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RESEARCH ARTICLE

INFLUENCE OF EXTRACT OF TUTSAN (*HYPERICUM PERFORATUM L*) ON CAUSE OF FUSARIUM ROOT DECAY IN BEET (*FUSARIUM OXYSPORUM*)

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ABSTRACT

The studies were conducted in the laboratory of plant protection at Konstantin Preslavski University of Shumen in 2015. Under in vitro conditions the influence of 7 concentrations of aqueous extract of tutsan leaves (*Hypericum perforatum* L.) on growth and aggressiveness of isolates of fusarium root decay in beet (*Fusarium oxysporum*) was investigated. The contents of the extract of the tutsan in a concentration 10,15 and 20 ml/l nutrition media increases as the growth of mycelium, as the aggressiveness of the pathogen. The content of the extract of tutsan in a concentration 10 ml/l nutrition media inhibits spore-formation of the agent during the period from 24h to 72h.

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INTRODUCTION

Beet root decay is pointed as the most harmful disease or beet growing (Toporovska, 1985; Umralina *et al.,* 1987; Dayakov, 1993; Schnaider and Poter, 1983). Studies of the pathogen in our country confirm the high harmfulness of intensive development of root decay during the sugar beet vegetation. For the climatic condition of the country as a part of the complex causes of beet root decay is determined the causative agent of fusarium decay - *Fusarium oxysporum*. This cause is highly injurious and can reduce the yield (Tanova, 2003). The studies show very low efficiency of the application of fungicides for control of pathogen (Tanova, 2003).This necessitated research work directing to search for alternatives. Проучват се Abiotic and biotic media factors are studied, that could affect the pathogenity and the aggressiveness of this pathogen (Tanova and Petrova, 2006; Chermenska, 2000; Curini *et al.,* 2003; Ujvary, 2002; Stadnik *et al.,* 2003). There has been established a relation between the acidity of the media and one-sided nitrogen fertilization, the use of herbicides and plant growth regulators and the development and the pathogenity of the causative agent (Tanova *et al.,* 1997; Tanova and Varbanova, 2002; Tanova, 2003a).

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The latest studies regarding alternative measures to restrict soil pathogens and in particular the causative agent *Fusarium oxysporum* are directed to study the biological and fungicidal activity of plant extracts and their secondary metabolites (Chermenska, 2000; Curini *et al.,* 2003). Some authors reported about the successful use of biologically active substances in extracts of juniper and tagetes to limit soil pathogens in vegetables (Mares *et al.,* 2002). Petrova and Tanova reported that the concentration of alcohol extract of oregano leaves – 10 ml/1 nutrition media inhibits growth and increases aggressiveness of cause of rhizoctonia root decay in sugar beet which very often is isolated at the same time with the cause of fusarium root decay (Dayakov, 1993; Tanova, 2003). In view the encouraging results from the effect of extract of oregano and demand for environmentally-friendly means we aimed to investigate the effect of extract of tutsan on the cause of fusarium root decay of the beet - (*Fusarium oxysporum*)

MATERIALS AND METODS

The studies were conducted in the laboratory of plant protection at Konstantin Preslavski University of Shumen in 2015. 14 days isolate of the pathogen, obtained from sugar beet plants with root decay was tested at in vitro conditions. The growth, sporulation in cultivation on nutrition media with the

addition of an aqueous extract from the leaves of tutsan (*Hypericum perforatum L.*) at various concentrations were studied, as well as the aggressiveness of the isolate, cultivated on a media with the addition of extract. For this purpose the extract obtained after aqueous extraction is added in a nutrition media – potato-dextrose agar (PDA), at concentration-Variant 1- 0,l ml/l; Variant 2- 0.5 ml/1; Variant 3-1.0ml/1; Variant 4-5.0ml/1; Variant 5- 10.0 ml/1; Variant 6- 15.0ml/l и Variant 7-20.0ml/l . The extracts are added in the ration drug/water- 1/1. The laboratory experience consists of 3 tests, each of which contains the above mentioned identical variants.

Test 1

On a potato-dextrose agar with added extract in the respective method of the agar block (Popkova, 1987). The cultures were cultivated in a thermostat at 24-26 °C for 14 days. Twice during this period извършени readings to determine the growth of the mycelium were performed, by measuring the diameter of the micelle colonies and quantify the dry mycelium mass by quantitative method.

Test 2

Mycelial-spore suspension was prepared by the method of Dragalski (Popkova, 1987), by culturing mono-spore culture on potato-dextrose agar. A diluted suspension with a titre 25 pcs. spores /1 visual microscopic field (25.10^3 pcs. spores per 1 ml suspension) was used. It is transmitted in solutions of the tested extract in relevant doses. Cultures prepared in this way are put in a thermostat at temperatures $26\text{-}28^0$C ($\pm 2^0$C) for 48 h. Spores germination is recorded after microscopy of the cultures (10 microscopic preparations for variant), after 24 h, 36 h and 48 h after incubation.

Test 3

To study the aggressiveness of the pathogen inoculation of the roots of sugar beet, Elite variety, with 14 days cultures of all nutrition media was carried out. On the 14 day of inoculation of infected roots the penetration rate is reported, depending on the diameter of the local damage (Schnaider and Poter, 1983). The quantity decayed mass is defined on the 21st day, according to the weighting method; the healthy mass of every infected root is previously separated. Data processing of the results from the studies were carried out in accordance with the appropriate statistical methods. After made statistical processings, the results are presented in tabular form.

RESULTS AND DISCUSSION

The results of the mycelium growth and the cause of fusarium root decay are given in the Table 1. The measured diameter of micelle colonies developed on nutrient media with participation of extract of tutsan (5,10,15 and 20ml/l) increases with concentration increasing. At the maximum concentration of 20ml/l the increasing is 32.5% and at minimum, respectively 18.9 %. In the accumulation of biomass we also found an increasing trend, but this indicator is weaker and reliable for the variant with highest contents of extract - 20ml/l.

We can conclude from the results obtained for the growth of mycelium that high concentrations of the extract in nutrition media increase the quantity of dry mass in the mycelium. These results are in close relation with the registered change in the cultural peculiarities of different variants. They consisted of change the colour and density of air mycelium. With increasing the concentration of extract the mycelium compacted and changes its colour from transparent to opaque white. This was most expressed for variants containing extract 10,15 and 20ml/l.

Table 1. Influence of extract of tutsan (*Hypericum perforatum L.*) on the growth of *Fusarium oxysporum*

Nutrition media with extract ml/1	Colonies diameter		Mycelium dry biomass	
	cm	Relative%	%/100g	Relative%
0.1	1.38	104.5	21.0	97.7
0.5	1.41	106.8	21.0	97.7
1.0	1.47	111.4	21.2	98.6
5.0	1.57	118.9 +	21.5	100.0
10.0	1.61	121.9+	21.9	101.9
15.0	1.66	125.7++	22.6	105.1
20.0	1.75	132.5++	23.5	109.3 ++
Control without	1.32	100	21.5	100.0
GD 0.1%	0.44	18.9	2.5	11.6
P %	1.12		2.26	

Table 2. Influence of extract of tutsan (*Hypericum perforatum L.*) on *Fusarium oxysporum* sporulation

Nutrition media with extract ml/1	Germination of spores %		
	after 24 h	after 48 h	after 72 h
0.1	18.00	19.38	19.70
0.5	18,81	19.41	19.58
1.0	17.56	18.00	18.45
5.0	16.65	17.00	17.86
10.0	15.88	16.54	16.50
15.0	15.60--	16.52	16.85
20.0	17.25	17.58	18.25
Control without extract	18.65	19.50	19.65
GD 1%	3.05	4.02	2.06
GD 0.1%	6.52	7.40	9.64
P %	2.85	3.27	5.01

Table 2 shows the results of the influence of extract of tutsan on the dynamics of sporulation of cause of fusarium root decay in beet. As can be seen from the results applied, the contents the extract of tutsan does not affect the dynamics of sporulation. However, in all variants there is a tendency for suppression of sporulation after 24 h, 48h and 72h. This is most highly expressed after 24th hour in all versions of the test. The strongest is the suppression of sporulation in the version with contents of extract of tutsan 15.0 ml/1.

In Table 3 are presented the results of the extract on the aggressiveness of the pathogen. They show that with increasing the concentration of the extract increases the aggressiveness of the cause expressed with increasing penetration rate in the tissues of roots and increased quantity decayed mass. For extract with contents 10,0, 15,0 and 20,0 ml/1 nutrition media the penetration rate increases by 4,5% for the first concentrations and by 9,0% for the maximum concentration used by us. The measured quantity decayed mass is biggest for variants 15 and 20 ml/1 and is increased respectively with 13,4 and 18,7 compared to control. The results of this study give reason to accept that the contents of the extract of tutsan at concentration 10; 15 and 20 ml/1

accelerates the penetration of the pathogen into the host tissues and increases its aggressiveness causing increasing the quantity decayed mass. Water extract of tutsan increases the pathogenity of the causative agent of fusarium root decay in beet.

Table 3. Influence of extract of tutsan (*Hypericum perforatum L.*) on *Fusarium oxysporum* aggressiveness

Nutrition media with extract ml/l	Rotten mass		Penetration rate	
	%/ 100g	Relativ.%	%/ 100g	Relativ. %
0.1	42.50	99.1	0.22	100
0 5	42 75	100 0	0 22	100
1 0	43 25	101 2	0 22	100
5 0	43 50	101 7	0 22	100
10 0	45 25	105 8	0 23	104 5 ++
15 0	48 50	113 4+	0 23	104 5 ++
20.0	50.75	118.7 +	0.24	109.0 ++
Control without	42.75	100	0.22	. 100
GD 5%	3 63	8 49	0 01	2 7
GD 1%	8 48	19 8	0 01	3 8
GD 0.1%	14.74	24.4	0.02	5.4
P %	2 84		0 93	

Conclusions

In conclusion, the results obtained from the present study lead to following conclusions:

The contents of the extract of tutsan at concentrations: 10,15 and 20 ml/l nutrition media increases as the growth of mycelium, as the aggressiveness of the pathogen. The contents of the extract of tutsan at concentrations: 10 ml/l nutrition media inhibits sporulation of the causative agent during the period from 24h to 72h. The results obtained can be useful for beet producers outside the conventional production technologies.

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