CAUSATIVE AGENTS OF BROWN STAINING OF LEAVES AND ROOT ROT OF TOMATOES IN CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract. On the basis of macro- and microscopic characteristics of fungi isolated from tomato plants with symptoms of the disease, it was found that the aerial part of the plant was affected by fungi Alternaria – *A. alternata, A. consortiale*, and Phytophthora – *P. infestans*; the subterranean part (root) was attacked by fungi *Fusarium* – *F. oxysporum* var. *orthoceras* and *F. solani*.. The research of the grade of attack on the foliage of plants in the process of growth has demonstrated its increase from the beginning of fruiting to the mass fruit ripening. This indicates the intensification of the disease state. The correlation coefficients of the grade of attacks of blight and fusariosis were low (with one exception); it can suggest that the resistance of tomato to the causative agents is under the individual genetic control.

Keywords: tomato, fungi, *Alternaria* spp., *Phytophthora* spp., *Fusarium* spp.

Rezumat. Agenţii cauzali ai pătării brune a frunzelor şi putrezirii rădăcinii la tomate în condiţiile Republicii Moldova. În baza caracteristicilor macro- şi microscopice ale fungilor izolaţi din plante de tomate cu simptome de boală, s-a constatat că partea aeriană a plantelor a fost afectată de fungii Alternaria – *A. alternata, A. consortiale* şi Phytophthora – *P. infestans*, iar partea subterană (rădăcina) a plantelor – de fungii Fusarium – *F. oxysporum* var. *orthoceras* şi *F. solani*. Cercetarea gradului de atac al aparatului foliar al plantelor în evoluţia creşterii – de la începutul fructificării până la coacerea în masă a fructelor a demonstrat creşterea acestuia, ceea ce denotă intensificarea stării de boală. Coeficientul de corelaţie a gradului de atac al alternariozei cu cel al fuzariozei a înregistrat (cu o singură excepţie) valori joase, ceea ce denotă că rezistenţa tomatelor la agenţii cauzali se află sub control genetic individual.

Cuvinte cheie: tomate, fungi, *Alternaria* spp., *Phytophthora* spp., *Fusarium* spp.

INTRODUCTION

Although tomatoes are cultivated in different climatic conditions, growth and development of this crop are affected by the strong influence of the limiting environmental factors, including fungal diseases and low temperatures at early stages of development (FOOLAD, 2007), so demonstrating that genetic resistance are diminished by the above-mentioned factors. Tomatoes are susceptible to more than 200 pathogens - fungi, bacteria, viruses, nematodes (FOOLAD, 2007; YANG et al., 2005).

At present, fungal pathogens *Fusarium* spp. and *Alternaria* spp. are remarked in the Republic of Moldova as biotic unfavourable factors supressing growth and development of cultural plants and being significantly extended. They provoke different diseases in a wide circle of species of agricultural and technical plants: tomato, wheat, sorghum, barley, sunflower, rapeseed, cotton, etc., being responsible for huge economic losses (ROTEM, 1994; XU et al., 2008). Diverse relationships are established between plant and pathogen, which are defined by resistance of the genotype, virulence of the fungus, environmental conditions, etc. (LUPAȘCU et al., 2015).

Fungal pathogen *Fusarium* spp. causes root and stem rot, and *Alternaria* spp., together with that, causes brown staining of leaves and fruits on tomato (ROTARU, 2011; GRIGORCEA et al., 2011).

In accordance with modern literature data it is known that the species *Alternaria alternata* manifests high frequency and aggressiveness, occupying ecological niches or decreasing the extension of the species *A. solani* (Ell. et Mart.), which is the basic species on tomatoes in many regions (KUSABA & TSUGE, 1995).

The aim of our research is to identify the fungal pathogens that cause leaf brown staining and root rot in tomatoes and to determine the degree of attacks of the causative agents to plants.

MATERIAL AND METHODS

Four parental forms – Gloria, Jubiliar, Atlasnii, Zastava, and reciprocal hybrids F_4 – Gloria x Jubiliar (oblong fruit – o.f.); Gloria x Jubiliar (spherical fruit – s.f.); Jubiliar x Gloria (o.f); Jubiliar x Gloria (s.f.); Gloria x Atlasnii; Atlasnii x Gloria (I); Atlasnii x Gloria (II); Gloria x Zastava; Zastava x Gloria –were taken as the material for research.

Small fragments of leaves, fruits, and roots with symptoms of the disease were used for the isolation of fungi and identification of species that provoked the disease in tomato plants. The fragments were sterilized in the 2 % solution of lime hypochlorite for 1-2 minutes, then rinsed 2-3 times in bidistilled water, pressed between two sheets of filter paper and placed on the medium with PDA (*Potatoes Dextrosis Agar*) in aseptic conditions using gas flame (Fig. 1).

A B C D

Figure 1. The isolation of pathogens from fragments of leaves (A), fruits (B, C), and roots (D) of diseased tomato (original).

The species of causative agents have been identified on the basis of macro- (Fig. 1) and microscopic characteristics (Fig. 2) using fungal guides (ELLIS, 1971; PIDOPLICICO, 1977).

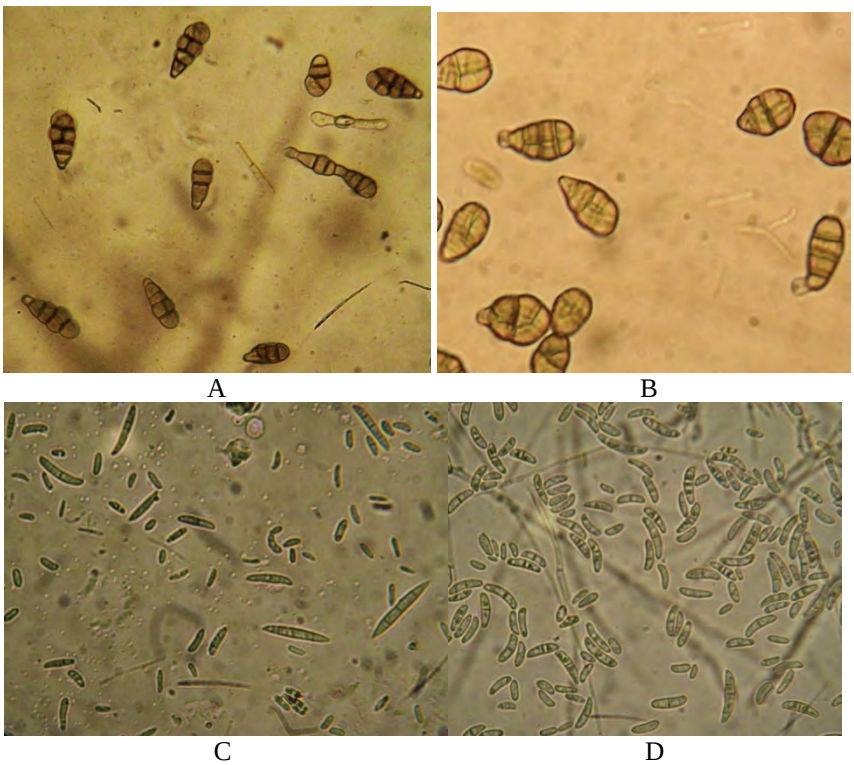
Evaluation of the degree of disease attacks was carried out in the field conditions based on symptoms of disease (brown stains, ulcers, necrosis) ranged in accordance with the scale of 6 grades, developed by the authors: 0 – healthy, immune (disease free), 1– highly resistant – HR (10 %), 2 – resistant – R (20 %), 3 – middle resistant – MR (30 %), 4 – sensitive – S (40 %), 5 – highly sensitive – HS (≥ 50 % of surface with symptoms of disease).

The obtained data obtained statistically processed using software package STATISTICA 7.

RESULTS AND DISCUSSION

Composition of the species involved in the developing of one or another disease is often different (XU et al., 2008). Moreover, high polymorphism of pathogenic agents, as a result of the liability of genetic and epigenetic systems, causes high adaptability of these agents to environmental conditions (YLI-MATTILA & GAGKAEVA, 2010). Taking this into account, one can understand that the studies of composition of fungi species, which cause diseases in tomato in the Republic of Moldova, are of interest.

The results of microscopic analysis of fungi showed that the aerial part of the tomato plant was affected by the fungi Alternaria – *A. alternata*, *A. consortiale*, and Phytophthora – *P. infestans*. The underground part of the plant (root) was affected by Fusarium fungi – *F. oxysporum* var. *orthoceras* and *F. solani* (Fig. 2).



A B C D

Figure 2. Microscopic aspect of the fungi *Alternaria. alternata* (A), *A. consortiale* (B), *Fusarium oxysporum* var. *orthoceras* (C), *F. solani* (D) (500^x) (original).

The data demonstrated that the highest percentage of the manifestation of brown leaf spots is achieved by the species *Alternaria alternata*, coming up to 39.6%, and of root rot – by *F. oxysporum* var. *orthoceras*, coming up to 40.7%, respectively (Table 1).

Table 1. Composition of the fungi species that provoked diseases on tomato in the field conditions (2015).

Species	Isolate, num.	Frequency,%
Brown leaf spots		
Altemaria alternata	36	39.6
A. consortiale	11	12.1
Phytophtora infestans	6	6.6
Root rot		
Fusarium oxysporum var. orthoceras	37	40.7
F. solani	1	1.0
Total	91	100.0

The fungus *Phytophtora infestans* was registered with a frequency of 6.6 %.

The research of the degree of attack on the aerial surface of plants demonstrated its increase from the beginning stage of fruiting to the stage of mass fruit ripening. This indicates the intensification of the disease state.

Parental varieties as well as hybrids F_4 manifested different types of reaction: resistant (Jubiliar, Zastava, F_4 Gloria x Jubiliar (I), F_4 Jubiliar x Gloria (I), F_4 Jubiliar x Gloria (II), F_4 Jubiliar x Gloria (III), F_4 Gloria x Atlasnii, F_4 Gloria x Zastava, F_4 Zastava x Gloria), middle resistant (Gloria, F_4 Gloria x Jubiliar (II)), sensitive (Atlasnii, F_4 Atlasnii x Gloria (I), F_4 Atlasnii x Gloria (II)). Immune, highly resistant or highly sensitive genotypes were not found. Hybrids F_4 had values of the attack nearest to the parent with smaller value (i.e. more resistant) except of the combination F_4 Atlasnii x Gloria (I) (Fig. 3).

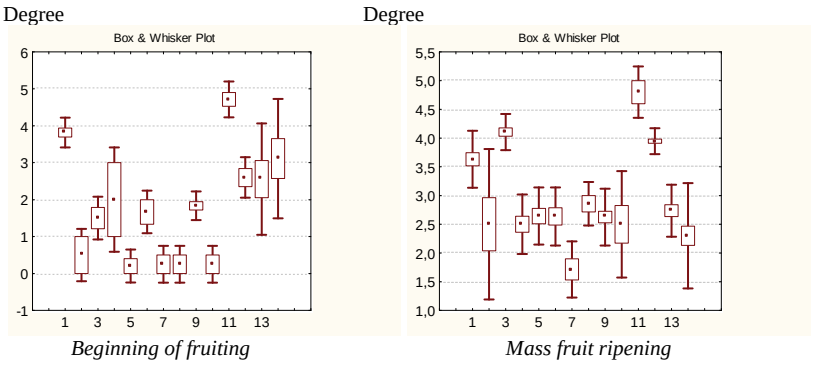


Figure 3. Brown leaf spots on tomato plants in the field conditions (2015).

Legend:
1 – Gloria; 2 – Jubiliar; 3 – Atlasnii; 4 – Zastava; 5 – F_4 Gloria x Jubiliar (I); 6 – F_4 Jubiliar x Gloria (I); 7 – F_4 Jubiliar x Gloria (II); 8 – F_4 Gloria x Jubiliar (II); 9 – F_4 Jubiliar x Gloria (III); 10 – F_4 Gloria x Atlasnii; 11 – F_4 Atlasnii x Gloria (I); 12 – F_4 Atlasnii x Gloria (II); 13 – F_4 Gloria x Zastava; 14 – F_4 Zastava x Gloria

The degree of fusariosis attack ranged within 2.58 ... 3.53 in parental forms and 1.26 ... 3.94 in reciprocal hybrids F_4, respectively. The following types of reactions were registered: resistant (F_4 Gloria x Jubiliar (I), F_4 Jubiliar x Gloria (I), F_4 Jubiliar x Gloria (II), F_4 Gloria x Jubiliar (II), F_4 Atlasnii x Gloria (I)), middle resistant (Jubiliar, Atlasnii, Zastava, F_4 Atlasnii x Gloria (II), F_4 Gloria x Zastava), sensitive (Gloria, F_4 Jubiliar x Gloria (III), F_4 Gloria x Atlasnii, F_4 Zastava x Gloria) (Fig. 4).

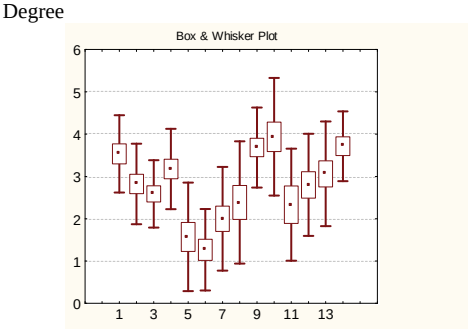


Figure 4. Root rot on tomato plants in the field conditions (2015).

Legend:
1 – Gloria; 2 – Jubiliar; 3 – Atlasnii; 4 – Zastava; 5 – F_4 Gloria x Jubiliar (I); 6 – F_4 Jubiliar x Gloria (I); 7 – F_4 Jubiliar x Gloria (II); 8 – F_4 Gloria x Jubiliar (II); 9 – F_4 Jubiliar x Gloria (III); 10 – F_4 Gloria x Atlasnii; 11 – F_4 Atlasnii x Gloria (I); 12 – F_4 Atlasnii x Gloria (II); 13 – F_4 Gloria x Zastava; 14 – F_4 Zastava x Gloria

The calculation of correlations between the degree of attack of the blight and of the fusariosis showed decreased levels of these coefficients except of the hybrid population F_4 Atlasnii x Gloria (I) (-1.0 *) (Table 2).

Table 2. Correlations between the degree of attack of the diseases on tomato plants.

Genotype/reciprocal hybrid F_4	**r**
	Brown leaf spots – fusariosis root rot
Gloria	0.11
Jubiliar	-0.22
Atlasnii	-0.04
Zastava	0.36
F_4 Gloria x Jubiliar (I)	-0.26
F_4 Jubiliar x Gloria (I)	0.45
F_4 Jubiliar x Gloria (II)	-0.00
F_4 Gloria x Jubiliar (II)	-0.54
F_4 Jubiliar x Gloria (III)	0.13
F_4 Gloria x Atlasnii	-0.40
F_4 Atlasnii x Gloria (I)	-1.0*
F_4 Atlasnii x Gloria (II)	0.18
F_4 Gloria x Zastava	0.37
F_4 Zastava x Gloria	-0.45

*-$p \leq 0.05$.

The results of the correlation analysis show that the resistance of tomato to fungi that produce brown leaf spots and root rot is under individual genetic control.

CONCLUSIONS

1. It was found that the diseases on tomato plants in the field conditions of the 2015 year were provoked by fungi Alternaria: *A. alternata, A. consortiale,* Phytophthora: *P. infestans,* and Fusarium: *F. oxysporum* var. *orthoceras, F. solani*; the highest frequency was registered by *A. alternata* (39.7%) and *F. oxysporum* var. *orthoceras* (40.7%).

2. Diseases on the foliage of tomato multiplied in the process of plants growth from the stage of beginning of fruiting to the stage of mass fruit ripening that indicated intensification of disease state.

3. The degree of infection of tomato plants ranged within 1.71 ... 4.8 and 1.2 ... 3.7 for brown leaf spots and fusariosis, respectively.

4. Correlation analysis has not demonstrated any dependencies between the degree of attacks of brown leaf spots and fusariosis; it can suggest that the resistance of tomato to the respective causative agents is under the individual genetic control.

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