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# The effectiveness of two methods used for isolating soil fungi

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## INTRODUCTION

With the increased interest in biodiversity and the ecology of soil fungi including the phytopathological role they play in such habitats, there is an urgent need to detect and describe all fungi which occur in soil. The known fungal soil microflora encompasses about 600 species. Each of the different methods has its advantages as well as its drawbacks. Therefore the results obtained do not, for the most part, represent the real qualitative and quantitative composition of the fungal communities. In order to find a method of isolation which would guarantee the reflection of the full fungal population in the examined biotop, the current study has been undertaken. The paper records the effectiveness of the two methods used for isolation of fungi from soil and evaluates the usefulness of each method for the specific tasks that arise in working with soil fungi.

## MATERIAL AND METHODS

Soil samples were taken from three locations in order to isolate the fungal flora. Samples were collected from a Scots pine (*Pinus sylvestris*) nursery, from a 17 year-old pine forestry and from a 70 year-old one in Zielonka forest district, sections 17,

41 g and 20 a, respectively (western Poland, near Poznań), in the beginning of May in 1992.

Type of soil

Section 17 – podzol (cryptopodzol) with a brown-gray, slightly clayed sand in the horizon $A_1$ – 0-25 cm, decalcification extends below 200 cm, pH at the depth of 10 cm is 6.61.

Section 41 g – podzol (cryptopodzol) with slightly clayed sand of various granulation with a small skeletal admixture in the horizon $A_1$ – 0-25 cm, humus stains reach the depth of 40 cm. Decalcification extends below 200 cm, pH at the depth of 10-15 cm is 3.95. The main tree is pine (afforestation 0.8), mingled with some single oak trees. The soil is covered mainly with *Deschampsia flexuosa* and single patches of *Brachypodium* and *Calamagrostis*.

Section 20 a – podzol (cryptopodzol), with brown-gray, slightly clayed sand in the horizon $A_1$ – 0-25 cm. Decalcification extends below 200 cm, pH at the depth of 10-15 cm is 3.74. 70 year-old pines (afforestation 0.6) grow in the area, admixture – – single oak trees. The forest floor is covered mainly with *Festuca ovina* and patches of *Luzula pilosa*, *Hippophaë* and tufts of moss (*Scleropodium purum*).

At each location the soil was taken from 6 different spots, 5-6 m apart from each other, from a depth of 10-15 cm, under almost aseptical conditions. The individual samples were poured together and dried in a clean workbench until the soil could be sieved through a mesh of 2 mm. Subsequently soil samples were mixed for 6 hours in an end over end laboratory rotator which guaranteed a perfect mixing. The three forest locations were so near to each other that the environmental conditions such as temperature and moisture can be assumed as identical.

The isolations were carried out according to two methods: method No. 1 (N i r e n b e r g and M e t z l e r, 1990) used in the Institute of Microbiology, Federal Biological Research Center of Agriculture and Forestry, Berlin, Germany, and method No. 2 which is the soil plate method of W a r c u p (1950) modified by J o h n s o n and M a ń k a (1961) and M a ń k a (1974), used at the Department of Forest Pathology, University of Agriculture, Poznań, Poland.

Description of method No. 1

A single soil particle calibrated to about 0.5 mm in diameter was taken from the soil sample and placed on the surface of the cooled SNA (medium by N i r e n b e r g, 1976) supplemented with antibiotics in the center of a 9 cm plastic Petri dish; 20 replicates were used per trial. For the first five days the plates were incubated at 17°C in darkness. Afterwards the plates were placed under continuous black light (P h i l i p s 40W/08) at 17°C for additional seven days. Subsequently they were transferred to the laboratory and kept under natural day-night-rhythm at about 22--25°C for ca. 30 days. After 5, 12 and 42 days the Petri dishes were inspected under the low power of the microscope (magnification x 100 or x 250). Most of the fungi could be identified in the plates, the others were transferred into plates with special media and then identified.

Description of method No. 2

First, 1 g of the soil was mixed by hand with 149 g of fine quartz-sand in a mortar for 10-20 seconds and then in a flask for further 2 minutes. Two flasks, each with 1 g of soil, were used. A portion containing 26 $mm^3$ of the mixture was transferred into an empty Petri dish and covered with a medium by J o h n s o n (1957), i.e. Czapek-Dox-agar, aureomycin, bengal rose. The plates were incubated at room temperature for 7-10 days. Individual colonies of fungi were transferred into test tubes with PDA for conservation and systematically identified. Experiments were conducted with 20 replications (10 Petri dishes from one flask).

The fungi were identified according to their morphology on SNA and PDA. *Aspergillus* and *Penicillium* species were additionally examined on Czapek-Dox--agar and malt-extract-agar, *Acremonium* species on 1 % carrot decoct agar and 2 % malt agar.

## RESULTS

The mycological analyses of 3 samples revealed the occurrence of 105 different fungal species belonging mostly to the *Zygomycetes* and *Deuteromycetes*. A few were *Ascomycetes*. The number of recovered species depended on the method used (Tab. 1).

The number of isolates obtained from the soils of pine nursery, 17 and 70 year--old pine forestries are given in Tab. 1.

Taking into consideration the number of species obtained, method No. 1 proved to be more efficient than method No. 2. As expected, the number of isolates was larger when method No. 2 was used.

31 species were recovered only once, 29 species in two soils and 16 species in all three examined soils, regardless of the method used. The last group included *Absidia cylindrospora, Acremonium bacillisporum, Cylindrocarpon destructans, Exophiala pisciphila, Mortierella vinacea, Oidiodendron griseum, Penicillium adametzi, P. chrzaszczi, P. daleae, P. janczewskii, P. restrictum, P. vinaceum, Sesquicillum candelabrum, Trichoderma virens* and *T. viride*. They are typical pine forest soil fungi and occur in this biotope in large quantities (G i e r c z a k, 1967; K w a ś n a, 1987; M a ń k a et G i e r c z a k, 1961; P r z e z b ó r s k i, 1982; S o d e r s t r o m, 1975).

Both methods demonstrated that there are fungi which colonize only certain habitats: *Coniothyrium fuckelii, Doratomyces stemonitis, Fusarium solani, Phoma terrestris, Trichocladium asperum, Trichoderma hamatum* were detected only in the nursery soil. In contrast *Mucor hiemalis* and *Penicillium simplicissimum* occurred only in the soil of the 17 year-old pine forestry and *Trichoderma polysporum* only in the 70 year-old pine forestry.

Table 1

Number of fungal isolates from forest soil (pine nursery, 17 year-old and 70 year-old pine forests)

| Species of fungi | Method No. 1 | | | Method No. 2 | | |
|---|---|---|---|---|---|---|
| | N | P 17 | P 70 | N | P 17 | P 70 |
| *Absidia cylindrospora* Hagem | 5 | 5 | 14 | | 6 | 22 |
| *Acremonium apii* (M.A. Smith et Ramsey) W. Gams | | 1 | | | | |
| *Acremonium bacillisporum* (Onions et Barron) W. Gams | 13 | 1 | 3 | | | |
| *Acremonium butyri* (van Beyma) W. Gams | | 1 | | | | |
| *Acremonium charticola* (Lindau) W. Gams | | | | 1 | 1 | |
| *Acremonium terricola* (Miller et al.) W. Gams | 1 | | | | | |
| *Alternaria alternata* (Fr.) Keissler | | 2 | | | | |
| *Aspergillus versicolor* (Vuill.) Tiraboschi | | | | | 22 | |
| cf. *Candida humicola* | | 2 | | | | |
| *Chaetomium cochlioides* Pall. | | | | 10 | | |
| *Chaetomium globosum* Kunze ex Steud | | 1 | | | | |
| *Chromelosporium fulvum* (Link) Mc Ginty, Hennebert et Korf | | 1 | | | | |
| *Chrysosporium merdarium* (Link ex Grev.) Carm. | 1 | | | | 1 | |
| *Cladosporium herbarum* Link ex Fr. | | | | | 1 | |
| *Coniothyrium fuckelii* Sacc. | 7 | | | 26 | | |
| *Cylindrocarpon cylindroides* Wollenw. var. *tenue* Wollenw. | 1 | | | 1 | 3 | |
| *Cylindrocarpon destructans* (Zins.) Scholten | 7 | 3 | 1 | | | |
| *Cylindrocarpon olidum* (Wollenw.) Wollenw. | 1 | | | | | |
| *Doratomyces stemonitis* (Pers. ex Fr.) Morton et G. Smith | 1 | | | 1 | | |
| *Exophiala dermatitidis* (Kano) de Hoog | | | 1 | | | |
| *Exophiala moniliae* de Hoog | | | 3 | | | |
| *Exophiala pisciphila* McGinnis et Ajello | 10 | 1 | 1 | | 1 | |
| *Exophiala salmonis* Carm. | 1 | | | | | |
| *Fusarium merismoides* Corda | 1 | | | | | |
| *Fusarium oxysporum* Schlecht. emend Snyder et Hansen | | | | 1 | | |
| *Fusarium solani* (Mart.) Appel et Wollenw. | 3 | | | 1 | | |
| *Fusarium torulosum* (Berk. et Curt.) Nirenberg | 1 | | | | | |
| *Geomyces pannorum* (Link) Singler et Carm. | 1 | | 3 | | 10 | 8 |
| *Geomyces pannorum* (Link) Singler et Carm. | | | | | | |
| var. *asperulatum* (Singler et Carm.) van Oorschot | | 1 | 5 | | | |
| *Geotrichum candidum* Link ex Leman | | 5 | | | | |
| *Gliocladium catenulatum* Gilman et Abbott | 3 | 1 | | 1 | | |
| *Gliocladium roseum* Bainier | 5 | 1 | | | | |
| *Gliomastix murorum* (Corda) Hughes var. *felina* (Mar.) Hugh | 4 | | | | | |
| *Humicola fuscoatra* Traaen var. *fuscoatra* | 1 | | | 1 | 1 | |
| *Mariannaea elegans* (Corda) Samson | 2 | | | | | |
| *Memnoniella echinata* (Riv.) Galloway | | | | | 2 | 4 |
| *Mortierella alpina* Peyronel | | 2 | | 1 | 3 | |
| *Mortierella exigua* Linnem. | 2 | | | | | |
| *Mortierella fatshederae* Linnem. | 1 | | | | | |
| *Mortierella gracilis* Linnem. | | 2 | 9 | | 1 | |
| *Mortierella humilis* Linnem. ex W. Gams | | 6 | 3 | | 1 | |
| *Mortierella hygrophila* var. *minuta* Linnem. | 1 | | | 3 | 1 | |
| *Mortierella isabellina* Oudemans et Koning | | 2 | | | 23 | 1 |
| *Mortierella nana* Linnem. | | | 2 | | 1 | 16 |
| *Mortierella spinosa* Linnem. | | 2 | | | | |
| *Mortierella turficola* Ling-Young | | | | | | 1 |
| *Mortierella vinacea* Dixon-Steward | 20 | 20 | 13 | 86 | 460 | 50 |
| *Mucor laxorhizus* Ling-Young var. *ovalispora* | 1 | 9 | | | | |
| *Mucor hiemalis* Wehmer | | 2 | | | 1 | |
| *Oidiodendron griseum* Robak | 7 | 15 | 11 | 1 | 20 | 8 |
| *Oidiodendron periconioides* Morrall | | | | | | 2 |
| *Paecilomyces farinosus* (Holm. S. F. Gray) A. H. S. Brown et Sm. | 2 | | | | | |

continued Tab. 1

| | | | | | | |
|---|---|---|---|---|---|---|
| *Paecilomyces* cf. *inflatus* Matsushima | 2 | 2 | | | | |
| *Penicillium adametzi* Zaleski | | 3 | 2 | 7 | 27 | 151 |
| *Penicillium arenicola* Chalabuda | | 1 | | | | |
| *Penicillium chrzaszczi* Zaleski | 1 | 1 | 7 | | 8 | |
| *Penicillium daleae* Zaleski | 15 | 15 | 13 | 5 | 86 | 24 |
| *Penicillium decumbens* Thom | 1 | | | | 12 | 4 |
| *Penicillium echinulatum* Raper et Thom ex Fassatiova | | | | | | 1 |
| *Penicillium glabrum* (Wehmer) Westling | | | 10 | | | |
| *Penicillium herquei* Bain. et Sartory | | | | | 11 | |
| *Penicillium janczewskii* Zaleski | 5 | 5 | 14 | 13 | 127 | 313 |
| *Penicillium janthinellum* Biourge | | 1 | | | | |
| *Penicillium miczynskii* Zaleski | | | 1 | | | |
| *Penicillium olsoni* Bain. et Sartory | 1 | | | | | |
| *Penicillium piscarium* Westling | 1 | 3 | | | | |
| *Penicillium raistrickii* G. Smith | | | | 1 | | |
| *Penicillium restrictum* Gilman et Abbott | 2 | | | 1 | 10 | 2 |
| *Penicillium roqueforti* Sopp. | | | | | 3 | |
| *Penicillium roseo-purpureum* Dierckx | | | | | | 12 |
| *Penicillium simplicissimum* (Oudem.) Thom | | 4 | | | 1 | |
| *Penicillium spinulosum* Thom | | 1 | 1 | | | |
| *Penicillium stoloniferum* Thom | | 1 | | | | |
| *Penicillium variabile* Wehmer | | | | 2 | 4 | |
| *Penicillium verruculosum* Peyronel | 3 | | | | | |
| *Penicillium vinaceum* Gilman et Abbott | 2 | 2 | 1 | 7 | 8 | |
| *Penicillium waksmani* Zaleski | 1 | | | | | |
| *Penicillium* sp. (*Biverticillata*) | 1 | | | | | |
| *Penicillium* sp. | | | | | 1 | |
| *Phoma eupyrena* Sac., | | | | 1 | | |
| cf. *Phoma terrestris* Hansen | 1 | | | 2 | | |
| *Pseudogymnoascus roseus* Raillo | 1 | | | 6 | 3 | |
| *Scolecobasidium constrictum* Abbott | 1 | | | | | |
| *Sesquicillium candelabrum* (Bonord.) W. Gams | 3 | 1 | 2 | 1 | 1 | |
| *Sporothrix schenckii* Hektoen et Perkins | 4 | | | 1 | | 1 |
| *Tolypocladium geodes* W. Gams | | 4 | 8 | | | 1 |
| *Torulomyces lagena* Delitsch | | | | | 15 | 1 |
| *Trichocladium asperum* Harz | 3 | | | 1 | | |
| *Trichocladium opacum* (Corda) Hughes | | | | 1 | | |
| *Trichoderma atroviride* Karsten | 1 | | | | | |
| *Trichoderma hamatum* (Bonord.) Bain. | 1 | | | 3 | | |
| *Trichoderma harzianum* Rifai | 1 | | 1 | | | |
| *Trichoderma koningii* Oudem. | | 3 | | | 7 | 1 |
| *Trichoderma "marseniae"* | 2 | | | | | |
| *Trichoderma polysporum* (Link: Pers.) Rifai | | | 1 | | | 2 |
| *Trichoderma* cf. *pseudokoningii* Rifai | 1 | 2 | | 1 | | |
| *Trichoderma strigosum* Bissett | 1 | | | | | |
| *Trichoderma virens* (Miller, Giddens et Foster) von Arx | 3 | | 2 | 1 | 2 | 3 |
| *Trichoderma viride* Pers: Fr. | 3 | 3 | 6 | 1 | 20 | 29 |
| *Trichothecium roseum* Link : S. F. Gray | 1 | | | | | |
| *Verticillium bulbillosum* W. Gams et Malla | | 4 | 1 | | | |
| *Verticillium suchlasporium* W. Gams var. *catenulatum* (Kamyschko ex Barron et Onion) W. Gams | | | 1 | | | |
| *Verticillium nigrescens* Pethybr. | | | | 3 | | |
| *Zygorhynchus moelleri* Vuillemin | 1 | 2 | | 2 | 3 | |
| Unidentified fungi | 1 | 1 | 2 | | | |
| Identified isolates | 166 | 144 | 140 | 194 | 908 | 657 |
| Found isolates | 167 | 145 | 142 | 194 | 908 | 657 |
| Number of species obtained by methods No. 1 and 2 | 56 | 43 | 30 | 33 | 38 | 23 |

Explanations: N – soil from pine nursery, P17 – soil from 17 year-old pine forestry, P70 – soil from 70 year-old pine forestry

Table 2

The 10 most often recovered fungi in three forest soils by two methods

| Location* | Fungi obtained with method No. 1 | Number of isolates | Fungi obtained with method No. 2 | Number of isolates |
|---|---|---|---|---|
| N | *Mortierella vinaceae* | 20 | *Mortierella vinaceae* | 86 |
| | *Penicillium daleae* | 15 | *Coniothyrium fuckelii* | 26 |
| | *Acremonium bacillisporum* | 13 | *Penicillium janczewskii* | 13 |
| | *Exophiala pisciphila* | 10 | *Chaetomium cochlioides* | 10 |
| | *Oidiodendron griseum* | 7 | *Penicillium adametzi* | 7 |
| | *Cylindrocarpon destructans* | 7 | *Penicillium vinaceum* | 7 |
| | *Coniothyrium fuckelii* | 7 | *Pseudogymnoascus roseus* | 6 |
| | *Absidia cylindrospora* | 5 | *Penicillium daleae* | 5 |
| | *Penicillium janczewskii* | 5 | *Trichoderma hamatum* | 3 |
| | *Gliocladium roseum* | 5 | *Verticillium nigrescens* | 3 |
| P 17 | *Mortierella vinaceae* | 20 | *Mortierella vinaceae* | 460 |
| | *Oidiodendron griseum* | 15 | *Penicillium janczewskii* | 127 |
| | *Penicillium daleae* | 15 | *Penicillium daleae* | 86 |
| | *Mucor laxorhizus* var. *ovalispora* | 9 | *Penicillium adametzi* | 27 |
| | *Mortierella humilis* | 6 | *Mortierella isabellina* | 23 |
| | *Absidia cylindrospora* | 5 | *Aspergillus versicolor* | 22 |
| | *Penicillium janczewskii* | 5 | *Oidiodendron griseum* | 20 |
| | *Geotrichum candidum* | 5 | *Trichoderma viride* | 20 |
| | *Penicillium simplicissimum* | 4 | *Torulomyces lagena* | 15 |
| | *Tolypocladium geodes* | 4 | *Penicillium decumbens* | 12 |
| P 70 | *Absidia cylindrospora* | 14 | *Penicillium janczewskii* | 313 |
| | *Penicillium janczewskii* | 14 | *Penicillium adametzi* | 151 |
| | *Mortierella vinaceae* | 13 | *Mortierella vinaceae* | 50 |
| | *Penicillium daleae* | 13 | *Trichoderma viride* | 29 |
| | *Oidiodendron griseum* | 11 | *Penicillium daleae* | 24 |
| | *Penicillium glabrum* | 10 | *Absidia cylindrospora* | 22 |
| | *Mortierella gracilis* | 9 | *Mortierella nana* | 16 |
| | *Tolypocladium geodes* | 8 | *Penicil. roseopurpureum* | 12 |
| | *Penicillium chrzaszczi* | 7 | *Geomyces pannorum* | 8 |
| | *Trichoderma viride* | 6 | *Oidiodendron griseum* | 8 |

N – soil from pine nursery, P17 – soil from 17 year-old pine forestry, P70 – soil from 70 year-old pine forestry

According to method No. 1, the frequency of occurrence of fungal isolates was highest in the nursery soil and was decreasing in the 17 and 70 year-old forestries respectively. According to method No. 2, the soil of the 17 and 70 year-old pine forestries contained more fungal isolates than the nursery soil. Both methods showed that the number of isolates of *Absidia cylindrospora*, *Penicillium adametzi*, *P. janczewskii*, *Trichoderma viride* increased in the soil with the age of the pine trees. *Mortierella isabellina*, *M. vinacea* and *Oidiodendron griseum* occurred most frequently in the soil of the 17 year-old pines. Other species, however, like *Cylindrocarpon destructans* and *Exophiala pisciphila* occurred more often in the soil of pine nursery than in the soils of older pine forestries.

Both methods demonstrated that the most common *Penicillium* species in pine forest soil in western Poland are *P. daleae* and *P. janczewskii*. According to method No. 1 and 2, the population of *P. daleae* seems to decrease in older pine forestries, if one does not take the nursery soil into consideration. *Penicillium janczewskii* occupied the soil of the 70 year-old forestry to the greatest degree. *Mucor laxorhizus* var. *ovalispora* did not grow or sporulate on the media. It was detected only near the soil particles used as inocula in method No. 1. It grew and sporulated only in contact with the soil particles or other fungi. Numerous trials to isolate the fungus failed.

## DISCUSSION

The isolation of soil fungi can be carried out with different methods. The most common are the soil-plate method of W a r c u p (1950) with its modifications and the soil washing technique by G a m s and D o m s c h (1967). However, there are other methods, such as the one described in this paper, eg. method No. 1 (N i r e n b e r g and M e t z l e r, 1990). All of them allow the isolation of soil saprophytes. The method that should be applied by certain soil projects depends on the type of soil and goals to be achieved.

Method No. 1 can only be used with soils that form aggregates and which can be calibrated by a sieve. Method No. 2 can be used on every soil.

With method No. 1, not only saprophytes can be detected but also hyperparasites of other fungi. *Mucor laxorhizus* var. *ovalispora* which could not be isolated, was quite often found. It is thought to be depended with its growth on another fungi. Also, fungi that grow very slowly or are suppressed by anaerobic conditions are recovered more easily by method No. 1. Species of the genus *Acremonium* are included in this category. Therefore a higher number of recorded species is detected by method No. 1.

Since with method No. 2 each viable fungal propagule, including conidia and spores, is recorded as an isolate in 26 $mm^3$ (= 0.03 g) of quartz sand containing 0.0002 g of soil, the output of well sporulating fungi is much bigger than with method No. 1. With the latter method one species is counted only once (as an isolate) per soil particle (= 0.04 g).

There are also differences in the order of occurrence of the fungal species by both methods (Tab. 2). Therefore we cannot say if method No. 1 can also be used for the evaluation of different soils according to the test of M a ń k a (1974). It estimates the antagonistic activity of soil fungi toward each other, i.e. interactions of the 10 or 15 most frequently occurring saprophytes with the most important plant pathogens are evaluated in vitro. The frequency of occurrence of the species is also an important factor in the test.

The results of investigations on the fungal soil flora by method No. 2, which has been used in Poland for many years, contributed to the acceptance of certain statements. One says, that the soil of pine forestries is occupied by a larger number of species and isolates than the nursery soil deprived of the pine litter.

The higher frequency of occurrence of such species as: *Absidia*, *Oidiodendron*, *Penicillium* and *Trichoderma*, in soils covered with pine litter, compared with areas without litter was already noticed by P r z e z b ó r s k i (1988). Trought the genus *Mortierella* is common in all soils of temperate zone, some of its species are only typical of pine forest soil (P r z e z b ó r s k i, 1982). These earlier results are confirmed also by the investigations here presented with method No. 2. With method No. 1, however, the largest number of species and isolates was found in the soil not covered with fallen needles. One could argue in favor of these results that nursery soils are quite different from the sandy soils of the pine forestries: they are richer in humus and nutrition. Since they are irrigated, the soils have a higher water content. Their pH degree value is higher. These circumstances may contribute to a larger number of species and isolates than are recovered with method No. 2.

One argument explains only why more species and isolates are found in the nursery soil than in the two others by method No. 1 and the other explains why fewer are found in the nursery soil by method No. 2. But they fail to explain why the two methods produce contradictory results on the nursery soil. There are two reasons that can be given:

1. Method No. 1 recovers more slow growing fungi (e.g. *Acremonium* species) than method No. 2. These species may colonize especially nursery soil.

2. Method No. 1 recovers less viable conidia than viable fungal mycelia. In contrast to method No. 1 with method No. 2 viable conidia are counted particularly. Since the nursery soil is much more cultivated than forest soil, the fungal growth is quite often intensively disrupted. Consequently the fungi tend to sporulate less.

The riddle might be solved by combining the two above presented points.

The composition of fungal communities in forest soils depends mostly on the presence of available nutrients and root exudates (S c h r o t h, H i l d e b r a n d t, 1964; R o v i r a, 1965) which affect the soil quality. In this case, the forest litter consisted mostly of pine needles. It is a constant and main source of specific organic substances which can favor or eliminate certain microorganisms, i.e. *Fusarium* species which do not occur in the pine forest soil due to the stimulation of chlamydospores and destruction of the hyphae by the organic acids produced in the process of decomposition of needles (M e n z i n g e r et al., 1966). Others, such as

*Trichoderma koningii* which colonize the pine needles (C h w a l i ń s k i, 1969) prefer such environmental conditions. Both methods confirmed these findings.

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## Efektywność dwóch metod izolowania grzybów z gleby

### Streszczenie

Porównano wyniki izolowania grzybów z gleby szkółkowej, spod 17-sto i 70-cio letniego drzewostanu sosnowego, wykonanej dwoma metodami: pierwszą stosowaną w Niemczech, polegającą na wykładaniu

pojedynczych grudek gleby jako inokulum, oraz drugą stosowaną w Polsce będącą zmodyfikowaną metodą Warcupa, a bazującą na rozcieńczeniu próbki glebowej. Metodą pierwszą otrzymano odpowiednio 56, 43, 30 gatunków i 167, 144, 140 izolatów, natomiast metodą drugą 35, 38, 23 gatunków i 194, 908, 657 izolatów. Obydwie metody pozwoliły na stwierdzenie w trzech badanych glebach powtarzającej się obecności 5 gatunków grzybów: *Mortierella vinacea, Penicillium daleae, P. janczewskii, Oidiodendron griseum* i *Trichoderma viride*.