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PATHOCOMPLEX OF PATHOGENIC FUNGUS IN TABLE CARROT AND RHIZOSPHERE DEPENDING ON A GROWING REGION

L.M. Sokolova, O.O. Beloshapkina, A.A. Rubtsov

In the pathocomplex of any agricultural crop, there are different micromycetes, the composition of which varies depending on weather and climatic conditions, seasonal changes. The fungus A. dauci (J. G. Kühn) Groves et Skolko is one of the most harmful pathogens on table carrots, affecting leaves, petioles, and stems. A. radicina Meier, Drechsler et E. D. Eddy damages carrot root crops during storage, causing a hard rot, coal-black, sharply delimited from healthy tissue.

These pathogens occur everywhere and simultaneously; n addition, the optimal conditions for the development of both pathogens are similar. Yield losses from Alternaria can reach 40–99 %. The complex of fungal species of the genus Fusarium is a ubiquitous soil pathogen of agricultural crops with a wide phylogenetic and ontogenetic specialization, which causes destructive vascular wilting, rot, and necrosis.

The most common are F. oxysporum and F. avenaceum, the latter can also exist as a saprotroph. As a result of the work done, the released mycelium for drawing up the palette is described. It was revealed that the colonies of fungi with light-colored mycelium (54.8%) were larger in the samples from the Voronezh region, which were based on species of the genus Fusarium. In the Moscow region with sufficient moisture, isolates of fungi with light-colored mycelium slightly dominated (43.7 %). In the Rostov region, the frequency of occurrence of isolates of fungi with dark-colored mycelium was on average 58.1%, dominated by fungi of the genus Alternaria.

Keywords: *carrot; regions of cultivation; fungi isolates; morphological and cultural peculiarities of un vitro*

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ПАТОКОМПЛЕКС ВОЗБУДИТЕЛЕЙ ГРИБНЫХ БОЛЕЗНЕЙ НА РАСТЕНИЯХ МОРКОВИ СТОЛОВОЙ И РИЗОСФЕРЫ В ЗАВИСИМОСТИ ОТ РЕГИОНА ВЫРАЩИВАНИЯ

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В патокомплексе любой сельскохозяйственной культуры присутствуют различные микромицеты, состав которых меняется в зависимости от погодно-климатических условий и сезонных изменений. Гриб A. dauci (J. G. Kühn) Groves et Skolko является одним из наиболее вредоносных патогенов на столовой моркови, который поражает листья, черешки и стебли. A. radicina Meier, Drechsler et E. D. Eddy повреждает корнеплоды моркови при хранении, вызывая сильную резко отграниченную от здоровой ткани угольно-черную гниль. Эти патогены встречаются повсеместно и одновременно. Кроме того, оптимальные условия для развития обоих возбудителей схожи. Потери урожая от грибов рода Alternaria могут достигать от 40% до 99%. Комплекс видов грибов рода Fusarium – это повсеместный почвенный патоген сельскохозяйственных культур с широкой филогенетической и онтогенетической специализацией, вызывающий деструктивное сосудистое увядание, гниль и некроз. Наиболее распространены F. oxysporum и F. avenaceum; последний также может существовать как сапротроф. В результате проделанной работы описан выпущенный мицелий для составления палитры. Установлено, что колонии грибов со светлоокрашенным мицелием (54,8%) были больше в образцах из Воронежской области, основу которых составляли виды рода Fusarium. В Московской области при достаточном увлажнении незначительно преобладали изоляты грибов со светлоокрашенным мицелием (43,7%). В Ростовской области частота встречаемости изолятов грибов с темноокрашенным мицелием составила в среднем 58,1%; преобладали грибы рода Alternaria.

Ключевые слова: *морковь; районы возделывания; изоляты грибов; морфокультуральные особенности in vitro*

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Introduction

The pathocomplex of any agricultural crop contains different micromycetes, the composition of which varies depending on weather and climatic conditions, seasonal changes, the cultivation technology used, plant resistance and many other factors [1; 2; 5; 8]. The dominant pathogens determine the development of the main diseases and the harm caused by them.

Table carrot (*Daucus carota* L. subsp. *sativus* Hoffm.) is grown worldwide to produce root crops rich in α - and β-carotenes [17]. The most common diseases in this culture are alternariosis and fusariosis. Mushroom *A. dauci* (J. G. Kühn) Groves et Skolko is one of the most harmful pathogens on table carrots, affecting leaves, petioles, and stems [14]. *A. radicina* Meier, Drechsler et E. D. Eddy damages carrot roots during storage, causing a hard rot, coal-black, sharply separated from healthy tissue [14]. *A. dauci and A. radicina* are found everywhere and often simultaneously. In addition, the optimal conditions for the development of both pathogens are similar. YIELD losses from alternarioses can reach 40%–99% [13; 18; 20].

The complex of fungal species of the genus *Fusarium* is a ubiquitous soil pathogen of agricultural crops with a wide phylogenetic and ontogenetic specialization. They cause destructive vascular wilting, rot, necrosis [2; 15; 16]. The most common *are F. oxysporum* and *F. avenaceum*. The latter can also exist as a saprotroph [4; 11]. Symptoms of fusarioses are manifested on table carrots more often during the storage period in the form of foci of dry light brown rot and ulcers on the surface of root crops [19].

The research **aims** (1) to identify micromycetes from table carrot plants and rhizosphere depending on the region of cultivation, (2) to determine the frequency of pathogens' occurrence and compare their prevalence by cultural and morphological characteristics.

The following are the research tasks:

1. Select the vegetable material of table carrots affected by fungal diseases from different ecological-geographical zones;
2. Select samples from different layers of the rhizosphere (soil);
3. Analyze and identify specified pathogens using phytopathological methods;
4. Describe the released mycelium to make a palette.

Scientific novelty

Highly aggressive causative agents of the genus Fusarium were identified in the Moscow and Voronezh regions. It is based on the data of long-term phytomonitoring on the increase in the soil and plant micromycetes harmfulness in various ecological-

geographical zones of carrot cultivation in Russia *Fusarium* were identified in the Moscow and Voronezh regions. It is based on the data of long-term phytomonitoring on the increase in the soil and plant micromycetes harmfulness in various ecological-geographical zones of carrot cultivation in Russia.

The main methodological principles for the isolation of soil micromycetes were determined and scientifically substantiated; the "palette" of myceliums for different ecological and geographical zones was compiled and comprehensively studied.

Materials and methods

The research used plant material (leaves, roots) of table carrots and soil samples of the rhizosphere taken from different soil horizons: 5, 10, and 20 cm. The selection of materials was carried out: at the Federal State Budgetary Scientific Institution of the All-Russian Scientific Research Institute of Vegetable Growing - a branch of the Federal Scientific Center of Vegetable Growing; at the Voronezh vegetable experimental station - a branch of the Federal Scientific Center of Vegetable Growing; at the Biryuchekutskaya vegetable experimental station - a branch of the Federal Scientific Center of vegetable growing. These regions differed significantly during the growing season in terms of temperature parameters and precipitation, as well as soil conditions:

Sampling, laboratory experiments, registration of fungal organisms was carried out using standard techniques, including: Methods of experimental study of microscopic fungi [10]; Rapid method for isolation in pure culture and characterization of fungi of the genus *Fusarium*, stir the table carrots [12]; the Methods of evaluation and source of breeding material to carrot disease resistance [3]; the evaluation of the stability of carrot to Fusarium dry rot [9]; Manual of diseases of agricultural crops [7]. Standard phytopathological methods (microscopic, wet chamber, microbiological) were used. The isolation of fungi into a pure culture was carried out on nutrient agar media: starvation agar, potato sucrose agar, Czapek's medium, carrot agar [6]. The researches were based on obtaining pure cultures of mycelium isolates of fungi from different regions, followed by a visual assessment of the ratio of dark and light-colored colonies.

Identification of the isolated micromycetes was carried out using determinants.

Results

The hypothesis was tested that specific epiphytic species of fungi (associated with the carrot plant or with the growing region) are localized in different regions of carrot growth.

The main feature that authors relied on in their research is the descriptive characteristic of pathogens in pure culture by the color and consistency of the mycelium on an agar medium. Fungi were divided into two main groups (light-colored and dark-colored) according to the intensity of color, which varied from white to almost black.

For fungi of the genus *Fusarium*, the mycelium is characterized by light-colored – white, white-pink, yellowish. In *Alternaria* sp. the mycelium is dark-colored – from yellow-brown or olive to dark gray, almost black. In *Penicillium* sp. – the mycelium is green, and yellow-green, gray-blue. Mycelium *Aspergillus* sp. can be dark yellow, green, dark gray, and black.

From the studied material (leaf plate, root crop, soil) selected in the conditions of the Moscow region (Table 1), 154 isolates were isolated into a pure culture. Among them, there are representatives of the genera *Fusarium, Alternaria, Acremonium prevailed*. Colonies of fungi with light-colored mycelium, having a white color, or white with a slight pink, yellow, or gray tint, accounted for 43.7% of the total number of isolates. Mycelium of fungi with a more intense color (green, yellow, gray) was observed in 37.1% of colonies. Dark-colored mycelium (dark gray, olive-brown, black-green) had 19.2%.

Table 1.

Species composition, frequency of occurrence, and color of the mycelium of pathogens (in the breeding fields of All-Russian Research Institute of Olericulture (ARRIO)

Species	Mycelium coloration	Frequency of occurrence, %
light-colored colonies		
Fusarium oxysporum	white	16.7
F. sambucinum	white	9.6
F. culmorum	white	15.2
F. heterosporum	white	9.1
F. moniliforme	white with a pink tinge	12.1
F. equiseti	white with a yellow tinge	7.6
F. culmorum	light yellow	6.1
Acremonium charticola	pale pink	6.0
Mycelia st.	white with a gray tinge	7.6
Gliocladium roseum	light pink	9.8
colonies of medium-intense color		
Aspergillus flavus	yellow-green	2.6
Aspergillus sp.	light green with various shades	13.6

End of Table 1.

Penicillium cumemberti	light green with various shades	1.3
P. chrysogenum	light green with a gray tinge	3.2
Penicillium sp.	green-yellow	11.7
Mycelia st.	green with various shades of gray-yellow	2.6
dark-colored colonies		
Aspergillus fumigatus	black and green	1.3
Aspergillus sp.	dark gray	7.8
Alternaria tenuis	brown	1.9
Alternaria sp.	olive-black	8.4

From samples of plant material of the Voronezh Experimental Station (Table 2), 62 isolates were isolated into a pure culture. Among them, there prevailed species with light-colored colonies (54.8%). The number of species with medium intensity mycelium coloration was 24.2%. The number of species with dark-colored colonies was 21.0%.

Table 2.

Species composition, frequency of occurrence, and color of the mycelium of pathogens (on the breeding fields of the Voronezh experimental station)

Species	Mycelium coloration	Frequency of occurrence, %
light-colored colonies		
Fusarium oxysporum	white	11.3
F. culmorum	white	8.1
Fusarium sp.	white with a pink tinge	19.4
Mortierella sp.	white with a gray tinge	6.5
Mycelia st.	white	9.7
colonies with more intense coloration		
Aspergillus sp.	dark yellow	4.8
Penicillium sp.	light green with various shades	6.5
Mucor sp.	gray	3.2
Mycelia st.	gray with different shades	9.7
dark-colored colonies		
Alternaria tenuis	brown	4.8
Alternaria sp	gray-black, olive-black	14.5
Aspergillus sp.	dark gray	1.6

From samples of plant material of the Biryuchekut experimental station (Table 3) 62 isolates were isolated into a pure culture. Among them, species with

dark-colored mycelium (58.1%) and species with medium-intensity mycelium coloration (32.5%) prevailed. The frequency of occurrence of colonies with white mycelium and its shades was slightly higher than 9%.

Table 3.

Species composition, frequency of occurrence, and color of the mycelium of pathogens (on the breeding fields of the Biryuchekutskaya experimental station)

Species	Mycelium coloration	Frequency of occurrence, %
light-colored colonies		
F. culmorum	white with a yellow tinge	4.8
F. moniliforme	white with a pink tinge	4.8
colonies with more intense coloration		
Trichoderma viride	green	3.2
Gliocladium sp.	green-yellow	4.8
Aspergillus sp.	light green with various shades	11.3
Mycelia st.	gray	3.2
Penicillium sp.	green with different shades	8.1
dark-colored colonies		
Alternaria sp.	gray-black, olive-black	22.6
Aspergillus sp.	dark gray	27.4
Macrosporium sp.	brown	8.1

Generalized data on the frequency of pathogens, depending on the geographical point from which the analyzed plant material was obtained, are presented in Table 4.

Table 4.

The frequency of mycelium coloration occurrence of pathogens depends on the geographical point of sampling from breeding sites

Geographic point	Frequency of occurrence of mycelium coloration, %		
	light-colored	average color intensity	dark-colored
ARRIO	43.7	37.1	19.2
Voronezh Vegetable Experimental Station (VES)	54.8	24.2	21.0
Biryuchekutskaya VES	9.6	32.6	58.1

It follows that most isolates of fungi with light-colored mycelium (54.8%) were present in samples from the Voronezh region. This indicator may indicate

that *Fusarium fungi predominate in the carrot pathocomplex in this region.* Samples from the Moscow region were also characterized, although slightly exceeding the frequency of occurrence of light-colored colonies by 43.7%. In the samples from the Rostov region, the frequency of occurrence of colonies of phytopathogenic fungi with dark-colored mycelium was the highest (58.1%). This indicator indirectly indicates the predominance of fungi of the genus *Alternaria in the carrot pathocomplex.*

As mentioned above, the climate of the region has an impact on soil-dwelling pathogens and on plants that affect plants. In the Voronezh region, there is a greater variety of fusarium fungi in humus-rich black-earth soils. In the Rostov region-more dark-colored, better adapted to periodic droughts and active solar insolation.

Fig. 1 shows the "palette" of unique myceliums of the Moscow and Rostov regions.

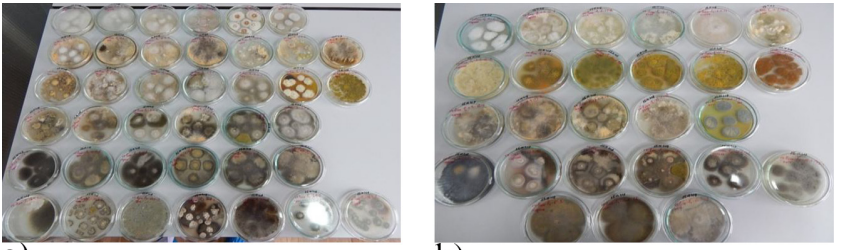

Fig. 1. Palette of unique colonies obtained in a) Moscow region and b) Rostov region

Discussion

There was an assumption that specific types of fungi, associated with the plant or with the region, are localized in different regions of growth, as well as an assumption was made. At the same time, the "profile of the region" (the number of variously and brightly colored mycelium) can be compiled without mandatory identification to the species or genus of fungi. It can be based on the percentage of differently colored unique colonies. The conducted experiment was intended to confirm or refute the research.

One of the most important signs is the color of the colony on the nutrient medium. The intensity of color in different species can vary from white to almost black and depends on the presence of pigments in the cell walls of fungi. *Fusarium* colonies are characterized by light-colored colonies – white or white with a pink, or yellowish, or bluish tint. *Alternaria* colonies are characterized

by dark-colored colonies – yellow-brown, olive, dark gray to black. For *Penicillium* colonies – green and yellow-green colonies of various shades from light to dark. *Aspergillus* colonies can be dark yellow, green, dark gray. The research aimed to (1) identify the ratio of light and dark-colored colonies isolated from soil samples and table carrot plants of various geographical origin and (2) determine the possibility of using the color palette of fungi colonies for the primary diagnosis of the origin of the analyzed samples.

Conclusion

A comparative analysis of the data obtained allows us to conclude that soil samples from the Biryuchekutskaya VES were characterized by a predominance of dark-colored mycelium 58.1%. The samples from more northern regions contained mainly light-colored fungi. Soil samples from ARRIO were characterized by a slight predominance of light-colored colonies of 43.7%. In soil samples from the Voronezh ARRIO, light-colored colonies of 57.8% clearly prevailed.

Based on the data, a "palette of mycelium fungi of the region" was compiled with photos of the mycelium of the dominant phytopathogenic fungi, released from carrots and its rhizosphere, based on the percentage of differently colored mycelium isolates typical for the region. The obtained results can be useful for the initial assessment of pathocomplexes of phytopathogenic fungi in different regions of the country, where the identification of pathogens is problematic due to the lack of specialists or appropriate equipment.

This work was carried out within the framework of a research project "Search, identification and creation of genetic sources and donors of valuable traits using traditional, phytopathological, biotechnological methods, and genetic labeling in the Moscow region."

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