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RESEARCH ARTICLE

STUDIES OF FUNGAL ISOLATES FROM SUGAR BEET RHIZOSPHERE

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ABSTRACT

In laboratory and greenhouse conditions in Episkop Konstantin Preslavski University of Shumen, during the period 2013-2014 studies were performed of fungal isolates from rhizosphere of "slaughtered" plants of sugar beet. The ability of a variety of sustaining media for the cultivation of the isolates and the influence of factors soil herbicide (Dual) and humidity of the environment for the growth and aggressiveness of the different isolates was studied. It was found that *Alternaria* ssp. Grows well on organic environment; corn agar can be referred to as a selective medium for *Pythium* ssp.; more aggressive cause of slaughtering is *Pythium* ssp., and weaker - *Alternaria* ssp.; their aggressiveness is maximized in the presence of the entire complex tested isolates; at 70% and 90% or soil humidity the herbicide dal in a dosage of 4500 ml/ha suppresses the aggressiveness of *Pythium* ssp. and stimulates that of *Alternaria* ssp.

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INTRODUCTION

In a significant number of agricultural crops a complex of soil fungi causing slaughtering, is established (Nipoti *et al.*, 1985; Stanychelini *et al.*, 1982). As aggressive cause of this complex are determined soil fungi of the genus *Pythium* (Dochenco, 1985; Pojayr, 1979; Pojayr *et al.*, 1986). Some methods for isolation of these fungi and nutrients for their best cultivation under laboratory conditions were developed (Ilieva, 1982). For the same species pathogenic pathogens in the early stage of development of sugar beet fungi the suppressive effect on growth and stimulating effect on pathogenity on the importation of some soil herbicides is established and then under certain parameters of soil moisture (Rudakov and Spiridonov, 1978; Tanova and Raykov, 1995; Schnaider and Dijst, 1992). Such studies are known and for the soil fungus, the cause of „slaughtering”, root rot and root decay during the vegetation and storage the sugar beet - *Rhizoctonia solani* Kuhn (Kowalik, 1984, Sullivan *et al.*, 1994, Tanova, 2002). Diseases that affect the root system and it's vary – root crop in sugar beets are from the ecologic-microbial type. They are regarded as a result combined of several interacting factors creating conditions for the aggressiveness of the complex of soil-dwelling microorganisms that cause relevant pathological syndromes (Naidenov *et al.*, 2001).

In this complex etiology of diseases of beet root system, most commonly from diseased plants are isolated the soil-dwelling species: *Pythium ultimum*, *Aphanomyces cochlioides* (Lysle, 1986 Olaya, 1994), *Rhizoctonia solani* Kuhn, *Sclerotium bataticola* (Toporovskaya, 1985; Garresus, 1995; Srivastava, 1996), *Phoma betae*, *Fusarium* ssp. (Wanghn *et al.*, 1993; Varbanov, 1987). There is also reported of increased aggressiveness of seed portable species of the genera *Alternaria* and *Mucor* primarily as cause of "slaughtering" to phase "crossing" of the beet (Varbanov, 1987). In our study we set the following objectives: to determine the aggressiveness of the isolates from some soil fungi, isolated from the rhizosphere of sugar beet and causing cutting; to determine the optimal environment for the growth of some of them and to ascertain the influence of soil moisture on the aggressiveness of isolates of *Pythium* and *Alternaria* against the most widely used herbicide for soil culture.

MATERIALS AND METHODS

The studies were conducted in the laboratory of plant pathology and academic hothouse of Episkop Konstantin Preslavski University of Shumen, in the period 2013-2014. There are used isolates of the genera *Pythium*, *Alternaria*, *Mucor*, *Fusarium*, *Rhizoctonia*, derived from beet plants to crossing phase. There were tested 7 types synthetic and organic sustaining media for cultivating isolates of *Pythium* ssp. and *Alternaria* ssp.: Barnes, Capek, water, root, potato, and

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oatmeal and corn agar. There is reported the influence on the growth of mycelium and the accumulation of biomass by measuring the diameter of the colony and by weighing samples (Nakova, 1988) on the 7th day of cultivation. The cultures on different sustaining media are made in five repetitions three times by the method of agar block incubation in a temperature at 22-24°C. The aggressiveness of the received isolates is tested under greenhouse conditions in pot experiments by artificial inoculating according to the established methods. There are sown for 100 seeds for option and the percentage of dead plants is reported. Inoculum is introduced into the bed through infected seed corn flour, as the seeds and soil previously are sterilized. The amendment of aggressiveness of isolates of *Pythium ssp.* and *Alternaria ssp.* is reported and under different variations of soil humidity at 50%, 70% and 90% soil humidity and in presence of the herbicide dual (metolachlor - 225 g/ kg) at a dose of 4500 ml/ha. For standard the version with 70% soil humidity without herbicide and inoculation is used.

RESULTS AND DISCUSSION

The results of the effect of culture medium on growth are shown in Table 1. The isolates of *Pythium ssp.* grows best on corn agar accumulating maximum fresh weight (8,59 g) and the highest percentage of solids in it (5.6) with a maximum diameter of the colony (66 mm - Table 1). Good growth shows isolate on oat and beet agar, as well as at dextrose nitrate media of Capek. Lower values than the control variant (water agar) for the diameter of the colony were measured in options on potato agar and close to control - for the diameter of the colony on the media of Barnes. The values for the accumulated fresh biomass and the percentage of dry matter in them of the variations therein on potato agar and Barnes media are lower than the other variants, but higher than the control. The results obtained show that *Pythium ssp.* digested protein and carbohydrate substances better than corn and oat agar potato agar.

Better is digested sucrose from beet agar than dextrose contained in the mineral media of Capek, and glucose in the media of Barnes. The isolate of *Alternaria ssp.* showed better growth on organic environment. The biggest diameter of the colony is reported on oatmeal agar (158, 6%), but the highest content of dry matter fungus accumulated on corn agar (6,20%). The media of Capek, suitable for the cultivation of a large number of soil fungi is also a good substrate for the growth and accumulation of biomass of *Alternaria ssp.*, while the media of Barnes is the most inappropriate of the tested as nutrient substrate.

Table 3. Influence of the soil's humidity and the herbicide Dual on the aggressiveness of *Pythium ssp.* and *Alternaria ssp.*

Variants	Germination plants, %		
	50 % USH	70% USH	90 % USH
I <i>Pythium ssp.</i>			
with dual 450ml/da	54,5	68,2	59,1
Without herbicide	30,0	55,7	51,5
Without herbicide and fungi	70,0	100,0	113,5
GD 5 %	9,1	9,1	9,1
II <i>Alternaria ssp.</i>			
with dual 450ml/ha	63,6	36,4	45,5
Without herbicide	31,0	45,5	54,5
Without herbicide and fungi	70,0	100,0	113,6
GD 5 %	8,2	8,2	8,2

In variants with simultaneous inoculation with complex of isolates the highest percentage of dead plants is reported (100% - Table 2). Slightly lower is the percentage from isolate of *Pythium ssp.* (96, 5), and lowest – for isolate of *Alternaria ssp.* (51.8). All tested isolates show high aggressiveness as agents of slaughtering up to crossing phase, as the later stages (1st pair of true leaves) do not cause damage to the root system. Such comparative studies of fungal isolates aggressiveness causing slaughtering at sugar beet in self and complex contamination, we do not found in our literature. For similar generic composition phytopathogens is reported in the environment of Chuyska Valley in Kazakhstan (Toporovskaya, 1985).

Table 1. Effect of different sustaining media on the growth of *Pythium ssp.* and *Alternaria ssp.* isolates

Sustaining media	Diameter of mycelium, mm		Fresh mass, g		Dry substance, %	
	<i>Pythium ssp.</i> (1)	<i>Alternaria ssp.</i> (2)	(1)	(2)	(1)	(2)
H ₂ O agar	21,2	43,00	0,05	0,19	2,43	1,90
Barne media	35,40	32,60	0,52	0,80	1,71	2,30
Chapek media	45,20	50,40	4,12	4,41	3,47	2,97
Potato agar	30,80	55,60	0,37	1,01	2,52	2,50
Oat agar	59,60	58,60	5,34	6,72	4,97	5,67
Maize agar	66,00	54,80	8,59	8,73	5,60	6,20
Beet agar	55,60	56,00	2,97	4,49	5,30	5,80

Table 2. Aggressiveness of fungal isolates, causing slaughtering in the sugar beet during different phenophases

Isolates tested	Dead plant % to the control		
	germ cotyledons	Cotyledons I pair of leaves	I pair of leaves, 5-th, 6-th leaf
<i>Pythium ssp.</i>	92,0	96,5	96,5
<i>Alternaria ssp.</i>	46,5	50,6	51,8
<i>Mucor ssp.</i>	50,0	54,1	54,1
<i>Fusarium ssp.</i>	73,3	74,1	74,1
<i>Rhizoctonia ssp.</i>	86,0	86,0	87,1
Complex			
<i>Pythium ssp.</i> , <i>Alternaria ssp.</i> , <i>Mucor ssp.</i> , <i>Fusarium ssp.</i> , и <i>Rhizoctonia ssp.</i>	100,0	100,0	100,0
Control – sterile soil	14,0	15,0	15,0

Testing the impact of soil moisture on the aggressiveness of isolates of *Pythium ssp.* and *Alternaria ssp.* at the background without herbicides and introducing dual 450 ml/da (Table 3) found that in variants with the diversion of the optimum value of the soil humidity (70%), the aggressiveness of *Pythium ssp.* increases, and the availability of Dual (4500 ml/ha) in all the variants of soil humidity suppresses this aggressiveness. The aggressiveness of *Alternaria ssp.* is highest in the variant with 50 % soil humidity, in the absence of herbicide. Increased soil humidity weakly suppressed the fungus aggressiveness, while Dual stimulates it.

Conclusions

Alternaria ssp. grows well on organic media. Corn agar can be referred to as selective media for *Pythium ssp.* More aggressive cause of slaughtering is *Pythium ssp.*, weaker - *Alternaria ssp.* Their aggressiveness is maximized in the presence of the whole complex isolates tested. At 70% and 90% soil humidity herbicide Dual at a dose of 4500 ml/ha suppresses the aggressiveness of *Pythium ssp.* and stimulates that of *Alternaria ssp.*

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