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Evaluation of Isolates of *Rhyzoctonia Solani* & *Fusarium Oxysporum* on Sugar Beet under Field Conditions

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1. INTRODUCTION

Root decay during the sugar beet vegetation appears periodically in the area of distribution of this culture and the most common consequences of this are greatly lowered productivity and quality, which is an economic risk to the industry (Tanova, 2003). Diseases of the root system of the crop, incl. the roots of beet are from ecological-microbial type. They are considered as a pathological syndrome, a combination of several pathogen and the most common are soil-dwelling microorganisms possessing the necessary pathological activity (Naidenov and Nechev, 2001).

Therefore, the etiology of root decay is specific and complex. According to Tanova (2003), root decay in sugar beet is caused by a complex microorganisms, and most aggressive pathogen determines the type of root decay. According to some authors the types of soil fungi of the genus Fusarium attack beet before the formation of root, but the symptoms occur in a particular combination of favorable factors for each type of media (Toporovskaia ,1985).

Fusarium decay is expressed in the tail decay from the central strand and is well expressed mikotraheozis. Most frequently from diseased plants are isolated species of Fusarium oxysporum (Bouchot, 1983) and the disease is provoked strong with moderate humidity and temperatures 22-30^{0}C. Fungi of the genus Fusarium infect plants earlier, before the formation of root and under favorable conditions the disease continues as an object of attack are roots, which form cavities filled with dense white to pink mycelium (Alhovskaya and Zagursky, 1985).

In a dry and hot climate, the soil pathogen Rhyzoctonia solani predominants in the complex of root decay of sugar beet (Tanova and Raykov, 2008). Usually complex causes of root decay in beet root system damage and other crops from beet crop rotation, which creates favorable conditions for the accumulation of contamination in the soil. Umralina and others. (1987) found differences in cultures and aggressiveness of Rhizoctonia solani isolated from sugar beet and conditionally divide them into two groups as defined anastomosis affiliation.

As regards the causative Fusarium oxysporum some differences in the aggressiveness of the resulting isolates are ascertained (Dayakov, 1993). It is well known that the interaction of the host plant and the pathogen occurs changes in the chemical composition and ferment system. The activity of the oxidase enzymes - catalase, peroxidase and polyphenol oxidase in diseased plant is changed depending on the aggressiveness of the pathogen and in practice are associated with the resistance of the plant (Christova, 1980).

The aim of this study is to test the aggressiveness of the isolates from Rhyzoctonia solani Kuhn and Fusarium oxysporum, obtained from sugar beet and establish their influence on the chemical composition and production of oxidase enzymes in a sick root.

2. MATERIAL AND METHODS

The study is conducted at the Laboratory of Phytopathology of Shumen University, the experiment field and beet technological laboratory of the Institute of Agriculture – the city of Shumen in 2014-2015 г. Five isolates were used: 3 of Rhizoctonia solani Kuhn and 2 isolates of Fusarium oxysporum. Inoculation was made under field conditions in parallel by two methods: individually, by placing a

block of agar culture pathogens at the head of root crop (Schnaider, 1983) and by infected meal of beet seeds buried at about 4-5 cm of root crop (150-200 g/m^2). The infection was made in the middle of July in an area of 2m^2 of each isolate in 4 repetitions. The degree of attack was calculated on 5 grade scale (Tanova, 2003). The aggressiveness of the pathogen was calculated as % diseased roots / m^2, during the vegetation and at harvest. To determine the activity of the enzymes peroxidase and polyphenoloxidase (defining the plant reaction to environmental conditions), pectinase and invertase (related to the production of sugar) modifications of known methods were used (Boyarkin, 1951; Boyarkin, 1954; Sapozhnikova, 1981; Helemskii et al., 1977). The sucrose content, mineral non-sugars, reducing substances and amino nitrogen was recorded in hot water extracts clarified with lead acetate (Bozhkov, 1972; Christova, 1980). The technological parameters; white sugar yield per unit of production and purity of heavy juice was calculated from the content of sucrose and non-sugar (Christova, 1980). Data on the chemical composition are presented as% to the average mass of beet (% smts).The analyses were performed immediately after the removal of samples contaminated with infected flour.

3. RESULTS AND DISCUSSION

The results for the aggressiveness of the isolates tested in two methods of field inoculation are presented in Table 1.

Table1. ***Aggressiveness*** *of Rhizoctonia solani and Fusarium oxysporum isolates after field inoculation of sugar beet*

Isolates	Inoculation with agar-block % diseased plants /m^2				Inoculation with infected powder % diseased plants/m^2			
	I term of reading	II term of readin g	III term of readin g	IV term of reading	I term of readin g	II term of readin g	III term of readin g	IV term of readi ng
R_1- *Rhizoctonia solani*	3.9	9.0	17.0	19.3	5.0	14.0	18.9	22.4
R_2- *Rhizoctoniasolani*	8.7	14.7	20.9	20.0	9.0	13.0	18.5	24.0
R_3- *Rhizoctonia solani*	10.2	17.4	34.0	32.0	15.2	25.9	20.3	35.0
F_1*Fusariumoxysporum*	3.0	3.4	12.5	17.5	12.5	11.9	14.3	21.7
F_2- *Fusariuoxysporum*	2.5	6.5	8.9	9.2	6.8	4.0	6.0	11.3
Control	1.7	1.5	3.2	5.8	1.7	1.5	3.2	5.8

The results in the Table show differences in the aggressiveness of all tested isolates. Isolates of *Rhizoctonia solani Kuhn*, regardless of the method of infection and the time of recording showed more expressed aggressiveness than those of these of *Fusarium oxysporum*. In inoculation using the method of Schnaider (1983), with agar block at the beginning of the reporting period the highest % diseased plants /m^2 is reported for isolate R_3 of *Rhizoctonia solani*- 10.2%, and the lowest for isolate F_2 of *Fusarium oxysporum*- 2.5%.

This trend continued throughout the study period, as the last reporting - extractions beet for isolate R_3 of *Rhizoctonia solani* are reported 32% diseased plants /m^2, and for isolate F_2 of *Fusarium oxysporum*- 9.2%. Reporting in inoculated plants in our method with infected flour show the same trend for higher aggressiveness of isolate R_3 of *Rhizoctonia solani* and lowest for isolate F_2 of *Fusarium oxysporum.*

This trend exists for the entire period of the study while in pulling the beet is found that for isolate R_3 % diseased plants/m^2 are 35, but for isolate F_2-11.3. The comparison in performance between two methods shows a trend in favour of inoculation with infected flour. The results of this test show that tested isolates differ in aggressiveness. Isolates tested of *Rhizoctonia solani* are more aggressive that those related to *Fusarium oxysporum*. Most aggressive isolate is R_3 of *Rhizoctonia solani*, and poorest - isolate F_2 of *Fusarium oxysporum*.

Table 2 presents the results of the chemical analysis of the experimental options.

Table2. *Influence of different isolates of Rhizoctonia solani and Fusarium oxysporum on the chemical structure and some technological indices of sugar beet (relative values)*

Isolates	Sucrose % % aAccording to K	Nonsugars, % of dry mass - % according to K					
		K	Na	amino-nitrogen	Reducing Substance	juice purity	output
R_1- *Rhizoctonia solani*	**83.4^{00}**	89.2	105.4	105.4	**198.7^{+++}**	**94.7^{00}**	**68.9^{000}**
R_2- *Rhizoctonia solani*	92.4	87.4	106.7	113.8	**162.2^{+++}**	**94.8^{00}**	**79.3^{00}**
R_3- *Rhizoctonia solani*	**78.2^{000}**	98.8	130.3	105.4	**144.8^{+++}**	**91.1^{000}**	**83.7^{0}**
F_1- *Fusarium oxysporum*	100.0	93.0	88.5	112.5	**134.3^{++}**	99.9	101.1
F_2- *Fusarium oxysporum*	95.0	91.7	96.2	108.0	**127.9^{++}**	99.3	94.4
Control	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Control value	**15.9**	**3.71**	**2.75**	**5.33**	**0.105**	**88.8**	**12.6**
GD – 5%	**9.0**	**24.9**	**40.5**	**28.0**	**18.5**	**3.4**	**13.2**
GD – 1%	**12.8**	**35.4**	**57.2**	**39.8**	**26.3**	**4.8**	**18.8**
GD – 0.1%	**18.5**	**51.2**	**83.3**	**57.6**	**38.1**	**6.9**	**27.2**

The results showed that the infected roots have altered chemical composition, depending on type of pathogen and the aggressiveness of the isolates thereof. The indicator sugar content measured in % deteriorates in *Rhizoctonia solani* isolates, as in strongest degree thus applies for isolate R_3 of this pathogen.

Isolates of *Rhizoctonia solani*- R_1, R_2 и R_3 increase significantly the quantity of reducing substances, highly lowering the purity of the juice and the yield of the sugar, wherein the infected roots are completely unsuitable for processing and the production of sugar. Isolates of species *Fusarium oxysporum*- F_1 and F_2 show trend to reduce the amount of sugar in infected roots, but they also reduce the quality of the product because it was established they highly increase the amount of reducing agents which is undesirable in sugar production.

The results of chemical analyses show that the studied isolates of Rhizoctonia root decay - *Rhizoctonia solani Kuhn* and Fusarium wilting and decay of root - *Fusarium oxysporum* strongly worsen the raw material of sugar beet and make it unsuitable for sugar production (Christova, 1980).

Table 3 presents the results of analyses of oxidase enzymes in the infected roots.

Table3. *Influence of different Rh. solani isolates and F.oxysporum isolates of the activity*

of some ferments in the sugar beet roots

Isolates tested	Peroxidase	Polifenoloxidase	Invertase
R_1- *Rh. solani*	**3.90^{++}**	2.86	**35.82^{+++}**
R_2- *Rh. solani*	**4.38^{++}**	2.89	**21,04^{+++}**
R_3- *Rh. solani*	**3.55^{++}**	2.82	**16.52^{+++}**
F_1 *F.oxysporum*	2.17	2.87	4.80
F_2 *F.oxysporum*	2.12	2.88	5.97
Control	1.60	2.88	2.13
GD- 5%	1.36	0.12	7.44
GD- 1%	1.93	0.17	10.58

Note: The ferments are shown as activity:

Peroxidase – ferment's activity as change of the optical density for 1 sec from 1 g fresh sample;

Polifenoloxidase – activity of the ferment as mmol oxidation for 1 min from 1 g fresh sample of ascor bic acid;

Invertase – activity of the ferment as mmol invertive sugar, formed for 1 min from 1 g fresh sample.

The interaction pathogen / host induces changes in the activity of some enzymes. Under the influence of isolates of *Rh. solani* - R_1, R_2 and R_3 reliably increases peroxidase and invertase activity, while that of polyphenoloxidase is on the level of control for all tested isolates (Table 3). Isolates of *F.oxysporum* do not cause chamge in the oxidase enzymes of infected roots. Slight increasing of pectinase activity, up to 10% was found only in isolate R_3.

Increasing the peroxidase and invertase activity to a significant level corresponds to decreasing in sugar content and increasing the reducing substances in roots. This leads to decreasing in both - yield of white sugar, and the purity of the juice.

Increasing pectinase activity suggests increasing of soluble pectin in beet (Хелемский et al., 1977), which together with reducing agents causes significant difficulties in beet processing. Data on chemical composition and activity of enzymes studied give reason to make some differentiation of isolates tested.

4. CONCLUSIONS

Tested isolates differ in aggressiveness as highly aggressive are isolates of *Rhizoctonia solani Kuhn*, and less aggressive are isolates of *Fusarium oxysporum.*

Isolates of *Rhizoctonia solani Kuhn* are arranged in aggressiveness in the following descending order: R_3; R_2 and R_1, and isolates of *Fusarium oxysporum*- F_2 and F_1.

Isolates of *Rhizoctonia solani Kuhn* worsen technological qualities of infected roots and raise their peroxidase and invertase activity.

Isolates of *Fusarium oxysporum* worsen in weaker level technological qualities of infected roots and do not affect the fermented activity.

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