Soil is inhabited by many saprobiontic organisms such as bacteria, fungi, and small invertebrates, which are responsible for biochemical and biogeochemical transformations of nourishing ingredients (mineral and organic) in soil [1]. Of all the saprobionts occurring in the soil, fungi play an essential role, not only in initiating soil processes, but also in maintaining its physical and chemical quality and, together with vegetation, influencing the health and sanitary state of the soil. Saprobiontic fungi, especially moulds existing in ecosystems where imperfect forms can be etiologic factors of human and animal mycoses, have particular ecological and medical importance. Over time, the imperfect forms of the fungi can become infectious for living organisms after being inoculated into the tissues of the host [2]. The dynamic development of moulds can be promoted by global warming and increases of humidity such as the flood in Poland in 2010.

The aim of our study was to evaluate the susceptibility of 17 mould fungi strains isolated from the soil of allotments in Łódź to 6 types of herbicide: Roundup Ultra 170 SL, Mniszek 540 SL, Casoron 6.75 GR, Basta 150 SL, Fusilade Forte 150 EC, and Starane 250 EC. Of these herbicides, only Casoron 6.75 GR is used in agriculture on the root system, whereas all the others are used on the leaf surface. Isolated strains of fungi belonged to 9 species: *Fusarium aquaeductuum, Fusarium oxysporum, Mucor indicus, Penicillium chrysogenum, Penicillium citrinum, Penicillium purpureogenum, Penicillium rugulosum, Penicillium spinulosum*, and *Scedosporium prolificans*. The herbicide Basta 150 SL is the strongest inhibitor of the growth of the examined fungi strains, as well as being the fastest herbicidal agent (2-5 days after use), and can be used to eradicate mould fungi in soil.

**Keywords:** fungi, herbicides, eradication
Table 1. Concentrations of herbicides used.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of herbicide</th>
<th>Concentration [mg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Roundup Ultra 170 SL</td>
<td>17,000 8,500 4,250 2,125 1,062 0,531 - - -</td>
</tr>
<tr>
<td>2</td>
<td>Mniszek 540 SL</td>
<td>54,000 27,000 13,500 6,750 3,375 1,687 - - -</td>
</tr>
<tr>
<td>3</td>
<td>Casoron 6.75 GR</td>
<td>6,750 3,375 1,687 0,844 0,422 0,211 - - -</td>
</tr>
<tr>
<td>4</td>
<td>Basta 150 SL</td>
<td>15,000 7,500 3,750 1,875 0,937 0,469 0,234 0,117 0,059</td>
</tr>
<tr>
<td>5</td>
<td>Fusilade Forte 150 EC</td>
<td>15,000 7,500 3,750 1,875 0,937 0,469 - - -</td>
</tr>
<tr>
<td>6</td>
<td>Starane 250 EC</td>
<td>25,000 12,500 6,250 3,125 1,5625 0,7812 - - -</td>
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</tbody>
</table>

Material and Methods

Seventeen fungal strains isolated from the soil of allotments in Łódź were chosen and used in the research. Isolated strains belonged to 9 species: Fusarium aqueductum, Fusarium oxysporum, Mucor indicus, Penicillium chrysogenum, Penicillium citrinum, Penicillium purpureogenum, Penicillium rugulosum, Penicillium spinulosum, and Scedosporium prolificans. Most commonly isolated were Fusarium aqueductum (4 strains), Penicillium citrinum (4 strains), and Penicillium chrysogenum (3 strains), whereas the species Fusarium oxysporum, Mucor indicus, Penicillium purpureogenum, Penicillium rugulosum, Penicillium spinulosum, and Scedosporium prolificans were represented by the one strain (Table 1) [4, 5]. The susceptibility of the strains was evaluated by agar-gel diffusion.

A 48-hour-old liquid culture on a Sabouraud medium was used in the study. 0.5 ml of this culture was added to the 5 ml of liquid Sabouraud medium. From that dilution, 1 ml of the culture was taken and cultured on a Petri plate (diameter 10 cm) containing 30 ml of 3% concentration of Sabouraud agar at pH=5.6. After 1 hour of incubation at 37°C, rows of 10 cm diameter were cut with a sterile tool. Each row was numbered, and to each was added 0.1 ml of one of the 6 herbicides. The plates were incubated for 48 hours at 37°C. The diameters of the inhibitory area of growth of the fungal strain around each dilution of each of 6 herbicides. The plates were incubated for 48 hours at 37°C. The diameters of the inhibitory area of growth of the fungal strain around each herbicide were measured in mm; an average measurement of two diameters perpendicular to each other was taken.

The minimal inhibitory concentration (MIC) was used as a measure of the antifungal activity of the herbicide, calculated from the curve of straight-line growth drawn in a semi-logarithmic scale according to the mathematical formula [6]:

\[
\log \text{MIC} = \log C_i + \frac{\log C_2 - \log C_i}{N_2 - N_i} \left(1 - \frac{N_i}{N_2}\right)
\]

\(N_i, N_2\) – arithmetical average of the two groups of the diameters of the inhibitory area of growth of the fungal strain

\[\log C_i, \log C_2\] – arithmetical average of logarithms of the two groups of the concentrations of the used herbicide.
Results

The in vitro Minimal Inhibitory Concentrations of 17 strains of the fungi isolated from the soil of allotments in Łódź, were evaluated using different concentrations of herbicides (Table 1). Concentrations of the herbicide Roundup Ultra 170 SL were between 17,000 [mg/ml] and 0.531 [mg/ml]. Concentrations of Mniszek 540 SL were between 54,000 [mg/ml] and 1.687 [mg/ml]. Concentrations of Casoron 6.75 GR were between 6.75 [mg/ml] and 0.211 [mg/ml]. Concentrations of Basta 150 SL were between 15,000 [mg/ml] and 0.059 [mg/ml]. Concentrations of Fusilade Forte 150 EC were between 15,000 [mg/ml] and 0.469 [mg/ml]. Concentrations of Starane 250 EC were between 25,000 [mg/ml] and 0.7812 [mg/ml].

For Roundup Ultra 170 SL, MIC measurements were between the minimal value 114.02 mg/l = 0.114 mg/ml (strain No. 16 Penicillium spinulosum) and the maximum value 2801.56 mg/l = 2.801 mg/ml (strain No. 5 Fusarium oxysporum). For Mniszek 540 SL minimal inhibitory concentration measurements were between the minimal value 603.94 mg/l = 0.604 mg/ml (strain No. 16 Penicillium spinulosum) to the maximum value 8947.5 mg/l = 8.947 mg/ml (strain No. 5 Fusarium oxysporum). For Casoron 6.75 GR MIC measurements were between 393.9mg/l = 0.394 mg/ml (arithmetical average for the species Penicillium chrysogenum) and 2936.3 mg/l = 2.936 mg/ml (strain No. 14 Penicillium purpureogenum). For Basta 150 SL MIC measurements were between 23.51 mg/l = 0.024 mg/ml (strain No. 17 Scedosporium prolificans) and 316.23 mg/l = 0.316 mg/ml (strain No. 6 Mucor indicus). For Fusilade Forte 150 EC MIC measurements were between 4,786 mg/l = 0.0048 mg/ml (strain No. 16 Penicillium spinulosum) and 3101.54 mg/l = 3.102 mg/ml (arithmetical average for the species Fusarium aquaeductuum). For Starane 250 EC MIC measurements were between 207.54 mg/l = 0.2075 mg/ml (strain No. 16 Penicillium spinulosum) and 3148.91 mg/l = 3.149 mg/ml (arithmetical average for the species Penicillium citrinum).

The arithmetical average of the two groups of the diameters of the inhibitory area of growth of the fungal strain varied, resulting from 10.5 to 32.0 mm based on the concentration and type of herbicide. Fungal growth inhibition was most commonly observed in the sixth concentration of a herbicide. The largest areas of inhibition of fungal growth were observed after treatment with Basta 150 SL. By the sixth concentration, 0.469 mg/ml, growth of all the strains was still inhibited.

Discussion

Fungi, being plant parasites, are susceptible to a range of chemical agents used in agriculture, including most herbicides, with some stimulating and others inhibiting their growth.

Hodges compared the influence of a number of herbicides on the growth, development, and germination of D. sorokiniana and D. teres [7], and found that Aminopielik D and 2, 4 – D and dicamba demonstrate the least antifungal activity toward D. sorokiniana in all used concentrations. Additionally, a study by Fabisiewicz and Mikolajksa on two fungal species, D. sorokiniana and D. teres, using 4 herbicides (Chwastox liquid, Chwastox M, Chwastox D, and Aminopielik D in concentrations from 1 to 1000 µg/g) showed that higher concentrations of herbicides inhibit growth of the mycelium as well as germination of spores to a greater degree. The least effective herbicide in inhibiting the growth of D. sorokiniana and D. teres mycelium is Aminopielik D, whereas the most effective is Chwastox D [8].

Our research included the herbicide Mniszek 540 SL, which has a similar chemical structure to 3 used herbicides: Chwastox (M, D and liquid) with the active agent dicamba, mecoprop, and MCPA. Mniszek 540 SL proved to be the least effective with respect to 8 of 9 examined fungal species: Fusarium aquaeductuum, Fusarium oxysporum, Mucor indicus, Penicillium chrysogenum, Penicillium citrinum, Penicillium purpureogenum, Penicillium rugulosus, and Scedosporium prolificans (Table 2). Penicillium spinulosum was the species most susceptible to Mniszek 540 SL.

According to Klimek et al., concentrations up to 25 mM of the herbicide glyphosate (Roundup) stimulate the growth of Penicillium chrysogenum strains on minimal medium (agar). Glyphosate in the medium was the only nitrogen source for strains of Penicillium chrysogenum. The authors suggest that Roundup may enter the fungi cells by simple passive diffusion, or inducible carriers and products of glyphosate breakdown (aminoacids) are used by mycelium cells; this may be confirmed by the fact that amino compounds, such as aminoacids and ammonia, have been isolated from mycelium instead of herbicide [9]. Our research showed Penicillium chrysogenum to be the second most susceptible species of all nine fungal species to Roundup Ultra 170 SL.

Araujo, Monteiro and Abarkeli studied in vitro the effects of glyphosate (Roundup) on microbial activity in two typical Brazilian soils, one with and one without a previous history of glyphosate application. A solution of technical glyphosate (analytical standard) sufficient to give a final glyphosate concentration of 2.16 mg/kg was added to 75.0 g sub-samples of soil. Biodegradation of glyphosate was evaluated by carbon dioxide evolution and soil microbial activity was evaluated by measuring fluorescein diacetate (FDA) hydrolysis over a period of 32 days. After 32 days of incubation, the number of actinomycetes and fungi increased while the number of bacteria showed a slight reduction. In addition, CO2 increased by10-15% and FDA hydrolysis increased by 9-19% in the presence of glyphosate when compared with the same type of soil that had never received glyphosate. An increase of 10-15% in the evolved CO2 suggests that soil microorganisms are able to use glyphosate as a carbon source. The authors observed that glyphosate quickly decomposed within 10 days of incubation [10].
Table 2. Comparison of values of MIC [mg/l] for used herbicides on 17 strains of fungi isolated from soil.

<table>
<thead>
<tr>
<th>No.</th>
<th>Fungi species</th>
<th>No. of strain</th>
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<th>x</th>
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<th>x</th>
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<td>Roundup Ultra 170 SL</td>
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<td>Basta 150 SL</td>
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</table>

**Table 2**: Comparison of values of MIC [mg/l] for used herbicides on 17 strains of fungi isolated from soil.
In our in vitro study, Roundup Ultra 170 SL proved to be the agent most strongly inhibiting the growth of 
Penicillium spinulosum, and to a lesser degree the growth of 
Fusarium oxysporum (Table 1).

Roundup Ultra 170 SL and Basta 150 SL are based on 
aminophosphate. Roundup consists of N-(phospho-
nomethyl)glycine as an isopropylamine salt, and Basta 
consists of ammonium gluphosynate. Despite the fact that 
both agents consist of aminophosphate, Basta 150 SL 
proved to be the more effective fungistatic agent against 8 
of 9 fungal species, as well as being the most effective of all 
6 herbicides.

The herbicide glufosinate-ammonium (GA) [butanoic 
acid, 2-amino-4-(hydroxymethylphosphinyl)-ammonium 
salt] was tested at concentrations from 2 to 2,000 g per ml 
for activity against growth and aflatoxin B1 (AFB) production 
by the mycotoxigenic fungus Aspergillus flavus 
Link:Fr by Tubajika and Damann. The highest concentration 
(2,000 mg per ml) reduced the colony diameter of A. 
flavus strain AF13 by 80%. AFB production was inhibited 
by 90% at this concentration. Reduction in mycelial dry 
weight and AFB1 production in response to GA application 
ranged from 17.2 to 97.1% and from 39.1% to 90.1%, respectively. Results indicate that GA has an inhibitory 
effect on growth and AFB1 production by Aspergillus 

El-Hissy and Abdel-Kader tested the effect of three 
doses of 5 pesticides: Ceresan and Orthocid (fungicides), 
VCS-438 (herbicide), Durban and Dipterex (insecticides) 
on the mycelial dry weight of Aspergillus fumigatus, 
Fusarium moniliforme, Penicillium italicum, and 
Sclerotium cepivorum after 2, 4, 6, 8, and 10 days of treat-
ment. Four pesticides (Ceresan, Orthocid, Durban, and 
Dipterex) induced inhibition of mycelial growth; low doses 
of VCS-438 were stimulatory to Fusarium moniliforme 
after 4 and 6 days, and to Penicillium italicum after 4 days. 
According to the authors, the rate of inhibition was influ-
enced by the type of fungus, the age of the mycelium, and 
the dose of the pesticide [12].

El-Said, Abdel-Hafez, and Saleem examined the effect 
of using two herbicides, Herbizid and Touchdown, on the 
mycelial growth and production of extracellular enzymes 
by fungal strains isolated from Egyptian soil. The authors 
treated in vitro fungal strains, Fusarium oxysporum, Mucor 
hiemalis, and Penicillium chrysogenum, with different con-
centrations of herbicides Herbizid and Touchdown at con-
centrations from 0.019 to 0.152 mg/kg soil. Lower doses of 
herbicides occasionally promoted extracellular enzyme 
production and mycelial growth of some fungi. The incor-
poration of 50 ppm of Herbizid and Touchdown signific-
antly activated amylase production by tested fungal 
strains. Treating the fungal strains Fusarium oxysporum, 
Mucor hiemalis, and Penicillium chrysogenum with 
“Herbizid” in concentrations of 50, 100, and 200 ppm 
increased Cx-cellulase production only in Penicillium 
chrysogenum. Lipase and protease production was always 
lower in the treated cultures than in the controls [13].

Gigliotti and Allievi evaluated and compared the toxic-
ity of Bensulfuron and Cinosulfuron on soil microbes at the 
normal field application concentration and 100 times high-
er. In both cases, sulfonylureas had no effect on the total 
number of bacteria and nitrifiers, or on the respiration 
activity in the soil, but they did decrease nitrification activity. In vitro 
toxicity tests carried out on representative soil micro-
bial strains using Bensulfuron at 50 mg/l showed some 
inhibition in 3 of the 17 bacterial strains and strong inhibi-
tion of almost all the 12 fungal strains. Cinosulfuron was 
found to have no effect on any of these strains in a previous 
study. Bensulfuron is potentially more toxic on soil het-
erotrophic microorganisms, but only at very high concen-
trations that are nearly impossible to reach though the usual 
aricultural applications [14].

Larson et al. tested the effect of glyphosate application 
on disease severity with certain isolates of Fusarium and 
Rhizoctonia in glyphosate-resistant sugar beet. Glyphosate 
at a concentration of 400 µg·mL⁻¹ inhibited the growth of 
tested strains, but only by 17% and 11%. This indicates that 
F. oxysporum tolerates glyphosate [15].

Korzycko-Lupicka and Sudol investigated the survival of 
autochthonous fungi in soil treated with 1 mM aqueous 
solution of glyphosate. It was shown that Fusarium strains 
have a high tolerance against the applied doses of 
glyphosate (0.5-2.0 mM). Glyphosate is used as a sole 
source of phosphorus by tested fungi and, at concentrations 
1.0-1.5 mM significantly increases the dry mass of tested 
fungi [16].

Vinther et al. examined the differences in microbial 
activity and soil characteristics due to the mineralization 
and sorption of two pesticides, glyphosate and metribuzin, 
in samples of sandy soil from Denmark. They focused on 
the clay and organic carbon content as well as soil respira-
tory and enzymatic processes and microbial biomass. The 
results indicated that the spatial variation of the soil param-
eters, particularly the content of organic C, had a major 
influence on the variability of the microbial parameters and 
and on sorption and pesticide mineralization in the soil. For 
glyphosate, with a co-metabolic pathway for degradation, 
the mineralization was increased in soils with high micro-
bial activity [17].

Evangelista, Cooper, and Yargeau examined the influ-
ence of the structure and presence of another source of car-on on the degradation of chlorophenoxyacids, such as 
mecoprop by microorganisms (including Aspergillus 
niger). Mecoprop was easily decomposed by microorgan-
isms and, similar to our observations, the authors conclud-
ed that it doesn’t inhibit the fungi growth [18].

Due to our research and analyzed literature of different 
authors, the issue of destroying soil fungi potentially path-
genic for humans is still current and worldwide. It is worth 
continuing research and seeking new herbicides whose low 
concentrations can have fungistatic potential and can be 
useful in agriculture.

Conclusions

1. Basta 150 SL proved to be the most effective of 5 her-
bicides used in agriculture applied to leaf surface
(Roundup Ultra 170 SL, Mniszek 540 SL, Basta 150 SL, Fusilade Forte 150 EC, Starane 250 EC) in inhibiting the growth of examined strains of mould fungi that are potentially pathogenic for humans.

2. The strongest inhibiting effect of Basta 150 SL, in contrast to the other herbicides used on the leaf surface, is connected with the presence of ammonium gluphosynate, which inhibits the synthesis of aminoacids such as glutamine.

3. Basta 150 SL is most effective in inhibiting the growth of examined strains of mould fungi as well as being the quickest-acting herbicide (2-5 days from applying); it is the only herbicide of the five tested that can be used to eradicate soil fungi.

4. Casoron 6.75 GR proved to be the weakest inhibitor of growth of the fungi mycelium, even though it was the only herbicide used on a plant’s root system.

5. *Penicillium spinulosum* proved to be the most susceptible fungi species to 4 of 6 examined herbicides, with the lowest MIC values after using Roundup Ultra 170 SL, Mniszek 540 SL, Fusilade Forte 150 EC, and Starane 250 EC.

References

3. BURZAWA A., SZWEDEK A. Saprobiontic fungi potentially pathogenic for human, isolated from soil of recreation area of Łódź. XLIII Polish and I International Conference of Students Scientific Association and Young Doctors, Annales Universitatis Medicae Lodzensis, Łódź, 46, 66, 2005.