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Short communication

FUNGAL PATHOGENS OF TOMATO IN SOUTH-WESTERN RUSSIA (KRASNODAR TERRITORY)

E.M. Chudinova[1], T.A. Shkunkova[1], S.N. Elansky[1,2]*

[1] Peoples' Friendship University of Russia, Moscow, Russia
[2] Moscow Lomonosov State University, Moscow, Russia

**corresponding author, e-mail: snelansky@gmail.com*

During a study of fungal diseases of tomato in the South of Russia (Krasnodar Territory) 56 fungal isolates associated with tomato fruits were obtained. Most of them belonged to the species *Alternaria alternata. Alternaria solani, Fusarium equiseti, Phomopsis phaseoli, Chaetomium cochliodes, Clonostachys* sp., *Irpex lacteus*, *Colletotrichum coccodes* were also identified. Laboratory experiments revealed that *Clonostachys* sp., *C. cochliodes, P. phaseoli, I. lacteus,* and *F. equiseti* developed well on the fruit's slices. *Fusarium equiseti* was the only species that can penetrate the tomato through epidermis and infect entire fruit. The most effective fungicide against *F. equiseti* was difenoconazole (EC_{50} = 0.08 mg/L); pencycuron was also effective (EC_{50} = 32.5 mg/L). Thiabendazole completely inhibited the growth of *F. equiseti* at the concentration 100 mg/L (EC_{50} = 47 mg/L).

Keywords: fungicides, tomato diseases, *Fusarium equiseti, Phomopsis phaseoli, Chaetomium cochliodes, Clonostachys* sp., *Irpex lacteus, Alternaria solani*

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Introduction

Climatic conditions allow the cultivation of tomato in open ground in the southern regions of Russia. In the Krasnodar Territory (2018) farmers grow tomato in open fields on an area of 750 hectares; the total yield is about 9 thousand tons (ab-centre.ru, small private gardens and greenhouses are not accounted). When grown in open ground, tomatoes are severely affected by diseases and pests. The most common diseases in the south of Russia and adjacent countries are late blight (caused by *Phytophthora infestans* (Mont.) de Bary), early blight (*Alternaria* spp.), Septoria leaf spot (*Septoria lycopersici* Mart.), Fusarium rot (*Fusarium* sp.), root and stem rot (*Pythium ultimum* Trow), powdery mildew (*Erysiphe communis* (Wallr.) Schltdl., *Oidium lycopersici* Cooke & Massee), white rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), gray mold (*Botrytis cinerea* Pers.), leaf mold (*Fulvia fulva* (Cooke) Cif. = *Cladosporium fulvum*), black tomato fruit rot (*Remotididymella destructiva* (Plowr.) Valenz.-Lopez, Cano, Crous, Guarro & Stchigel = *Phoma destructiva*) (Agaev et al., 2014).

In addition to the aforementioned widespread phytopathogenic microorganisms, new ones are currently appearing. They can cause diseases similar in symptoms. These microorganisms may differ in pathogenicity and resistance to fungicides. The use of effective fungicide preparations is the basis of high-quality tomato protection. That is impossible without the monitoring of tomato pathogens. The aim of our work was to analyze tomato fungal pathogens to search for new species atypical for Southern Russia.

Materials and Methods

The paper represents the results of a study of mycobiota associated with affected tomato fruits in two studied fields of the Krasnodar Territory (Slavyansk-na-Kubani district). There were many plants with lesions caused by insects consequently colonized by bacteria and fungi, as well as plants with fungal, bacterial damage, mixed lesions, and lesions resulting from

sunburn. For the analysis of fungal pathogens, fruits with brown spots or whitish mycelium without punctures of the surface caused by insects were selected. In each case one fruit per plant was taken. Fruits were washed carefully, and surface was sterilized with 70% alcohol. Their slices were placed in moist chambers. Mycelium or spores were taken from alive tissue using needle under the microscope and placed on a Petri dish with wort agar mixed with penicillin. Further, axenic cultures of fungi were analyzed according to cultural-morphological characteristics. To confirm the results of cultural-morphological identification of species, sequencing of species-specific DNA region (ITS1-5.8S-ITS2, primers ITS5-ITS4, White et al., 1990) was performed for all isolates except small-spores *Alternaria*.

Pathogenicity tests were conducted on symptomless, detached green tomato fruits, with surface sterilized using ethanol (70%) and on slices of these fruits. Sterilized fruits were washed in three changes of distilled water. Agar plugs with fungal mycelium was placed in the center of the slice or on the surface of the fruit. Control fruit or slice was inoculated with a small piece of agar only. The fruits were then incubated in a plastic container at 23 °C with wet paper placed on the bottom. Slices were incubated at the same temperature in Petri dishes on the glass lying on the wet paper. Tested fruits and slices were examined for mycelium development for 7 days after inoculation.

Estimation of fungicidal activity was carried out on Petri dishes with different concentrations of the studied fungicides. A block of colonized agar was placed in the center of Petri dish with hard oat medium of four gradually increasing concentrations of active compound: 0.1; 1.0; 10.0 and 100.0 mg/l. The medium without the fungicide was used as a control. Two perpendicular diameters of each colony were measured when diameter of control colony was 70–80% from radial size of Petri dish. After the measurements average diameter for each isolate was calculated. The effective inhibitory concentration EC_{50}, i.e. the concentration of a fungicide in the medium needed to reduce the radial growth of a colony by half in comparison to fungicide-free control, was determined.

Results and Discussion

During this study 56 fungal isolates were obtained. The vast majority (44 isolates) belonged to the species *Alternaria alternata* (Fr.) Keissl. Other species, such as *Alternaria solani* Sorauer, *Colletotrichum coccodes* (Wallr.) S. Hughes, *Fusarium equiseti* (Corda) Sacc., *Phomopsis phaseoli* (Desm.) Sacc., *Chaetomium cochliodes* Palliser, *Clonostachys* sp. and *Irpex lacteus* (Fr.) Fr. were also isolated (table 1).

Table 1. Fungal species, isolated from tomato fruits

Species name	Number of strains	GenBank accession number
Alternaria alternata	44	Not tested
Alternaria solani	2	KY496637*
Colletotrichum coccodes	2	MT292616*
Fusarium equiseti	1	MT588081
Phomopsis phaseoli	2	MH412692*
Chaetomium cochliodes	3	MT279444*
Clonostachys sp.	1	MT588112
Irpex lacteus	1	MT276332

* – sequences of all strains were identical

Fusarium equiseti is widespread on tomato in Asian countries (Akbar et al., 2018), but its distribution in Russia has not been studied. *Phomopsis phaseoli* is one of the common pathogens of soybeans; this fungus was first discovered on tomato (Elansky et al., 2019). The basidiomycete *I. lacteus* is a wood white rot fungus, which has not been recorded as tomato pathogen. Soil saprotrophic fungi *Chaetomium cochliodes* Palliser and *Clonostachys* spp. form antagonistic relationship with many soil microorganisms. One of *Chaetomium* species is used in the commercial preparation of Ketomium, which inhibits the growth of pathogens of many significant crops, including tomato (Soytong et al., 2001). Strains of the genus *Clonostachys* are widely used in biotechnological applications (Borges et al., 2015).

Some isolated fungal species had never been typical tomato pathogens in Russia. Since these fungi were isolated from affected tomato fruits, we evaluated their ability to develop on tomato fruits and slices in a moist chamber. According to our experiments, *Clonostachys* sp., *C. cochliodes, P. phaseoli,* and *I. lacteus* were not able to penetrate the tomato epidermis and infect fruits, but they developed well on fruits' cuttings. On day 7 after infection with *Clonostachys* sp., a lesion of 18±2 mm was formed on tomato slices (average diameter for 3 tested slices ± standard deviation). Inoculation with other fungal species was also resulted in lesions: 15±3 mm (*C. cochliodes*), 25±3 mm (*P. phaseoli*), 29±4 mm (*I. lacteus*). Apparently, these fungi can parasitize on tomato fruits when a crack occurs on their surface. *Fusarium equiseti* showed high aggressiveness in slices test, after 7 days the tomato slices were completely braided with its hyphae. *Fusarium equiseti* was the only tested pathogen that can infect the tomato fruits through the epidermis.

In the present study *F. equiseti* was first discovered on tomato in Russia. We tested its susceptibility to the following fungicides: difenoconazole (preparation Score), thiabendazole (Tecto) and pencycuron (Prestige) (table 2). The most effective fungicide was difenoconazole (EC_{50} = 0.08 mg/L). This drug

Table 2. The diameter of the colonies of *F. equiseti* in Petri dishes with medium containing fungicides

Fungicide	Colony diameter* at different active compound concentrations (mg/L)					EC_{50}**, mg/L
	0	0.1	1	10	100	
Diphenoconazole	45±2	20.5±1	6.5±0.5	4±1	Not tested	0.08
Thiabendazole	47±2	Not tested	42±5	36±4	0	47
Pencycuron	47±2	Not tested	41±5	26±3	10±1	32.5

* – average diameter for 3 tested Petri plates (mm) ± standard deviation,

** EC_{50} – the concentration of fungicide (active ingredient) in the medium needed to reduce the radial growth of a colony by half in comparison to fungicide-free control.

is used for treatment of vegetative tomato plants against early blight. Thiabendazole is recommended for sterilization of storages. Our data showed that at a concentration of 100 mg/L it completely inhibits the growth of *F. equiseti*. Pencycuron is effective against *F. equiseti* (EC_{50} = 32.5 mg/L) and can be recommended for the treatment of tomato seeds (Catalog..., 2020).

Our research provided new information on the mycobiota of tomato fruits in Southern Russia, the main potato producing region of the country. Several new affected tomato fruits with fungal species were found. Detection of new pathogens showed the need for disease monitoring and optimization of disease control management.

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Краткое сообщение

ГРИБНЫЕ ПАТОГЕНЫ ТОМАТА НА ЮГО-ЗАПАДЕ РОССИИ (КРАСНОДАРСКИЙ КРАЙ)

Е.М. Чудинова[1], Т.А. Шкункова[1], С.Н. Еланский[1,2]*

[1] Российский университет дружбы народов, Москва, Россия

[2] Московский государственный университет имени М.В. Ломоносова, Москва, Россия

** ответственный за переписку, e-mail: snelansky@gmail.com*

При изучении грибных болезней томата в Краснодарском крае из пораженных плодов были выделены в чистую культуру 56 штаммов грибов. При анализе видовой принадлежности коллекционных изолятов культурально-морфологическими и молекулярными методами оказалось, что большая их часть принадлежала виду *Alternaria alternata*. Также были идентифицированы *Alternaria solani, Fusarium equiseti, Phomopsis phaseoli, Chaetomium cochliodes, Clonostachys* sp., *Irpex lacteus, Colletotrichum coccodes*. Лабораторные эксперименты по заражению ломтиков плодов томата показали, что *Clonostachys* sp., *C. cochliodes, P. phaseoli, I. lacteus, F. equiseti* способны их успешно заражать. *F. equiseti* оказался единственным видом из исследованных, способным заражать неповрежденные плоды, проникая через эпидермис. Оценка восприимчивости *F. equiseti* к фунгицидам выявила, что наибольшей эффективностью отличался дифеноконазол (EC_{50} = 0.08 mg/l). Пенцикурон также показал хорошую эффективность (EC_{50} 32.5 mg/l). Тиабендазол полностью ингибировал рост колонии *F. equiseti* при концентрации 100 mg/l (EC_{50} = 47 mg/l).

Ключевые слова: фунгициды, болезни томата, *Fusarium equiseti, Phomopsis phaseoli, Chaetomium cochliodes, Clonostachys* sp., *Irpex lacteus, Alternaria solani*

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