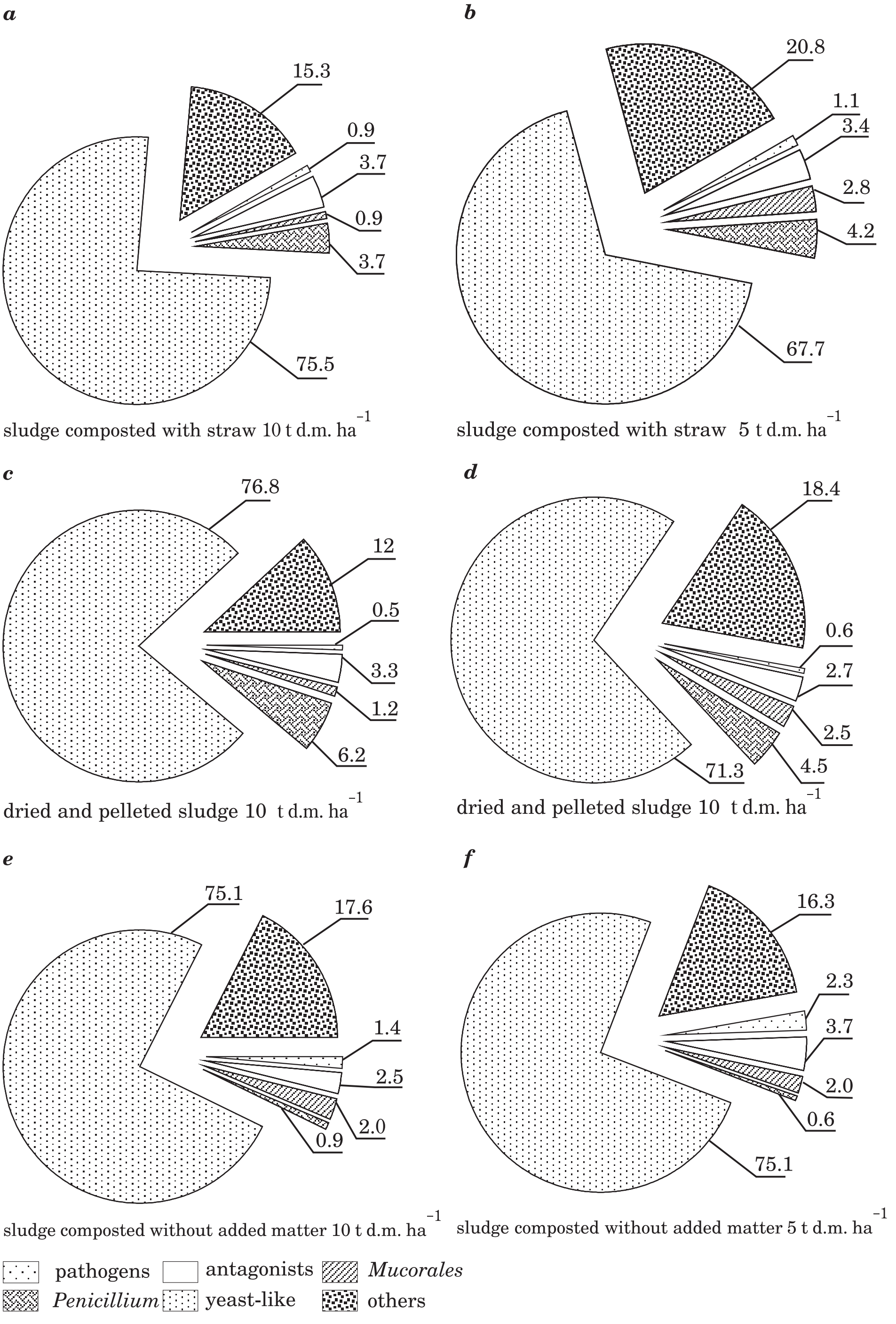

Fig. 4. Fungi isolated from soil fertilized with composted sewage sludge (%)

applied in a single dose under fodder barley (Figure 2a). For the phytosanitary safety of crops, it is desirable to maintain dynamic changes of fungal populations in the soil. Organic fertilizers added to soil create favourable conditions for the development of antagonistic fungi (HOITIN, BOEHM 1999, PANDEY et al. 2006), especially the ones belonging to the genus (BULLOCK et al. 2002). Such fungi produce antibiotic and parasitic effects on pathogenic organisms, which means that they can be employed for the biological control of plant pathogens (PAPAVIZAS 1985, NELSON et al. 1983, ZAK et al. 2003). Soil cropped with *Brassicaceae* has been demonstrated to contain a growing population of the antagonists *Streptomyces* spp., which produce secondary metabolites inhibiting the development of plant pathogens, e.g. *Rhizoctonia solani* (MAZZOLA, MULLINIX 2005).

Among the saprotrophic fungi, the species of the order *Mucorales* produced the most numerous populations in the soil amended with the municipal waste compost added in a dose of 10 t d.m. ha^{-1} (6.4%, Figure 2a). In the treatments fertilized with composted sewage sludge and FYM (Figures 3c,d, 4a-f), the highest counts of saprotrophs were isolated from the soil amended with a double application of the fertilizers, except sewage sludge composted with no added substances. Species of the genus *Penicillium* had a similar share in the total number of fungi as saptrotrophs, and their development was promoted by the soil environment amended with a single dose of FYM or dried and pelleted sludge (Figures 3c, 4c). The saprotrophs of the genus *Penicillium*, analogously to those of the genus *Aspergillus*, as well as the pathogens belonging to *Fusarium* spp. are the most common components of fungal assemblages dwelling in soil enriched with organic fertilizers (SARAIVA et al. 2004).

Recapitulating, the positive influence of farmyard manure as well as sewage sludge or composted municipal waste applied as soil amending substances on the structure of soil fungal assemblages is noteworthy. These fertilizers have reduced the development of potential plant pathogens but stimulated that of antagonistic fungi.

CONCLUSIONS

In brief, the experiment has demonstrated a suppressed growth of populations of pathogenic fungi in soil amended with farmyard manure or organic fertilizers. The highest count of fungal colonies was obtained from the soil enriched with dried and pelleted sludge. At the same time, the soil from this treatment presented the most desirable phytosanitary conditions, i.e. a small count of pathogens but a high number of antagonistic fungi.

REFERENCES

Awad N.M., Fawzy K.S.M. 2004. *Assessment of sewage sludge application on microbial diversity, soil properties, and quality of wheat plants grown in a sandy soil.* Ann. Agric. Sci., (Cairo), 49(2): 485-499.

Barakat R.M., Al.-Masri M.L. 2009. *Trichoderma harzianum in combination with sheep manure amendment enhances soil suppressiveness of Fusarium wilt of tomato.* Phytopathol. Mediterranea, 48(3): 385-395.

Bullock L.R., Barker K.R., Ristaino J.B. 2002. *Influences or organic and synthetic soil fertility amendments on nematode trophic groups and community dynamics under tomato.* Appl. Soil Ecol., 21: 233-250.

Cespedes-Leon, M.C., Stone A. G., Dick R. P. 2006. *Organic soil amendments: impacts on snap bean common root rot and soil quality.* Appl. Soil Ecol., 31:199-210.

Chernin L., Chet L. In: Burns R. G., Dick R. P. 2002. *Enzymes in the environment. Activity, ecology and applications*. Marcel Dekker Inc., New York, Basel.

Cooperband L. 2001. *Intermediate-term effects of building soil carbon on soil properties and crop production using paper-mill residues.* Proc. of the Wisconsin's Annual Potato Meeting, Madison, Wisconsin.

Cotxarrera L., Trillas-Gay M.I., Steinberg C., Alabouvette C. 2002. *Use of sewage sludge compost and Trichoderma asperellum isolates to suppress Fusarium wilt of tomato.* Soil Biol. Biochem., 34(4): 467-476.

Dhingra O. D., Sinclair J. B. 1995. *Basic plant pathology methods*, Appendix A, 345-394. Lewis Publishers. CRC Press. Boca Raton, FL, USA.

Dissanayake N., Hoy J. W. 1999. *Organic material soil amendment effects on root rot and sugarcane growth and characterization of the materials.* Plant Dis., 83: 1039-1046.

Górska E. B., Stępień W., Russel S. 2007. *Wpływ dodatku osadu ściekowego, kurzeńca i kompostu Dano na aktywność mikrobiologiczną gleby i plony buraka ćwikłowego [Effect of added sewage sludge, poultry manure and Dano compost on the soil microbial activity and edible beetroot yield].* Zesz. Probl. Post Nauk Rol., 520: 287-293. (in Polish)

Hoitink H. A. J., Boehm M. J. 1999. *Biocontrol within the context of soil microbial communities a substrate-dependent phenomenon.* Ann. Rev. Phytopathol., 37: 427-446.

Kundu P. K., Nandi B. 1985. *Control of Rhizoctonia disease of cauliflower by competitive inhibition of the pathogen using organic amendments in soil.* Plant Soil, 83: 357-362.

Lazarovitz G., Hill J., Patterson G., Conn K. L., Crump N. S. 2007. *Edaphic soil levels of mineral nutrients, pH, organic matter, and cationic exchange capacity in the geocaulosphere associated with potato common scab.* Phytopathology, 97(9): 1071-1082.

Lewis J. A., Lumsden R. D., Millner P. D., Keinath A. P. 1992. *Suppression of damping-off of peas and cotton in the field with composted sewage sludge.* Crop. Prot., 11: 260-266.

Mazzola M, Mullinix K. 2005. *Comparative field efficacy of management strategies containing Brassica napus seed meal or green manure for the control of apple replant disease.* Plant Dis., 89: 1207–1213.

Mills D. J., Hoffman C. B., Teasdale J. R. 2002. *Factors associated with foliar disease of staked fresh tomatoes grown under differing bed strategies.* Plant Dis., 86: 356-361.

Nelson E.B., Kuter G.A., Hoitink H.A.J. 1983. *Effects of fungal antagonists and compost age on suppression of rhizoctonia damping-off in container media amended with composted hardwood bark.* Phytopathology, 73: 1457-1462.

Pandey A.K., Gopinath K.A., Chattacharya P., Hooda K.S., Sushil S.N, Kundu S., Selvakumar G., Gupta H.S. 2006. *Effect of source and rate of organic manures on yield attributes. Pod yield and economics of organic garden pea (Pisum sativum subsp. hortense) in north west Himalaya.* Ind. J. Agric. Sci., 76(4): 230-234.

PAPAVIZAS G.G. 1985. *Trichoderma* and *Gliocladium. Biology, ecology and potential for biocontrol.* Ann. Rev. Phytopath., 23: 23-54.

PRATT R. G. 2008. *Fungal population levels in soils of commercial swine waste disposal sites and relationships to soil nutrient concentrations.* Appl. Soil Ecol., 38(3): 223-229.

PUGET P. CHENU C., BALESDENT J. 2000. *Dynamics of soil organic matter associated with particle-size fractions of water-stable aggregates.* Eur. J. Soil Sci., 51: 595-605.

SARAIVA V.P., ARAUJO E., ARAUJO-FILHO J.O.T., BRUNO G.B., BRUNO R.L.A., COELHO R.R.P. 2004. *Populations of three fungi in soils treated with different forms of cattle manure and sown with carrot.* Proc. of Interamerican Soc. Trop. Horticul., 47: 43-44.

SERRA-WITTLING C., HOUOT S., ALABOUVETTE C. 1996. *Increased soil suppressiveness to Fusarium wilt of flax after addition of municipal solid waste compost.* Soil Biol. Biochem., 28: 1207-1214.

SIKORA L. J., STOTT D. E. 1996. *Soil organic carbon and nitrogen*. In: *Methods for assessing soil quality.* SSSA Special Publication 49, Madison, WI, 247-271.

STONE A. G., VALLAD G. E., COOPERBAND L. R., ROTENBERG D., DARBY H. M., JAMES R. V., STEVENSON W. R., GOODMAN R. M. 2003. *The effect of organic amendments on soil borne and foliar diseases in field-grown snap bean and cucumber.* Plant Dis., 87: 1037-1042.

TRANKNER A. 1992. *Use of agricultural and municipal wastes to develop suppressiveness to plant pathogens*: 35-42. In: *Biological control of plants diseases*. TJAMOS E.C., PAPAVIZAS G.C., COOK R.J., eds. Plenum Press, New York.

TSROR /LAHKIM/ L., BARAK R., SNEH B. 2001. *Biological control of black scurf on potato under organic management.* Crop Protect., 20: 145-150.

VAN BRUGGEN A. H. C., SEMENOV A. M. 2000. *In search of biological indicators for soil health and disease suppression.* Appl. Soil Ecol., 15: 13-24.

WEYMAN-KACZMARKOWA W., WÓJCIK-WOJTKOWIAK D., POLITYCKA B. 2002. *Greenhouse medium enrichment with composted pig slurry; effect on the rooting of Pelargoniom peltatum Hort. Cuttings and development of rhizospere microflora*. Pol. J. Environ. Stud., 11: 67-70.

ZAK R.D, HOLMES W.E, WHITE D.C, PEACOCK A.D, TILMAN D. 2003. *Plant diversity, soil microbial communities and ecosystem function: Are there any links?* Ecology, 84: 2042-2050.