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Joanna DŁUŻNIEWSKA[1] and Ryszard MAZUREK[2]

EFFECT OF EXTRACTS OF SOILS FROM VARIOUS DISTANCES FROM BLACK LOCUST (*Robinia pseudoaccacia*) SHELTERBELTS ON *Trichoderma* FUNGI

WPŁYW WYCIĄGÓW Z GLEB Z RÓŻNEJ ODLEGŁOŚCI OD ZADRZEWIEŃ ROBINII AKACJOWEJ (*Robinia pseudoacacia*) NA GRZYBY Z RODZAJU *Trichoderma*

Abstract: The aim of the paper was determining the effect of extracts of soils from various distances from black locust shelterbelts on growth, spore germination and antagonism of selected *Trichoderma* spp. isolates. The soil was characterized by the highest organic carbon content in the 0–2 m zone, whereas total nitrogen content was decreasing with the distance from the shelterbelts. The extract of soil from the zone situated 0–2 m from black locust significantly decreased growth rate and stimulated formation of sporogenous hypha. Extracts of soils originating from various distances from black locust shelterbelts caused changes of *Trichoderma* spp. activity towards *B. cinerea*.

Keywords: *Trichoderma* spp., black locust

Fungi of the *Trichoderma* genus play an important role in agricultural environment, because they commonly settle the soil and act antagonistically towards numerous pathogens causing plant diseases. Common occurrence of these fungi is connected with their easy utilization of carbon and nitrogen from various available sources. Demand for carbon and energy is covered by mono- and polysaccharides but also by purines, pyrimidines, fatty acids and even by methanol and methylamine. The sources of nitrogen are ammonia, amino acids, urea, nitrates(V) and nitrates(III). Differences in the species composition of *Trichoderma* spp. in soil and their antagonistic effect in the first place depend on the soil physico-chemical properties. The most important factors comprise: the temperature, humidity, nutrient content, soil pH and presence of other

[1] Department of Agricultural Environment Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 00, email: rrdluzni@cyf-kr.edu.pl

[2] Department of Soil Science and Soil Protection, University of Agriculture in Krakow, al. A. Mickiewicza 21, 31–120 Kraków, Poland, phone: +48 12 662 44 00, email: rrmazure@cyf-kr.edu.pl

microorganisms. Like a majority of soil microorganisms, *Trichoderma* fungi are sensitive to changes of their living conditions and provide early evidence of even slight changes in soil long before the changes of chemical composition and soil physical properties actually take place [1–4].

One of plants frequently occurring in the shelterbelts is black locust. The plant affects an increase in nitrogen content in soil and its shelterbelts and plays a humus forming role. Moreover, the roots of black locust release substances with allelopathic effect. This plant's impact on the soil environment is visible primarily in the arable field zones and it decreases with increasing distance from them [5, 6].

The paper aimed to determine the effect of soil extracts from soils originating from various distances from black locust shelterbelts on the growth and germination of spores and on the antagonism of selected *Trichoderma* spp. isolates.

Material and methods

The following species of antagonistic fungi were selected for the research: *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai, *Trichoderma viride* Pers. ex Gray and pathogenic: *Botrytis cinerea* Pers. and *Rhizoctonia solani* Kühn.

The soil material was collected from the arable field in Krolewice village situated on the Proszowicki Plateau. The arable field adjoined the black locust shelterbelt, 8–10 m high and about 30 years old. The soil samples from which the microorganisms were isolated were collected from the zones at the distance of : I – 0–2 m from the shelterbelt, II – 4–6 m and III – 10–12 m. The soil material was classified as typical brown soil developed from loess and revealed granulation of clay silt. Surface soil samples collected in Krolewice revealed the highest content of organic carbon (19.88 g $\cdot$ kg^{-1}) in the 0–2 m zone and the lowest (13.14 g $\cdot$ kg^{-1}) in the 10–12 m zone (Table 1). Total nitrogen content in the soils was decreasing with the distance from the shelterbelts (from 1.70 g $\cdot$ kg^{-1} in 0–2 m zone to 1.66 g $\cdot$ kg^{-1} in 10–12 m zone). Acid reaction was assessed in all soil samples, but the highest pH values were registered in the zone neighboring black locust trees (pH 5.75).

Table 1

Selected properties of soils sampled from particular zones

Distance for black locust [m] – zones	$C_{org.}$ [g/kg]	N_{total} [g/kg]	C/N	pH_{KCl}	pH_{H_2O}
0–2	19.88	1.70	11.71	4.87	5.75
4–6	16.87	1.69	9.99	4.72	5.67
10–12	13.14	1.66	7.93	4.63	5.64

Soils extracts were prepared by pouring 90 cm^3 of sterile distilled water over 10 g of soil. The solution was shaken for 24 hours, then filtered through filter paper and passed through bacteriological filters. The extracts prepared in this way were added to PDA medium in the amount allowing to obtain 20-fold dilution. The effect of the extracts on *Trichoderma* fungi growth rate was determined by inoculation of selected *Trichoderma* ssp. inoculum on glucose-potato medium (PDA) with added soil extracts. Incubation was conducted until the moment when mycelium overgrew the whole Petri dish in the control combination (PDA medium without the soil extract supplement). The growth rate index was computed on the basis of results obtained from daily measurements of the colonies' diameters [7]. *Trichoderma* spore germination ability in the soil extract solutions was also assessed using the method presented in this paper. Spore collected from the two-week-old *Trichoderma* spp. cultures were placed in the soil extracts. After 48 hours of incubation germinating process was stopped by adding a drop of formalin. Subsequently the process of spore germination was assessed on the scale and the spore germination index was computed on the basis of the obtained results [7].

The experiment was conducted in 4 replications and the results were elaborated using ANOVA for two-factor experiments (factor A – soil extraction, factor B – *Trichoderma* fungus). The significance was verified by the Duncan test.

The effect of soil extracts on the interrelations between antagonistic fungi treated with the tested factors and *Botrytis cinerea* and *Rhizoctonia solani* was assessed with the biotic series method after Manka [8]. *Trichoderma* fungus inoculum was inoculated in the center of a Petri dish, on PDA medium with added soil extracts and 2 cm away from a pathogenic fungus (4 replications). In the control combinations the medium did not contain soil extracts. After 10 days of incubation each combination was assessed on a scale considering three parameters: the degree to which one fungus colony was surrounded by the other, the inhibition zone and the diminishing of a colony. The highest assessment on the 8 point scale denoted a total lack of growth of one fungus. If the *Trichoderma* spp. fungus was dominating, it was assigned "+" sign (positive effect), when the pathogenic fungus was dominating, "–" (negative result) sign was used. If no prevalence of any colony was visible, the "0" mark was assigned. The obtained marks jointly gave the individual biotic effect (IBE), illustrating the effect of *Trichoderma* spp. on the pathogen growth.

Results and discussion

The obtained results point to existing differences in the tested *Trichoderma* spp. isolates' influence on organic matter content in soil. The most sensitive isolate was *T. harzianum.*

The tested soil extracts did not affect the increase in *T. pseudokoningii* or *T. viride* fungi colony (Fig. 1). On the other hand it was noticed that the extract of soil from the zone situated 0–2 m from black locust markedly decreased the growth rate of *T. harzianum*.

It was also noted that the studied extracts caused a decline in the spore germination index of *T. pseudokoningii* and *T. viride* isolates (Fig. 2). On the other hand the soil extract from the 0–2 m zone from the shelterbelt stimulated the process of sporogenous hypha formation in *T. harzianum* isolate.

Extracts from soils situated at various distances from black locust did not diminish *Trichoderma* spp. antagonism towards *R. solani* (Table 2). On the other hand, these extracts caused changes in *Trichoderma* spp. activity towards *B. cinerea*. It was noticed

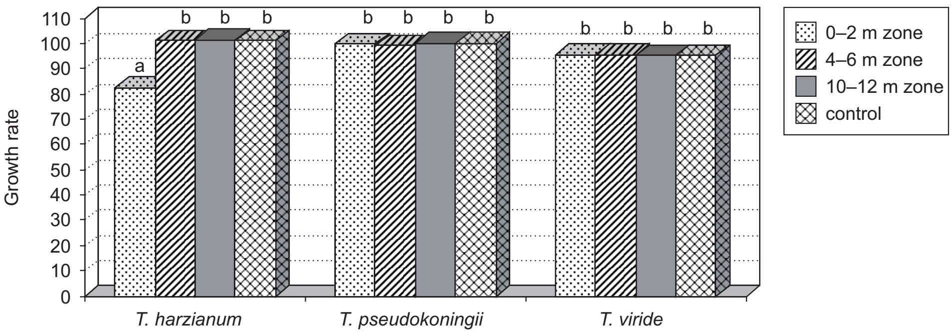

Fig. 1. Growth rate of *Trichoderma* fungi on medium with addend soil extracts
* Columns marked with the same letters do not differ significantly acc. to Duncan test (p = 0.05)

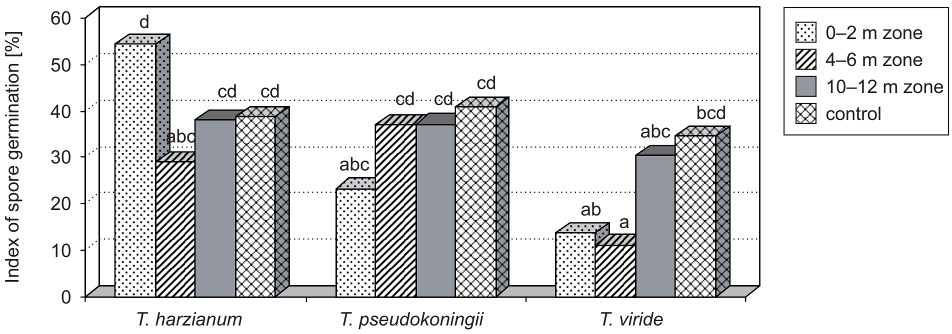

Fig. 2. *Trichoderma* fungi spore germination in soil extracts
* Columns marked with the same letters do not differ significantly acc. to Duncan test (p = 0.05)

that the extract from the soil situated at the distance of 10–12 m from black locust caused a decrease in IBE to +3. However, the activity of *T. pseudokoningii* and *T. viride* isolates was increasing under the influence of soil extracts from the 0–2 m zone from black locust.

Table 2

The impact of soil extracts on individual biotic effect of *Trichoderma* fungi on pathogens

Extract from soils distanced from black locust shelterbelts	*T. harzianum*		*T. pseudokoningii*		*T. viride*	
	Botrytis cinerea	*Rhizoctonia solani*	*Botrytis cinerea*	*Rhizoctonia solani*	*Botrytis cinerea*	*Rhizoctonia solani*
0–2 m zone	+6	+7	+7	+8	+7	+6
4–6 m zone	+5	+8	+5	+8	+2	+8
10–12 m zone	+3	+8	+6	+8	+2	+8
Control without extract	+6	+8	+5	+8	+1	+6

The results obtained in this work confirm numerous reports that microorganism activity depends on many physical and chemical properties of soil, including also mechanical composition, pH, contents of nitrogen and organic matter. Reaction close to acid creates better conditions for *Trichoderma* fungi development [1–4, 9, 10]. Life and activities of soil microflora are strictly connected with plant life. Microorganisms are especially influenced by the rhizosphere, where root secretions of individual plant species modify soil microorganism communities and pathogen antagonist relationship. Black locust roots secrete allelopathic substances, such as robinetin, myricetin and quercitin [6].

Black locust shelterbelts affect also nutrient content in soil. In the zone most distanced from the trees the amount of organic carbon and total nitrogen content were decreasing. The greatest amount of leaves, branches and pods find their way to the area 10 m distant from the shelterbelts. Fallen black locust leaves revealing a low C:N ratio are fast decomposed providing an additional source of nitrogen in soil [5]. It should be added that black locust lives in symbiosis with *Bacilus radiola* nodule bacteria and *Rhizobium* bacteria, therefore it markedly affects an increase in nitrogen concentrations in the substratum. Nitrogen is transformed into humus substances with the highest degree of humification [5, 11]. At growing nitrogen concentrations in substratum a reaction of forming conidia and chlamydospores was observed [9].

Higher content of plant residue in the soil surface creates better conditions for *Trichoderma* development. These fungi are characterized by a great ability of activating and absorbing nutrients from soil in comparison with other microorganisms. The efficient utilization of available nutrients is the basic ability of *Trichoderma* spp. for ATP acquisition from the metabolism of various carbohydrates originating from polymers widely dispersed in the soil environment, such as cellulose, glucan and chitine [10]. Increased soil abundance in carbon causes that the C:N ratio is more advantageous for the development of fungi revealing antagonism towards pathogens including *Trichoderma* fungi. A low C:N ratio causes the disappearance of the competitive effect between fungi responsible for many diseases and organisms revealing considerable fungistatic activity at a high C:N ratio [12, 13].

Plant cover and soil environment are factors which may modify development and antagonistic activity of *Trichoderma* spp. Therefore, the application of these fungi in agricultural practice for plant protection against pathogens meets obstacles.

Conclusions

Black locust (*Robinia pseudoacacia*) caused changes in carbon and nitrogen content in soil and affected mycelium growth, spore germination and antagonism of *Trichoderma harzianum*, *T. pseudokoningii* and *T. viride*.

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[1] Katedra Ochrony Środowiska Rolniczego,
[2] Katedra Gleboznawstwa i Ochrony Gleb
Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie

Abstrakt: Celem pracy było określenie wpływu wyciągów z gleb pochodzących z różnych odległości od zadrzewień robinii akacjowej na wzrost, kiełkowanie zarodników i antagonizm wybranych izolatów *Trichoderma* spp. Gleba charakteryzowała się największą zawartością węgla organicznego w strefie 0–2 m, a wraz z odległością od zadrzewień malała zawartość azotu ogólnego w glebie. U izolatu *T. harzianum* wyciąg z gleby ze strefy położonej 0–2 m od robinii znacznie obniżał tempo wzrostu i stymulował proces tworzenia strzępek kiełkowych. Wyciągi z gleb pochodzących z różnych odległości od zadrzewień robinii akacjowej powodowały zmiany aktywności *Trichoderma* spp. w stosunku do *B. cinerea.*

Słowa kluczowe: *Trichoderma* spp., robinia akacjowa