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Species composition and phytotoxic activity of fungi participating in the formation of mycobiota of tomato plant cultivated in Azerbaijan

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Abstract

As a result of the mycological analysis of samples taken from the surface and underground parts of different types of tomato plants cultivated in some ecologically different areas of the Republic of Azerbaijan carried out in 2014-2018, 38 species of fungi were identified and taken to the pure cultures. It became clear that from the registered fungi 5 species belong to the department of fungi-like organisms(Chromista), 3 species to the Zygomycota, 31 species to the Ascomycota. It has been established that the registered fungi are in different trophic relationships with the host plant and although most of them have both conditionally saprotrophs and biotrophy, the true biotrophy of 6 species are the exception. While many of fungi involved in the formation of mycobiota of tomato have phytotoxic activity, among them are also found fungi Trichoderma koningii which synthesized biologically active metabolites in the cultural solution which causes to increased the overall productivity of the plant.

Keywords: tomato, mycobiota, ecologo-trophic relation, phytotoxic activity, total productivity

Introduction

Fungi, characterized by the ability to spread anywhere where organic matter is present also cause various pathologies in both vegetative and generative organs of plants. As a result, the overall productivity of the plant decreases, the quality of the product gets negatively changes, and the aesthetic appearance of the plant and its productivity deteriorates [1]. Thus, fungi that cause different pathologies in plants [4], including those used for practical purposes has always been differed by its relevance and were the subject of separate research. On the other side, a mysterious world and carrying a wide range of biological properties of fungi have always led them to become the main object of various studies [2, 15-16]. One of these areas is the study of their phytopathological aspects. Although nearly two centuries have passed from the suggestion as a precautionary measure of Nayt to use sustainable sorts for the prevent pathologies caused by microorganisms, including fungi today has shown that the study of pathogenic microorganisms, as well as researches conducted in connection with the preparation of combat measures against them, keeps its actuality with

full power [19, 21, 23]. This can be explained by the fact that the influence of the anthropogenic factor on the environment in recent years is rising and as a result of this process, the environmental situation is changing not only locally or regionally already changes globally. Unfortunately, in most cases, this change is observed in the deterioration direction. According to this change, occurrence the evolution of fungus closely with the evolution of host organisms, from time to time leads to the formation not only morphological but also biologically different forms, as well as the forms that are resistant to the used (physical and chemical) fighting methods [6, 19].

As a small part of the globe, the nature of the Republic of Azerbaijan is also under the influence of changing environmental conditions of the world that is globalizing day by day. As a result of the restoration of Azerbaijan's independence, emergence qualitative has changed in the economy which necessitated to the reconsideration of measures carried out related to phytopathological researches [11]. As a result of changes in the country's transition to the market economy, the transfer of lands to private ownership and formed private farming instead of state and collective farms have also changed the phytosanitary situation in the country. As a result, the old system, its infrastructure, as well as the old communication mechanisms designed to secure the material and technical base broke up. As a result, the deterioration of the country's phytosanitary status and as a result of this, the spread of the diseases have significantly increased the likelihood formation of new diseases.

There are various factors that influenced the formation of a phytosanitary state of this or that area [12]. Among these, the diseases caused by microorganisms, in particular, fungi are of particular importance both for the spread range and for the degree of hazard [1, 4, 15-16]. Thus, the fungi differ from the other pathology carriers by adapted abilities, the number of pathologies caused by them, and strong fermentative activity.

Phytopathological researches in the world have been studied until the early 19 th century in the context of mycology researches carry out based on pathologies caused by fungi. Over time, research has been grouped under the level of development of science. The analogy situation began to be observed in Azerbaijan too and research in this area has begun after in the second half of the twentieth century. Although many of the first studies were episodic and covered a specific group of illnesses but systematic studies were also included. Research carried out related diseases like as rust, smut and so on can be an example of this.

Nevertheless, phytopathological researches [7] conducted in Azerbaijan today can not be considered as sufficient to assess the phytosanitary status of the country. More precisely, today the study of fungal diseases in Azerbaijan is an open and interesting object for research.

Occupy an important place in the Azerbaijani economy of the agrarian sector and providing of the country population with plant food products today is one of the issues of any country, including our state [11]. One of these products is a tomato, which characterized by the medicinal significance and most important components of food ration of people. The presence of valuable nutrients in tomatoes, cultivated in any area of Azerbaijan characterized by the fact that phytopathogenic microorganisms form on this which has been repeatedly confirmed in studies conducted from time to time [7]. The spread rate of the pathologies registered in the tomato plant, as well as species composition of pathogens, vary depending on the environmental conditions. For this reason, sometimes to the studied of a phytopathological aspect of a concrete phyto- or agro- senosis has become necessary to return over time. On the other side, their comprehensive study is essential for the preparation of effectual measures against the pathogens registered in a concrete plant.

Taking into consideration all the above, the presented study was intended to the investigated the species composition of fungi involved in the formation of mycobiota and phytotoxic activity, as well as influence on the productivity of tomato plant cultivated in Azerbaijan.

Materials and Methods

Samples for the research were taken from the vegetative and generative organs, as well as from the soil of the rhizosphere of tomato plants cultivated in ecologically different regions (Absheron, Aran, Guba-Khachmaz and Lankaran economic regions) of Azerbaijan. Sampling, passportization and preparation for laboratory analysis have been implemented based on mycology and phytopathological methods [5, 8-9] currently used for this purpose.

The processing of taken samples was carried out in the Scientific-Research Institute of Vegetable of Ministry of Agriculture of the Republic of Azerbaijan, as well as in corresponding laboratories of the Institute of Microbiology of the NAS of Azerbaijan.

For the taking to the pure culture of fungi were used from the nutrient medium: malt juice agar (MJA), rice agar(RA), starchy (SA) and potato agar(PA), Chapek agar(CA), and Capek-Doks agar(CDA). Preparation of mediums, sterilization and to pour of medium to the Petri dishes, transfer of taken samples to the nutrient environment, obtaining clean cultures were carried out in according to the methods [9, 13].

Identification of fungi conducted to the pure culture and their disease were carried out according to the determinant [10, 17, 22] which compiled according to the morphological and physiological symptoms of fungi, as well as visual and microscopic images of the pathologies they have committed.

During the study of the ecophysiological characteristics of fungi for the grow were used liquid Czapek and cultivation was carried out at a temperature of $26-28^{\circ}$ C for 7 days [9]. Growth assessment was based on the dry weight of the biomass formed by fungi, which was reached up to the permanent weight at the 105° C temperature.

During improving the ability of germination of tomato seeds were used aqueous extract obtained from respectively plants and fungi as well as from cultured solutions obtained after 7 days of cultivation and the process was carried out in the following sequence: The visually healthy 100 seeds of the plant were put into controlled solutions in room temperature for 24 hours. Then these seeds are placed on the filter paper soaked with water used for irrigation, and the growth of seeds monitored for 5 days. As control was used those seeds which soaked only with water used for irrigation.

Results and Discussion

During research conducted in 2014-2018, have been processing about 250 samples taken from the surface and underground parts of various tomato plants cultivated in the wild condition in different ecologically areas of the Republic of Azerbaijan and were identified that about 38 species of fungi involved in the formation of mycobiota of this plants. It became clear that from them 5 species (*Phytophthora capsici*,

P.drechsleri, P.infestans, P.parasitica, Pythium debryanum) belong to the fungi-like organisms (Chromista), 3 species (Mucor hiemalis, Rhizopus oryzae, Rh.stolonifer) to the Zygomycota, 31 species(Alternaria alternata, A.solani, Aspergillus niger, Aspergillus rugulosus, Botrytis cinerea, Cercospora Cladosporium fuligena, fulvum, C.tenuissimum, Colletotrichum coccodes. C.lagenarium, C.dematium, C.gloeosporioides, Didymella lycopersici, Erysiphe communis, Fusarium moniliforme. F.oxysporum, F.solani, Glomerella cingulata, Leveillula taurica, Penicillium corvlophilum, P.cyclopium, P.oxalicum, Phoma destructiva, Rhizoctonia solani, Sclerotium rolfsii, Scl.sclerotiorum, Septoria lycopersici, Stemphylium solani. Trichoderma hamatum, T.koningii, Verticillium dahliae) to the department of Ascomycota. Among of a small part of the recorded fungi, especially those found in the samples taken from spread vegetative and generative organs of the plant belonging to the true biotrophs are small and the number of such species is 6, 5 of which belong to the fungi-like organisms, and 1 (E.communis) to telemorphs of sac fungi. However, the vast majority of registered fungi more or less degree are related to pathogenicity. Thus, saprotrophy or biotrophy most of the registered fungi don't carry true character and they receive the organic matter needed to perform vital functions both from living creatures and organisms that have lost their viability. For this reason, almost all of the fungi recorded in the tomato plant can be characterized as organisms that have the potential to negatively affect.

It should be noted that many fungi recorded in the vegetative, and generative organs of the tomato plant also have toxicity properties, and have the ability to synthesize mycotoxins that can lead to dangerous complications for living things, primarily for human health [14]. For example, Alternaria alternata, Aspergillus niger. Fusarium moniliforme. F.oxysporum, F.solani, P.cyclopium, P.oxalicum, Verticillium dahliae so on fungi can be an example of this. If we add that to the above, then the necessity of registration of these issues during the cultivation of tomato plant, firstly of fungi involved in the formation of the mycobiota tomato and the assessment ecobiological features according to the specific characteristics of local conditions are not in doubt.

Interestingly, some metabolites of fungi associated with more or less to pathogenicity have the ability to stimulate the growth of plants, as well as antibacterial and antifungal activity. For example, species from the genus of Fusarium, Trichoderma and so on have the ability to synthesize phytohormones [3, 18, 20] that stimulate the growth of plants, at the same time, there are antagonistic relationships between them, which is due to the presence of metabolites with antifungal activity among the metabolites of the Trichoderma genus.

Fungi, which have these features, also participate in the formation of the mycobiota of the tomato plant. Therefore, at the next stage of the research was also considered appropriate to evaluate the phytotoxic activity of some fungi strains involved in the formation of mycobiota of tomato. Clarification of this issue was carried out based on the germination of the seeds tomato.

From the obtained results became clear that the effect of fungi on the germination ability of the seeds involved in the formation of the mycobiota of tomatoes is also different (tab. 1). As seen, the number of germinating seeds compared to control at the influence of the majority controlled fungi decreases. From the effects of fungi such as Fusarium moniliforme, F. solani, P. cyclopium and Verticillium dahliae in the number of germinating seeds occurs significant reduction which allows to note that these fungi have high phytotoxic activity. In culture solutions (CS) obtained from the other fungi also observed a certain amount of decreases which allows to note that they also have phytotoxic activity though relatively weak. This case is not only observed in fungi T. koningi, T. hamatum, G. roseum. Thus, even the culture solution obtained from the fungi T. koningii compared to the control causes to increase the number of germination seeds by 8.7%. This indicator is 3.7% in the T. hamatum, and 4.4% in the G. roseum. In our view, the increase in the number of germination seeds is due to the presence of biological activity metabolites (BAM) in the culture solution (CS) of these fungi. ts effect on the growth, development, and ultimately productivity of the plant is one of the issues of interest. Therefore it was also considered reasonable to clarify this issue at the next stage of the research.

 Table 1. Influence of culture solution obtained from fungi involved in the formation of mycobiota of the tomato plant to the germination of plant seeds.

N	Fungi species	The total number of used seeds (unit)	Number of germinated seeds (unit)	Growing share seeds(%)
1	Alternaria alternata	150	102	68.0
2	A. solani	150	107	71.3
3	Aspergillus niger	120	90	75.0
4	Aspergillus rugulosus	120	95	79.2
5	Botrytis cinerea	150	101	67.3
6	Cercospora fuligena	120	94	78.3
7	Cladosporium fulvum	120	88	73.3
8	C.tenuissimum	120	93	77.5
9	Fusarium moniliforme	150	75	50.0
10	F. oxysporum	150	70	46.7
11	F. solani	150	72	48.0
12	Gliocladium roseum	140	127	90.7
13	Mucor hiemalis	120	97	80/8
14	Penicillium corylophilum	130	106	81.5
15	P.cyclopium	130	86	66.2
16	P.oxalicum	130	92	70,8
17	Rhizopus oryzae	140	110	78.6
18	Rh.stolonifer	140	117	83.6
19	Trichoderma hamatum	120	108	90.0
20	T.koningii	120	114	95.0
21	Verticillium dahliae	120	68	56.7
	N zar t	150	130	86.7

In this case, plant seeds, more precisely those of the "Leyla" sort before planted soaked in the culture solution of mentioned *T.koningii* for 24 hours and the process is evaluated until the fruit is taken. During the evaluation of the plant were used the size of the growth, the start of the flowering phase, as well as the amount of formed common product. At this stage of research, for the compare also were used from strains of same fungi taken to the pure culture collected by the Microbiological Biotechnology Laboratory of ANAS Institute of Microbiology from the areas (forest lands rich in plant residues, from weak polluted soils) not related to the cultivation of tomato plant. From the result of the research conducted on this issue, become

clear that the presence of BAS in the CS of fungi also retains its influence on the subsequent stage of development of tomato and the quantitative indicator of this effect is also related with the place where were separate used strains (tab. 2). As seen, obtained resultsconfirms once again to the presence of biologically active metabolites in the CS of tested fungi. So that, regardless of the isolated place during use of culture solution obtained from strains of fungi *T. koningii* are observed changes in the growing size of plants, as well as in its overall productivity, more precisely occurs an increase. This also allows to successfully used them in the future as a source for stimulating preparations.

Table 2.nfluence of strains of fungus *T.koningi* isolated from different areas
to the total productivity of the tomato plant.

	Fungi species (number of strains)	solated space	The criteria used during to evaluate the overall growth of the tomato plant.		
			Growth dimensions of the plant (start/end, sm)	Starting flowering (days)	The total amount of product belonging on the unit individual of the plant(kg)
1	T. koningi (5)	Taken from the samples of the tomato plant	12/92-96	30-34	3,4±0,15
2	T. koningi (3)	From the forest soil rich with plant remnants	12/88-93	31-34	3,1±0,12
3	T. koningi (3)	Taken from the soils lowly contaminated with chemical production products	12/87-92	32-36	3,2±0,15
4	Control		12/86-91	32-35	3,0±0,14

It would be advisable, to touch issue which draws attention from the results presented mentioned (tab. 2), and related to the place where separated used strains. As seen, all signs during the used of strains isolated from the areas related to tomato are higher compared to both control and other variants. This fact once again allows to note the presence of the ecological compatibility factors among the living things in the same environment, and the need to take into account this fact during the production of such products. Thus, from the carried out of research became clear that in the formation of mycobiota of tomato plant cultivated in the condition of Azerbaijan involved 34 species of fungi which, 6 of them are true biotrophs, but others more or less degree prone to the pathogenicity. Among the registered fungi along with fungi which have high phytotoxic activity, are also found those who synthesize biologically active metabolites capable to increase the growth and overall productivity of the plant which can be successfully used in the future in the production of stimulating preparations(grow, productivity, so on.).

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