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INVESTIGATION OF THE CORRELATION BETWEEN PHYTOPATHOGENIC ACTIVITY OF FUSARIUM SOLANI AND ABILITY TO PRODUCE MYCELIAL LECTINS

Rishat S. Muhammadiev [1*], Tatyana V. Bagaeva [2]

Kazan Federal University, Kremlyovskaya str.420008, Kazan, Russian Federation

Abstract:

A comprehensive study of eighteen isolates of micromycetes belonging to species F. solani on the phytopathogenic activity and the ability to produce common and surface lectins of mycelium was carried out. It is shown that among studied F.solani strains, 44% of isolates belong to phytopathogens, and 56% to saprophytes. The phytopathogenic effect of these strains is manifested, both at the level of germination of seeds, and in the development of plants. The intensity of plant damage by individual strains was 59%. F.solani strains are capable of infecting various plant objects (vegetable and cereals), but a more intense effect was observed in vegetable crops. Fusarium blight was accompanied, mainly, by destruction of the root and root zone of plants.

Significant differences in the activity of total and surface mycelial lectins in the F.solani strains studied were found. The correlation of phytopathogenic activity of micromycetes with the degree of activity of their surface lectins is shown. Phytopathogenic strains of F.solani micromycetes differ from other strains by the ability to form surface mycelial lectins with higher hemagglutination activity.

Keywords: micromycetes, Fusarium, phytopathogenicity, lectins, activity.

Corresponding author:

Rishat S. Muhammadiev,

Kazan Federal University,

Kremlyovskaya str.420008, Kazan,

Russian Federation

QR code

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INTRODUCTION:

The most common and phytopathogenic micromycetes, causing various diseases of plants, include fungi of the genus *Fusarium*. Micromycetes of the genus *Fusarium* cause special attention of the researchers by the fact that they mainly affect the most important agricultural plants. These micromycetes can affect not only the vegetative, but also the reproductive organs of plants, causing their withering, damage, rot and death. As a result of infection of plants, yield decreases, this subsequently leads to significant economic losses. On the other hand, fusarium damage can lead to the accumulation of secondary metabolites of mycotoxins, which cause severe mycotoxicosis, in grains, vegetables and fruits [15,10,20,2]. In this regard, one of the main directions of modern agrobiotechnology is to control the spread of plant diseases. To this end, various methods of analyzing pathogenic and saprophytic strains of microorganisms are used, however, they do not provide a complete and rapid picture, so the need for development of new methods of analysis arises.

The works of a number of authors studying the lectins of micromycetes found that they not only play an important role in the processes of morphogenesis and fungal development, but also participate in the adhesion, pathogenesis of the plant, mycopathism [11,9,13,12]. Lectins participate in interactions between prokaryotes and eukaryotes, play the role of signaling molecules in the formation of response reactions of the organism [3,12]. It could be assumed that the main feature of phytopathogenic and saprophytic forms of fungi can be their ability to synthesize active lectins.

The objective of this research was to establish the relationship between the phytopathogenic activity of micromycetes of the genus *Fusarium* and the activity of lectins they produce.

MATERIALS AND METHODS:

In our study we used 18 micromycetes of species *F.solani* isolated from soil samples and plant objects: isolates *F. solani 1, F. solani 8* - from internal and surface tissues of tubers of potatoes of the variety Nevsky, other isolates from sod-podzolic soils of different regions of the Republic of Tatarstan.

Cultivation of micromycetes was carried out on a potato-glucose nutrient medium containing potatoes and glucose at a concentration of 200 and 20 (g/ll), respectively. The temperature of cultivation of isolates is 28°C. Cultivation was carried out for 7 days. To determine the phytopathogenic activity of the strains studied, spore suspensions with a titer of 10^5 macronidia/ml were obtained by flushing them off the surface of the mycelium after growth of the micromycete on a dense agar medium.

To determine the effect of the phytopathogenic potential of the micromycete isolates on seed germination, growth and weight of plants, we used the seeds of winter soft wheat of the variety Kazan 560 and the planted pea of the variety Albuman in our experiments. Before sowing, the seeds were sterilized with a 2% solution of potassium permanganate for 15 minutes, then washed with distilled water and used in experiments. Experimental variants of seeds were grown in conidia suspension for 14 days, seeds grown on tap water served as controls [7].

Plants were germinated in a phytotron in cuvettes at 23±2°C and a 12-hour light period with 100 W/m^2 illumination for 14 days. Analysis of the appearance of sprouts was carried out daily. On day 14, the height of the aerial part, the length of the roots, and their weight were measured.

Assessment of the damage caused by root rot of winter wheat and inoculum was assessed on a four-point scale [8, 1]. The intensity of development of root rot and the prevalence of the disease were determined according to generally accepted formulas [5].

To determine the activity of surface and mycelial lectins, a culture of microorganisms was grown on the above liquid nutrient medium. The biomass of the mycelium was collected by filtration through a nylon tissue, followed by removal of the culture fluid residues by washing the mycelium repeatedly with sterile distilled water and then with 20 mM Tris-HCl buffer solution (pH 7.3).

The general mycelial lectins were obtained by the method described earlier [14].

Surface lectins were prepared by adding a 20 mM Tris-HCl buffer solution (pH 7.3) to the micromycete mycelium by the method described in the work [16].

The activity of lectins was determined by direct hemagglutination reaction (DHGR) with native red blood cells of human blood of the first group. Erythrocytes for DHGR were obtained by the method proposed by Lutsik et al. [4].

The titre of hemagglutinating activity was expressed as the maximum dilution or minimum concentration

of lectin in solution, at which the visible hemagglutination reaction of erythrocytes was observed [19].

RESULTS AND DISCUSSION:

One of the main physiological properties of micromycetes of the genus *Fusarium* is their pathogenicity. It is known that fungi of the genus *Fusarium* are a heterogeneous group of microorganisms, which include explicit parasites of plants that develop, as a rule, in the vascular system of plants, facultative parasites that live a saprophytic life and parasitize on weakened plants (pathogens of various rot), and true saprophytes, living only in the soil and feeding on dead plant residues [6].

The results of the experiments showed that *F.solani* micromycetes isolates were characterized by different phytopathogenic activity against agricultural plants of peas and wheat (Figure 1).

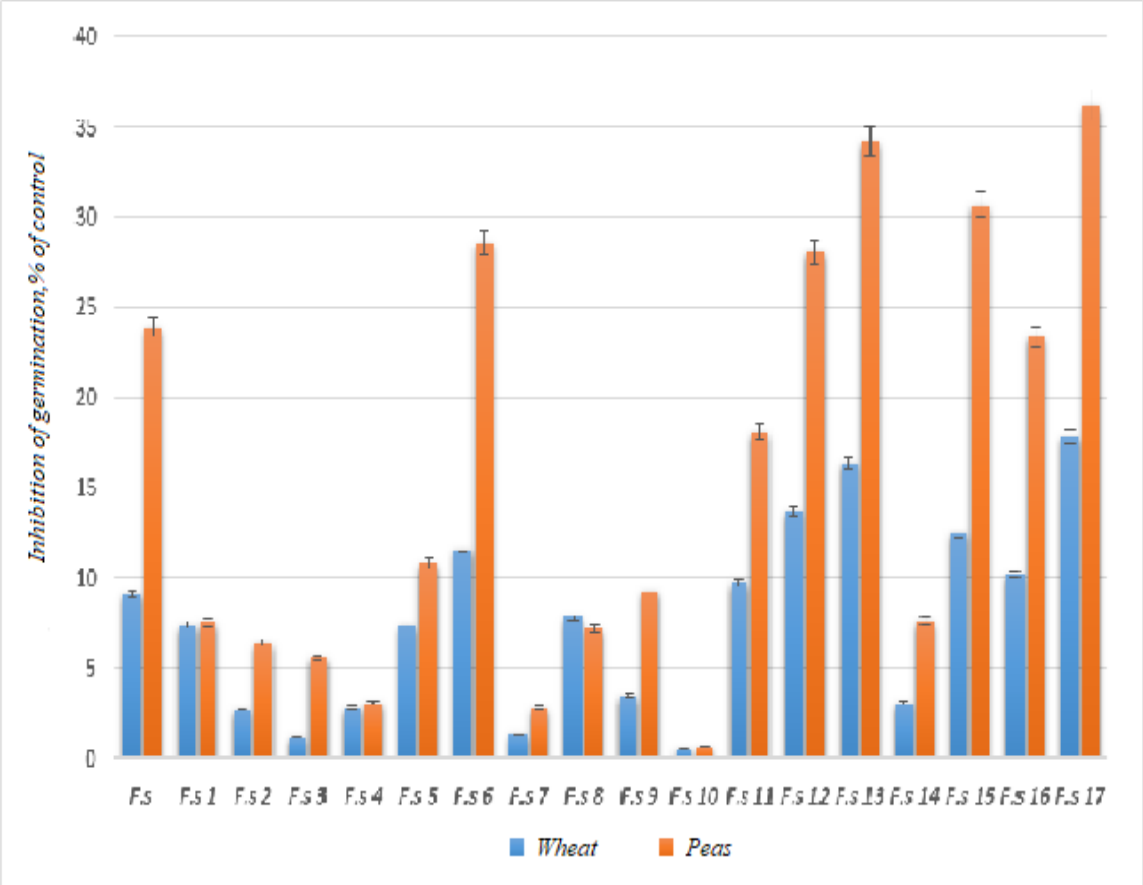

Figure 1 - Effect of a spore suspension of *F. solani* isolates on the germination of wheat and pea seeds.

As can be seen in Figure 1, *F.solani, F.solani 6, F.solani 12, F.solani 13, F.solani 15,* and *F.solani 17* strains showed the greatest phytopathogenic effect, having reduced the germination of wheat and pea seeds by 9.1-36.2%. The remaining strains - *F.solani1, F.solani 2, F.solani 3, F.solani 4, F.solani 5, F.solani 7, F.solani 8, F.solani 9, F.solani 10, F .solani 11, F.solani 14* and *F.solani 16* - limited seed germination from 0.5 to 23.4%.

Measurement of the length of seedlings and roots of plant seeds confirmed the intra-stress difference in phytopathogenic properties in these micromycetes (Table 1). It should be noted that all isolates of *F. solani* influenced the growth and development of pea plants to a greater extent than of wheat. At the same time, the action of micromycetes was directed on the underground part of 14-day plants compared to their aerial part, which is typical for micromycetes of the genus *Fusarium*.

Table 1: Development of plants from seeds artificially infected with *F.solani* micromycetes*

Species, strain	Winter wheat		Green pea	
	Length of sprouts, mm	Length of roots, mm	Length of sprouts, mm	Length of roots, mm
Control, water	194.5 ±3.8	111.3 ±3.5	166.0 ±2.9	99.6 ±3.0
F.solani	183.2 ±3.2	94.6 ±2.1	145.3 ±3.9	73.2 ±2.2
F.solani 1	188.7 ±2.4	97.7 ±2.6	162.2 ±2.8	82.3 ±2.5
F.solani 2	192.4 ±2.7	105.6 ±3.8	160.1 ±3.4	90.0 ±3.2
F.solani 3	193.9 ±3.4	110.9 ±2.9	163.0 ±2.6	99.1 ±1.8
F.solani 4	193.5 ±2.3	108.0 ±3.1	164.5 ±2.9	96.1 ±3.5
F.solani 5	188.6 ±3.5	98.4 ±1.1	158.4 ±2.3	85.4 ±2.6
F.solani 6	180.7 ±2.6	92.9 ±1.9	140.6 ±1.8	64.2 ±2.8
F.solani 7	193.3 ±2.2	108.1± 1.6	164.0 ±3.3	95.4 ±2.0
F.solani 8	189.8 ±3.7	98.5 ±2.2	161.4 ±3.5	87.1 ±1.9
F.solani 9	191.6 ±2.9	103.4 ±2.8	160.5 ±2.5	87.0 ±2.4
F.solani 10	194.3 ±2.5	110.3 ±1.8	165.7 ±3.2	98.7 ±2.1
F.solani 11	186.7 ±3.9	93.7 ±3.2	153.2 ±3.0	77.2 ±3.3
F.solani 12	179.3± 2.1	84.9 ±2.3	140.9 ±2.1	65.8 ±2.7
F.solani 13	174.7 ±2.7	79.4 ±1.7	136.3 ±2.6	55.4 ±2.0
F.solani 14	192.9 ±3.8	105.5 ±2.0	162.3 ±3.7	89.1 ±2.6
F.solani 15	181.5 ±3.1	93.2 ±2.9	141.6 ±2.5	61.0 ±3.1
F.solani 16	180.7± 2.8	95.3 ±2.9	141.3 ±2.2	76.3 ±2.5
F.solani 17	169.5 ±3.4	77.7 ±2.2	129.8 ±3.8	54.9 ±2.7

*The data reflect the effect of micromycetes on the growth and development of 14-day plant seedlings

F.solani, F.solani 6, F.solani 12, F.solani 13 , F.solani 15 and *F.solani 17* strains showed the greatest reliable effect on plants, inhibiting the growth of the underground part by an average of 15-30% for seedlings of wheat, by 27-51% for pea seedlings. The growth of the aboveground part of the plants was inhibited by 6-22%.

The treatment of winter wheat seeds with a spore suspension of macroconidia of these micromycetes significantly reduced the biomass of plant seedlings within 8-16% of control (unprocessed seeds), and roots by 16-37%. A more marked decrease in the biomass of seedlings, namely by 17-28% and root biomass by 36-61%, was observed in peas.

F.solani 1, *F.solani 2, F.solani 3*, *F.solani 4*, *F.solani 5*, *F.solani 7*, *F.solani 8*, *F.solani 9*, *F.solani 10*, *F.solani 11, F.solani 14 and F.solani 16* strains had a negligible negative effect on the development of experimental plants, reducing the growth of their aerial part by an average of 1-6% for wheat germs and 1-14% for pea seedlings. Root growth was reduced in wheat by 1-15%, in peas by 1-23%. The biomass of sprouts and roots decreased by less than 24%.

The main indicator of phytopathogenicity of *F.solani* strains is their ability to cause root rot in a plant. Determination of the parameters of root rot of plant seedlings showed that the intensity of damage to pea roots by isolates of *F.solani, F.solani 1, F.solani 6, F.solani 8, F.solani 12, F.solani 13, F.solani 15* and *F. solani 17* was 1.1-2.9 times higher compared to winter wheat (Table 3). The intensity of damage to the roots of wheat with these strains was 10.8-62.1%, and peas 6.1-25.0%.

The spread of root rot was correlated with the indications of the intensity of the damage to the plants.

Table 3: The intensity of damage and the spread of root rot of plants with their seeds infected with *F.solani* micromycetes

Species, strain	Intensity of damage, %		Spread of disease, %	
	wheat	pea	wheat	pea
Control, water	0.03 ±0.001	0.06 ±0.003	1.8 ±0.1	2.4 ±0.1
F.solani	9.9 ±0.4	11.2 ±0.7	38.3 ±1.5	42.3 ±2.1
F.solani 1	7.1 ±0.3	12.0 ±0.6	32.3 ±1.8	42.5 ±2.2
F.solani 2	2.7 ±0.2	4.3 ±0.3	18.8 ±0.8	26.1 ±1.4
F.solani 3	0.2 ±0.01	0.4 ±0.002	11.7 ±0.6	13.3 ±0.7
F.solani 4	0.5 ±0.02	1.1 ±0.6	13.6 ±0.5	21.0 ±1.6
F.solani 5	6.8 ±0.4	8.1 ±0.5	27.3 ±1.0	35.4 ±1.8
F.solani 6	17.4 ±1.3	45.7 ±2.4	50.5 ±2.2	70.1 ±2.7
F.solani 7	2.3 ±0.1	4.5 ±0.2	22.7 ±1.1	31.5 ±1.3
F.solani 8	6.1 ±0.3	10.8 ±0.5	33.1 ±1.7	44.3 ±2.0
F.solani 9	4.2 ±0.2	7.6 ±0.3	30.1 ±1.5	35.3 ±1.6
F.solani 10	0.03 ±0.002	0.06 ±0.003	1.8 ±0.09	2.4 ±0.1
F.solani 11	6.5 ±0.4	9.7 ±0.4	28.3 ±1.5	36.4 ±1.8
F.solani 12	20.5 ±1.1	44.8 ±2.5	48.2 ±2.4	75.8 ±2.5
F.solani 13	23.2 ±1.3	57.7 ±2.4	53.6 ±2.8	81.5 ±3.1
F.solani 14	3.4 ±0.1	5.2 ±0.03	24.8 ±1.1	28.5 ±1.7
F.solani 15	17.7 ±0.8	52.0 ±2.1	47.8 ±2.1	77.1 ±3.0
F.solani 16	5.9 ±0.2	10.1 ±0.5	33.1 ±1.6	39.6 ±2.1
F.solani 17	24.9 ±1.3	59.1 ±3.0	51.1 ±2.4	76.9 ±2.8

Comparing the phytopathogenic properties of *F.solani* micromycetes, we can conclude that 44% of the strains studied had phytopathogenic properties (strains *F.solani, F.solani 1, F.solani 6, F.solani 8, F.solani 12, F. solani 13, F.solani 15, F.solani 17*). They reliably reduced the germination of seeds and the development of seedlings. The degree of damage to the root system of pea plants by rot, with the action of these strains, was more than 11%, with the spread of the disease of more than 42%. The remaining 56% of the strains (*F.solani 2, F.solani 3, F.solani 4, F.solani 5, F.solani 7, F.solani 9, F.solani 10, F.solani 11 and F.solani 14, F.solani 16*) did not have a significant effect on the germination and growth of plants. The intensity of root damage of plants with the *F. solani* micromycetes did not exceed 10.1%, which indicates the absence of development of this disease in test plants.

As known from the literature, phytopathogenic species of micromycetes synthesize more surface proteins and proteins involved in intercellular recognition [17,18]. One could assume that one of the distinguishing features of saprophytic and phytopathogenic strains of micromycetes may be the presence of lectins.

The main feature of lectins is their ability to interact specifically with certain carbohydrate residues on the surface of erythrocytes, couple them, causing a hemagglutination reaction.

Determination of the total content of lectins in the mycelial of *F.solani* revealed capability of their synthesis in many micromycetes. Among the 18 strains studied, the extract from the isolate mycelium of *F.solani 6* (titer of 4096 units) and *F.solani* (titer of 2048 units) showed the greatest lectin activity (Table 4). The activity of surface mycelium lectins was significantly lower than the total activity of mycelium lectins and was retained only in strains having a higher overall activity.

Table 4: The activity of surface lectins of *F.solani* micromycetes mycelium

No.	Genus, species	Mycelial lectins, GA titre	Surface lectins, GA titre
		Blood type O	Blood type O
1.	*F.solani*	2048	164
2.	*F.solani 1*	1,024	16
3.	*F.solani 2*	4	-
4.	*F.solani 3*	256	8
5.	*F.solani 4*	128	2
6.	*F.solani 5*	-	-
7.	*F.solani 6*	4096	128
8.	*F.solani 7*	64	2
9.	*F.solani 8*	1,024	32
10.	*F.solani 9*	16	-
11.	*F.solani 10*	32	-
12.	*F.solani 11*	-	-
13.	*F.solani 12*	256	32
14.	*F.solani 13*	64	8
15.	*F.solani 14*	-	-
16.	*F.solani 15*	512	32
17.	*F.solani 16*	8	-
18.	*F.solani 17*	128	16

SUMMARY:

Analyzing the haemagglutinating activity of general mycelial and surface lectins with the phytopathogenic properties of *F.solani* micromycetes, we found the relationship between these indicators. The higher the phytopathogenicity of the studied isolate is, the more active the lectins synthesized by this micromycete are. Thus, *F.solani 6* micromycete strain, which had a

strong phytopathogenic effect on seed germination of test objects (seed germination - 11.6 and 28.5% for wheat and pea, respectively), damage or rot of their root and root parts (7.1 and 7.9% for wheat, 15.3% and 18.8% for peas) and the root system (16.5% and 17.2% for wheat, 35.5 and 43.6% for peas), had the most active lectins, both the mycelium in general, and the lectins of its surface. Strains that lacked lectin activity or were insignificant (*F.solani 5, F.solani 10, F.solani 11, F.solani 14*) did not affect the germination of plant seeds and were not capable of damaging them.

CONCLUSION:

Thus, the established relationship between the activity of micromycete lectins and their phytopathogenicity makes it possible to recommend this indicator of lectin activity as an additional method of accelerated determination of the relation of the micromycetes of the genus *Fusarium* to phytopathogenic or saprophytic species.

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