Effect of Copper Sulfate on the Soil Fungal Community Structure

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Studies of fungi communities in soil were carried out in laboratory model experiments set on slightly podzolized garden soil. Copper sulfate was introduced into soil at a rate of about 65 mg Cu/kg, which represented a mean dose in garden soils in Lithuania. Control – Soil without copper sulfate addition was used as a control. The total number of 378 colonies of fungi was isolated from the soil samples. The isolates represented 29 species of 15 genera. Control soil was characterized by different composition of potentially phytopathogenic fungi from the genera *Athelia, Fusarium, Pythium, Rhizoctonia* and *Verticillium*. Fungal community structure varied in both soils and depended on exposure time. After 2, 4 and 6 months fungi of potentially phytopathogenic genera were predominant in control soil. Species of *Trichoderma* genus constituted only 5.3–7.2%, while almost 54.6% of isolated fungi were species of potential pathogens. Introduction of copper ions into soil stimulated activity of fungi from genera of *Acremonium, Cylindrocarpon* and *Nigrospora*. They may be pathogenic under favorable conditions. Strong soil antagonists to plant pathogens – strains from the genus *Gliocladium* – were more active in soil with copper sulfate added.

Key words: soil pollution, microorganisms, heavy metals

INTRODUCTION

Plant protection against diseases by biological and chemical methods influences the activity of both pathogens and saprophytic microorganisms. Fungal succession in soil occurs in response to nutrition potential, pollution and microbial competition [14]. Copper-containing compounds have a long history of successful use as agricultural fungicides and are now being recognized as very hazardous pollutants. Copper salts are widely used to control bacterial and fungal diseases in agriculture [13]. Basic cupric calcium sulfate (Bordeaux mixture) was introduced a century ago as an antifungal agent in crop spraying. Above micronutrient levels, the fungicidal nature of copper compounds is commonly assumed, but certain fungi have the ability to withstand and grow under concentrations that are toxic to most other organisms [6, 22]. Agrochemical agents change the soil ecological balance either by directly affecting non-target soil organisms or by changing the soil physico-chemical characteristics, which in turn dictates the composition of the soil biota.

In our earlier study [18] we found that biotic activity between *Trichoderma harzianum* Rifai and thirteen test fungi was dependent on copper sulfate or copper oxychloride concentration in Czapek's agar. A considerable negative effect of *Fusarium oxysporum* Schleckt. on the growth of *Trichoderma harzianum* was detected on the media containing copper sulfate at a concentration of 0.0015 M. Copper sulfate at a concentration of 0.001 M stimulated the growth of *Botrytis cinerea* Pers. ex Fr Copper compounds changed a relationship between all studied fungi in our earlier study.

Tests carried out under laboratory conditions on rich media provoke fast unnatural growth of microorganisms and show no real interaction between them. Therefore, the objective of the study was to evaluate the effect of copper sulfate on soil fungal community. Changes of interrelation between saprophytic and potentially phytopathogenic fungi in soil with high copper sulfate concentrations were studied.

MATERIALS AND METHODS

Studies were carried out in laboratory model experiments set on sandy podzolic soil.

Sandy garden soil which during 18 years had not been treated with any pests was studied.

From earlier investigations it followed that those soils were characterized by a different composition

of potentially phytopathogenic fungi [10, 11]. Garden soil samples were averaged and then screened with a 2 mm-mesh sieve. Copper sulfate was introduced into the soil in selected amounts of about 65 Cu^{2+} mg/kg, which represented a mean dose in garden soil where copper compounds were used for plant disease protection. The initial content of copper in soil samples was from 5.6 to 6.9 mg/kg. After mixing the soil with copper sulfate and moisturing it up to 65% of total water capacity, samples were placed in glass vessels (3 kg soil in each). Incubation was carried out at 18 °C for 6 months, mixing the soil from time to time.

Viable counts of soil fungi were determined by placing 0.1 ml of 10-fold dilutions onto Malt extract agar. The colonies were counted and the identification was done by macro- and micromorphological observations in microcultures after subculture on Czapek's, Sabouraud or potato dextrose agar, according to the keys: Domsch et al., (1980) [6], Gilman (1945) [7], Nelson et al., (1983) [16], Raper and Thom (1968) [20], Rifai (1969) [21].

Soil pH and the species composition of fungi were estimated after 0, 2, 4 and 6 months of soil exposure. Identification of isolates was done and on plates commonly used diagnostic medium. 50 lupine (*Lupinus luteus* L.) seeds per container were sown into control soil and soil with copper sulfate addition. Seed gerimination, root length and healthiness in tread soils were detected. This procedure was repeated at the beginning of study and 2, 4 and 6 months after addiing Cu ions into soil.

RESULTS AND DISCUSSION

Fungal complexes in various soils are not accidental groups of separate microorganisms, but are formed on the basis of the interaction of species under certain soil conditions [2, 8, 15, 17, 24]. Development of these complexes depends directly on soil peculiarities and on soil pollution. Microbial biomass in soils contaminated with metals are less efficient in the utilization of the substrates for biomass synthesis. High metal concentrations in soil can slow down fungal destructive capacity and the ability to penetrate into plants and cause their diseases.

Mycological analysis of soil pointed a different reaction of a soil mycoflora to introduction of copper sulfate commonly used in Lithuania. Copper sulfate did not cause significant quantitative changes of mycoflora in soil. The total count of fungal population was from 2.5×10^5 to 3.4×10^5 c.f.u. g^{-1} d. m. of soil. Soil fungi communities from two soil samples did not differ considerably in their qualitative structure, and isolates were numerous in both of them.

Species variety of fungi was found under study. The total number of 378 colonies of fungi was isolated from the soil. The isolates represented 29 species of 15 genera. Fungi of the genus *Penicillium* Link ex Fr. were most numerous (12.9%), while almost 8.4% of isolated fungi belonged to species of the genus *Trichoderma* Pers. ex Gray. Fungi of the genus *Fusarium* Link. ex Fr. constituted 9.2%. Fungi of the genera *Athelia* Pers., *Sclerotinia* Fuckel and *Verticillium* Nees ex Link were isolated from both soils. Introduction of copper sulfate into soil induced selection within micromycete communities. A mutual action of microorganisms under natural conditions depends on many factors both of biotic and abiotic origin. Copper compounds are among them. Mycoflora selection due to copper sulfate was mainly based on a decrease of potentially phytopathogenic population with an increase of occurrence frequency of potential antagonists and saprophytes that can become pathogenic (Fig. 1).

The fungi were divided into five groups: I – species of the genus *Trichoderma*; II – potential phytopathogens (fungi of the genera *Fusarium, Pythium, Rhizoctonia, Sclerotinia, Verticillium*); II – saprophytes that can be pathogenic under favorable conditions (fungi of *Acremonium* Link ex Fr., *Cylindrocarpon* Wollenw., *Nigrospora* Zimmerm., *Trichotecium* genera); IV – species of the genus *Gliocladium* Corda (potential antagonists); V – fungi of the genera *Aspergillus* Mich. ex Fr., *Penicillium, Mortierella* Coemans, *Mucor* Mich. ex St.-Am., *Zygorrhinchus* Vuill. and other fungi.

No antagonistic relations between fungal species from control soil grown on malt agar were observed. *Trichoderma* constituted only 5.3–7.2%, while almost 40% of isolated fungi were species of potential phytopathogens. Their amount in isolated community increased with time. Constant temperature and humidity improved the activity of potential pathogens. The activity of the fungal community from soil with copper sulfate changed during exposure. Potential pathogens lost their activity – their percentage decreased from 26.4% at the beginning of this study to 14.8% after 6 months. The percentage of saprophytes (group III) significantly increased (from 5.2% in the first control to 10.2, 18.9 and 22.4% after 2, 4 and 6 months, respectively). Increase of potential pathogen population observed in soil supplemented with copper sulfate was caused by *Gliocladium* Corda and *Trichoderma* species. The percentage of *Trichoderma* species in soil with copper sulfate was 2–5 times higher than in control soil.

Decrease of occurrence frequency of *Trichoderma* strains and increase of potential phytopathogens influenced lupine seed germination in the study soils

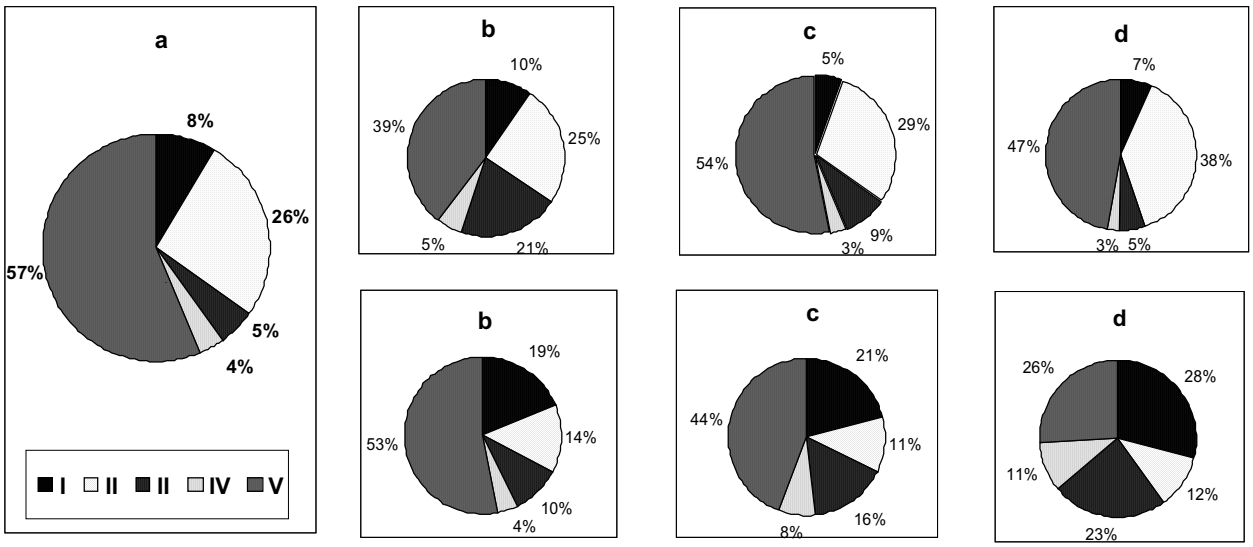

Fig 1. Percentage of fungus genera in soil samples: *a* – soil at the beginning of study (control), b – after 2, c – after 4 and d – after 6 months' exposure under laboratory conditions. Soil without copper sulfate addition at the top and soil with copper sulfate addition at the bottom

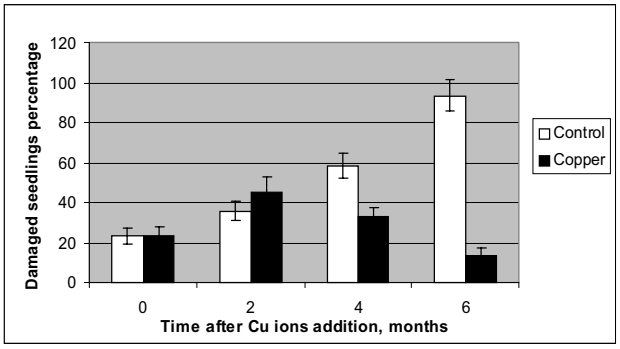

Fig. 2. Percentage of damaged and not germinated lupine seeds in garden soil with (Copper) and without (Control) copper sulfate addition.
Data are average % ± SEM of four replicates of each variant, 50 individuals per replicate

(Fig. 2). Analysis of seed germination and sprouts quality showed that at the beginning of the study 22.2% of seeds were damaged in both soil samples. About 43.5% of lupine seeds were damaged in soil with copper ions and 37.6% in the control soil after 2 months of exposure. The percentage of diseased seeds in the control increased with soil exposure time and reached 100% after 6 months. Potential pathogenic species were more frequent in the control soil (Fig. 1). *Fusarium oxysporum, F. culmorum* (Wm. G. Sm.) Sacc., *Athelia rolfsi* Pers., *Rhizoctonia solani* J. G. Kuhn and *Verticilium albo-atrum* Reinke et Berthold were isolated from seeds and sprouts grown in the control soil. The percentage of damaged seeds in soil with copper sulfate after a 6-month exposure decreased to 13.2% (Fig. 2). *Trichoderma viride* was the predominant fungus in those soil samples. It constituted about 24.5% of the total count of fungi. Fungi of the genera *Calonectria* and *Nigrospora* were noticed on damaged seed sprouts in soil after 4 months of exposure. Fungi from the genera *Acremonium* and *Cylindrocarpon* were common on diseased seeds and sprouts in soil after 6 months of exposure. Fungi from that genus can be phytopathogenic under favorable conditions.

Results obtained in our study showed that *Trichoderma* (especially *T. viride* and *T. harzianum*) were more active antagonists against phytopathogenes and influenced growth of saprophytes. The population of potential antagonists in both types of soil included mainly *Gliocladium* species such as *G. catenulatum* Gilm. and *G. roseum* Bain., and *Penicillium* spp. (most often *P. frequantans* Westling and *P. brevicompactum* Dierckx). Most of these fungi are able to destroy the remnants of plants, tissues of roots and can cause rot.

Results of numerous tests have proved that metals have a diversified influence on microorganisms. Their effect depends on the kind of microorganisms, metal concentration and time of exposure. A change in biological activity may be connected with a possible decrease in microflora enzymatic activity due to heavy metals and lowering the level of biochemical reaction by metals [3]. A study of Jaworska and Dlueniewska [9] showed that lead ions stimulated effectively *T. viride* towards *Rhizoctonia solani* and *Botrytis cinerea*. Our earlier studies [18] where copper ions caused changes in an individual biotic effect of *T. viride* and *T. harzianum* have revealed that the biological activity of the genus *Trichoderma* can change under the influence of ions of this metal.

Both abiotic and biotic factors of soil environment may influence the antagonistic effect of other organisms on pathogenic fungi. Results of investigations reveal that microorganisms are sensitive to heavy metals in the environment. Metal ions may influence the growth rate, mass, sporulation and their enzymatic activities of produced fungal mycelium [5, 9]. *Trichoderma* is well involved in the natural cycles in soil ecosystem and has been proved to be useful in fungal disease control of cultivated plants [1, 23]. *Trichoderma* actively prevents plants from disease attacks, increases and stabilizes soil fertility and stimulates plant growth. The positive effects on health status and crop growth are demonstrated on practical application level, in field crops, in growing media and in reactivating biological activity in soil after steam treatment [1, 9, 12, 19, 23].

The results of the present experiments show that increased copper concentrations in soils change the fungal community structure and the level of soil phytosanitary.

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D. Pečiulytė

VARIO SULFATO PRIEDŲ ĮTAKA DIRVOŽEMIO MIKROMICETŲ KOMPLEKSŲ STRUKTŪRAI

S a n t r a u k a

Mikromicetų komplekso sudėties kitimą dirvožemyje nustatėme laboratorinėmis sąlygomis. Tyrime lengvos granuliometrinės sudėties dirvožemį. Į dirvožemio mėginius įterpėme 65 mg/kg vario (vario sulfato druskos pavidalo). Šis kiekis atitinka natūralią vidutinę vario koncentraciją sodų dirvožemiuose Lietuvoje. Lygiagrečiai tyrėme dirvožemio mėginius be vario priedų (kontrolė). Iš dirvožemio išskyrėme 378 mikromicetų štamus, kurie buvo priskirti 29 rūšims iš 15 genčių. Dirvožemio mėginiai 6 mėnesius buvo laikomi pastovios drėgmės (65%) ir temperatūros (18°C) sąlygomis. Dirvožemio mikromicetų kiekis (gyvybingų pradų skaičius lygus 2,8 x 10^5/g dirvožemio) per 6 tyrimo mėnesius išliko beveik nepakitęs, tačiau keitėsi rūšių populiacijų tankio santykis ir mikromicetų komplekso sudėtis. Kontroliniame dirvožemyje buvo gausu fitopatogeninių mikromicetų (*Athelia, Fusarium, Pythium* ir *Verticillium* genčių rūšių), kurių pradų kiekis didėjo ir po 6 mėnesių sudarė apie 54,6% bendro mikromicetų kiekio. *Trichederma* genties mikromicetų kiekis sumažėjo ir sudarė tik 5,3–7,2% bendro mikromicetų skaičiaus. Vario sulfato priedai dirvožemyje veikė visas jame esančias mikromicetų rūšis – skatino *Trichoderma* genties grybų aktyvumą, slopino fitopatogeninių mikromicetų gyvybingumą. Pasikeitė populiacijų tankis ir aktyvumas tų mikromicetų, kurie palankiomis jiems sąlygomis gali sukelti augalų ligas (*Acremonium, Cylindrocarpon* ir *Nigrospora* genčių rūšys). Stipriai veikiantys augalų ligų sukėlėjus antagonistai, *Gliocladium* genties mikromicetai, buvo aktyvesni už kitus mikromicetus, išskyrus juos iš dirvožemio su vario sulfato priedu.