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The Quarantine Control and the Methods of Disinfection of Tomato and Cucumber Seeds in Different Regions of Russian Federation

F K Alimova, N G Zakharova, Mako Ghulam Asghar, A N Fattahkova and A V Garusov*

Department of Biochemistry, the Kazan University, Kazan 420008, Russia

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Quarantine phytopathogenic analysis of different sorts of tomato and cucumber seeds used in greenhouses in Russian Federation was studied. The common number of fungi and bacteria colonizing the outside and inside of seeds were determined. The characterization of micromycetes and bacteria complex disseminating the seeds was established. The phytotoxic infectious microorganisms were isolated. The negligible effect of some physical and chemical factors in seeds disinfectant was shown. The high antagonistic activity against phytopathogens as a new method of biocontrol with *Trichoderma harziamum* was proposed.

Key words: Quarantine control, Phytopathogens, Sorts, Tomato seeds, Cucumber seeds.

Introduction

Under conditions of concentration and specialization of agriculture the widespread plant infections defeat the enormous economic harm (Khanzada *et al* 1988; Grondona *et al* 1997; Duffy *et al* 1997). The infected seeds, remains of infected plants, infected soil, and the pests (nematodes, louses, pathogenic flies) are the main sources of plant diseases spreading (Vasile 1992; Yarchan 1993; Duffy *et al* 1996; Shtienberg and Elad 1996). The seeds poisoning leads to decrease viability and sprouting of the plant. The sowing with infected seeds is resulted as plant disease and crops damage (Zvaygintsev 1991; Grondona *et al* 1997). The recent chemical methods of seed treatment in greenhouses are forbidden in Tatarstan Republic since 1991. To control seed-borne pathogens and produce healthy seeds new biological methods must be developed. Biological control of plant pathogens has been mostly used then others classical methods. Research on controlling plant pathogens by biological means has been directed to manipulation of the environment by introduction of antagonists, especially *Trichoderma* species (Jarvis 1989; Yarchan 1993; Orlikowski 1995; Orlikowski *et al* 1995). In our previous work the new isolates of *Trichoderma harzianum* from greenhouse soil in Tatarstan Republic with high competitive and growth stimulating characteristics to plants were selected (Alimova and Zakharova 1994; Alimova *et al* 1996). The aim of our present study is to investigate the quarantine procedure for detecting the pathogenic microflora colonizing the tomato and cucumber seeds (the main greenhouse sorts) in Russia and to see the effect of *Trichoderma*. Treatment of phytopathogens selected from seeds to produce healthy seeds.

*Author for correspondence

Materials and Methods

The tomato seeds, sorts 'Krasnaya strela' from (Czuvash Republic), 'Krasnaya strela' (Tatarstan Republic), cucumber seeds, sorts of 'TCXA-575', (Timiryazev Agricultural Academy) 'Belaya dacha', 'Gladiator', 'Estafeta' were used to isolate the microorganisms colonizing the seeds. Determination of phytopathogens in different sorts of tomato and cucumber seeds was performed according to the recommendation of International Asociation in seeds testing (The Law of Russian Federation 1988; Khanzada *et al* 1988; Popkova 1988; Elad *et al* 1993; Zutra 1992; Atlas and Bartha 1997).

For disinfection the seeds were incubated at 50-52°C for 72 h. To prevent the fungal and bacterial growth, the seeds pretreated by heating, were incubated with dry trimethyl thiocarbamoyl disulfide (TMTD) (4g kg^{-1}) (Palkin 1991) for 3 weeks before sowing. Observation on the structure of micromycete association and the growth rate was tested as described (Popkova 1988; Zvaygintsev 1991; Atlas and Bartha 1997).

To investigate the inner microflora the surface disinfection procedure was performed as described here. The seeds were washed with water (10 h), then treated with 0.1% Ag NO_3 (2 min), with 0.5% $KMnO_4$ (5 min), and with 96% ethanol (30 sec). The seeds washed in sterile water were grounded and water suspension of these were inoculated on Dextrose Agar, Czapek Agar and beef Broth (Popkova 1988; Boberto *et al* 1995).

The isolation and cultivation of *T. harzianum* G-432 from greenhouse soil was performed as described (Alimova and Zakharova 1994; Alimova *et al* 1995).

The antagonistic activity of *Trichoderma harzianum* G-432 against selected phytopathogens was investigated. The analyzed micromycetes were inoculated by prick plating on czapek medium (at 30 mm between them). The growth rate (mm/h) and the cooperation type of phytopathogen colony and *T.harzianum* were detected after 3-6 days of the growth and reaction type was detected as described (Simonian and Mamikonian 1982; Camporota 1985; Grondona *et al* 1997), where reaction types are: A-combined growth of phytopathogen and antagonist; D-antagonstic activity: *E.mycoparasitism*.

Results and Discussion

All selected isolates of fungi and predominant bacteria were determined up to species and it has been established that tomato and cucumber seeds were disseminated with micro-organisms from different taxa. The common number of fungi and bacterial colonizing the seeds were shown in Table 1. The tomato and cucumber seeds of various sorts were disseminated to a different degree. Maximal number of mycoflora was observed in cucumber seeds of sort 'Estafeta', and in tomato seeds of 'Majyskiy' sort. The considerable number of bacteria were obtained in cucumber seeds of 'Gladiator' sorts. The bacteria were a predominant group colonizing all tested seeds. It was shown that common number of fungi on the external surface of seeds exceeded the number inside of the seeds (Table 5).

Five fungal species and four bacterial species were isolated from the cucumber seeds Sort 'TCXA-575' (Table 2). The seeds of cucumber 'Gladiator' sort were characterized with high diversity of epiphyte microorganisms (9 fungal species and 8 bacterial species), (Table 2). The seeds of sort 'Estafeta' were disseminated with 11 fungal species and 8 bacterial species (Table 2). The tomato seeds of sort 'Krasnaya strela' (Tatarstan Republic) were shown to be disseminated on high degree (24 spceies of microorganisms) with wide diversity of microorganisms (12 species (Table 3).

Table 1

The common number of fungi and bacteria colonizing the different sorts of tomatoes and cucumbers seeds

	Tomatoes and cucumbers sorts				
Number of micro-organisms in 1g of seeds	Cucumber seeds TCHA-575 Belaya dacha	Cucumber seeds Gladiator	Cucumber seeds Estafeta	Tomato seeds Krasnaya strela	Tomato seeds Majyskiy
Population of fungi propagules	210	380	520	230	310
Population of bacteria	935	5200	4080	2118	4323

According to the literature report (Zvaygintsev 1991; Atlas and Barta 1997) every ecological niche contains the wide set of fungi and bacteria, which take different roles in the complex. Some may be typical in those conditions and they take a leading position in the community, but others may be accidental. Observation on some complexes structure was performed to determine the position of every species in fungi, bacterial community, and the functional role of every species in plant pathogenesis. The frequency of species determination criterion was used. The typical predominant species and genera taking the leading position define the character of community (frequency of species detection 60-100%). The frequency of species detection for frequent species and genera was calculated as 30-60%, for rare species and general as 10-30%, and of accidental species and general less than 10%.

The fungi in genera *Aspergillus* and *Penicillum* were determined to be predominat genera defining the character of micromycete community that colonizing the tomato and cucumber seeds of various sorts (Table 2 and 3). The frequency of this fungi species detection on the cucumber seeds of tested sorts was calculated as 60-100%.

Aspergillus niger colonizing all tested cucumber seeds was observed as the predominant species (Table 2 and 3). Fungi of the genera *Aspergillus* and *Penicillum* were shown to cause the sprouts disease and root rot in conditions of mass accumulation. It was known that these fungi usually accompany with root rot. The greenhouse soil is very favourable for plants and for fungi too. The *Mucor* spp, and *Rhizopus* spp. participating fungi with genera *Aspergillus* and *Pencillum* can cause the mass withering of plants. Many species of these genera can produce toxins and biologically active substances stoping the growth of roots and whole plant. In some cases the fungi cause the real reason of disease and prevent the correct choice of protection means (Zvaygintsev 1991; Atlas and Bartha 1997).

It was observed that bacterial spp. of *Bacillus* were prevalent in all tested cucumber seeds (frequency of species detection 100%). *Bacillus megaterium. Bacillus subtilis* were selected as predominant species, *Bacillus mycoides* and *Bacillus idosus* were observed as frequent species (Tables 2 and 3).

The bacteria of genera *Pseudomonas* and *Erwinia* taking the leading position among Gram negative bacteria colonizing the seeds (Table 2 and 3).

Phytopathologic analysis of various sorts of cucumber seeds revealed that *Acremonium* (Sort 'Gladiator') *Oospora lactis* (Sort 'Estafeta') *Cephalosporium* (Sort 'Estafeta') (Table 2 and 3) were the main infectious agents *Oospora lactis* was shown to be the predominant species in microbial

complex, but other infectious agents causing cucumbers disease *Cephalosporium* and *Acremonium* were the minor components in the community.

It was shown that tomato seeds of sort 'Krasnaya strela' in (Czuvash Republic) were disseminated with micro-organisms on different degree (Table 3). *Aspergillus niger* and *Aspergillus flavus* were isolated from the seeds as predominant species. The other fungi and bacteria were observed as rare and accidental species.

Analysis of tomato seeds of 'Krasnaya strela' sort (Tatarstan Republic) revealed the opposite picture (Table 3). Fungi of genera *Aspergillus* and especially *Penicillum* were isolated as predominant microflora defining the characteristic of fungi community. It is shown that the fungi infect plant at the period of plant reproducing and propagate at the storage condition 20°-25°C (Zvaygintsev 1991).

The phytopathogenic fungi *Fusarium oxysporum, Stemphilium, Oospora lactis* and *Sclerotinia sclerotinorum* as infectious agents from the tomato seeds (sort 'Krasnaya strela' Tatarstan Republic) were isolated (Table 3). The bacterial infectious agent of genera *Pseudomonas* and *Erwinia* were observed on tomato seeds (Table 3).

To decrease dissemination in the seeds of Tatarstan Republic heating and treatment of cucumber seeds with TMTD was used (Palkin 1991). It was shown that heating decreased the fungi number at very low degree on the out side and inside of

Table 2

The species richness in fungi and bacteria complex isolated from the cucumber seeds

Microorganism	A			B			C			D			Toxin
	1	2	3	1	2	3	1	2	3	1	2	3	producing
Fungi													
Aspergillus niger	100	-	100	-	-	-	-	-	-	-	-	-	+
A. flavus	100	-	-	-	-	50	-	-	-	-	-	-	+
A. nidulans	-	100	-	-	40	-	-	-	-	-	-	-	+
A. fumigatus	-	-	60	-	-	-	-	20	-	-	-	-	+
A. ustus	-	-	60	-	-	-	-	-	-	-	-	-	+
Penicillum cyclopium	60	-	70	-	-	-	-	-	-	-	-	-	+
P. asterosporum	-	-	-	40	40	-	-	-	-	-	-	-	+
P. glancus	-	-	70	-	-	-	-	-	-	-	-	-	+
P. oxalicum	-	-	-	-	-	40	-	-	-	-	-	-	+
P. fullutanum	-	-	60	-	-	-	-	-	-	-	-	-	+
Arthrobotrys spp	-	-	-	-	-	-	-	-	-	10	-	-	+
Mucor spp	-	100	-	-	-	40	-	-	-	-	-	-	-
Geotrichum spp	-	-	-	-	50	-	-	-	-	-	-	-	+
Acremonium spp	-	-	-	-	-	-	-	20	-	-	-	-	+
Rhodorula spp	-	100	-	-	-	-	-	-	-	-	-	-	-
Oospora lactis	-	-	70	-	-	-	-	-	-	-	-	-	-
Cephalosporium spp	-	-	-	-	-	-	-	-	30	-	-	-	+
Bacteria													
Bacillus megaterium	-	-	60	50	40	-	-	-	-	-	-	-	-
B. cereus	-	-	-	-	-	50	-	-	-	-	-	-	-
B. subtilis	60	-	-	-	50	40	-	-	-	-	-	-	-
B. mycoides	-	-	-	-	30	-	-	-	-	-	-	10	-
B. idosus	-	-	-	-	40	-	-	-	-	-	-	-	-
B. mesentericus	-	-	-	-	-	-	-	-	-	-	-	10	-
Pseudomonas spp	-	-	-	40	-	-	-	30	30	-	-	-	-
Micrococcus roseus	-	-	-	-	-	-	-	20	-	-	-	-	-
M. albus	-	-	-	-	-	-	-	25	-	-	-	-	-
Flavobacterium spp	-	-	-	-	-	-	-	-	30	-	10	-	-
Erwinia herbicola	-	-	-	-	-	-	-	30	-	-	-	-	-

A, Typical, predominant 60-100%; B, Typical, frequent 30<60%; C, Typical, rare 10<30%; D, Accidental <10%. 1, TSXA-575; 2, Gladiator; 3, Estafeta.

seed. The treatment of cucumber seeds with fungicide TMTD was more effective only against the epiphyte microflora (Table 5). The number of inner microflora was negligible after the treatment with TMTD. It can be explained that TMTD is the fungicide with contact action.

All tested seeds of tomato and cucumber were used in greenhouses of Tatarstan Republic, where the fungicides were forbidden from 1991.

The screening of species *Trichoderma* was performed to produce biopreparation trichodermin. The strain *T. harzinum* G-432 with high antagonistic activity and high viability in soil under the anthropogenic factor was isolated (Alimova and Zakhrova 1994; Alimova *et al* 1996).

It was shown that *T. harzianum* G-432 possessed high antagonistic activity against all isolated from the tested seeds phytopathogens (Table 4). The maximal growth inhibition after 3 days (C%) of cultivation and sharply decrease of growth rate were determined in *Fusarium moniliforme, Phoma* spp., *Aspergillus niger, Ascohyta pisi* and *Acremonium fuscum.* Observation on the reaction type revealed that the main type

Table 3

The species richness in fungi and bacteria complex isolated from the tomato seeds

Microorganisms	A		B		C		D		Toxin
	1	2	1	2	1	2	1	2	producing
Fungi									
Aspergillus. niger	100	-	-	-	-	-	-	-	+
A. flavus	-	-	-	-	30	-	-	-	+
A. ustus	-	-	-	-	-	-	-	-	+
A. clavato-nanica	-	-	-	-	-	-	-	-	+
A. fumigatus	-	-	50	-	-	-	-	-	+
A. nidulans	-	-	-	-	20	-	-	-	+
Penicillium spinulosum	-	-	-	40	-	-	-	-	+
P. wortmanii	-	-	-	40	-	-	-	-	+
P. janthnellum	-	-	40	40	-	-	-	-	+
P. miczynskii	70	-	-	-	-	-	-	-	-
P. rosea-purpureum	70	-	-	50	-	-	-	-	+
P. lanosa-viride	100	-	-	-	-	-	-	-	+
P. griseolum	60	-	-	-	-	-	-	-	+
P. fellutanum	-	-	50	-	-	-	-	-	-
P. fuscus	-	-	-	-	-	-	10	-	-
P. camemberti	-	-	-	-	30	-	-	-	-
P. oxalicum	-	-	40	-	-	-	-	-	+
Mucor sp.	-	-	-	-	30	-	-	-	-
Aureobasidium	-	-	-	-	-	-	-	-	-
Pollulans	-	-	-	-	20	-	-	-	+
Stemphilium sp.	-	-	-	-	30	-	-	-	+
Fusarium oxysporum	-	-	-	-	30	-	-	-	+
Oospora lactis	-	-	40	-	-	-	-	-	-
Sclerotinia	-	-	-	-	-	-	-	-	-
Sclerotivorum	-	-	-	-	-	20	-	-	+
Bacteria									
Bacillus megaterium	-	-	-	-	30	20	-	-	-
B. cereus	-	-	-	-	20	-	-	-	-
B. mesentericus	-	-	-	-	-	-	10	-	-
B. subtilis	-	-	-	-	-	20	-	-	-
Pseudomonas sp.	-	-	-	-	20	-	-	-	-
Erwinia herbicola	-	-	-	-	40	20	-	-	-
Flavobacterium sp.	-	-	-	-	-	-	10	-	-

A, Typical, predominant 60-100%; B, Typical, frequent 30<60%; C: Typical, rare 10<30%; D, Accidental <10%. 1, Krasnaya strela (Tatarstan); 2, Krasnaya strela (CZU, Vash).

Table 4

The growth rate, antagonistic activity, reaction type and the colonization intensity in *Trichoderma harzianum* against the phytopathogens

Plant Pathogen	C,%,*** on 3 day	Reactio n type	mm/h,** *T. harzianum*	mm/h,* test organism	**RI, % 7 days
Alternaria alternata	20	E	0.446	0.016	78.5
Alternaria brassical	20	E	0.446	0.016	69.6
Botrytis cinerea	33	A	0.346	0.027	88.8
Bipolaris species	10	D	0.375	0.0083	94.0
Cephalosporium	37	E	0.375	0.030	42.0
species	23	E	0.521	0.019	91.7
Colletotrichum corda	33	E	0.446	0.027	68.7
Acremonium species	27	E	0.488	0.021	91.1
Fusarium solani	7	D	0.383	0.055	88.2
Fusarium oxysporum	67	E	0.383	0.050	69.0
Fusarium moniliforma	30	E	0.333	0.025	47.0
Fusarium graminiarum	7	D	0.450	0.055	97.0
Phytophtora species	23	E	0.458	0.019	92.2
Rizoctonia	27	E	0.504	0.062	86.2
Verticillium	80	E	0.471	0.072	75.1
Phoma	60	D	0.500	0.031	80.2
Aspergillus niger	75	E	0.458	0.060	92.4
Ascochyta pisi	85	E	0.479	0.050	68.0
Acremonium fuscum					

*-test-the growth rate of phytopathogens on the medium with *Trichoderma harzianum*, **-control-the growth rate of phytopathogens on the medium without *Trichoderma harzianum*; ***-C=100 DT/DE (Camporota; 1986); DT-growth of phytopathogen in *Trichoderma harzianum* direction after 72 hr (cm); DE-distance between two fungi (cm); **-RT, 100 (R_2-R_1)/R_2 (Grondona *et al* 1997) - the growth rate of *Trichoderma harzianum* (control) - 0.25 mm h.

Table 5

The effective of tomato seeds and treatment with fungicide TMTD of cucumber seeds on the fungi number (cfu/1g seed)

Sort	External microflora		Internal microflora	
	Untreated	Treated	Untreated	Treated
Tomato 'Krasnaya strela'	310	290	200	200
Cucumber 'Estafeta'	480	400	40	35
Cucumber 'Gladiator'	300	250	80	70

of interaction of *Trichoderma harzianum* G-432 with *Phytopathogens* were mycoparasitism (E type) and antibiotic activity (D type). The antagonistic activity (RI%) was stable after 7 days of cultivation (Table 4). The maximal growth inhibition in all tested phytopathogens was observed.

It is established from the results, that bicontrol of phytopathogens against tomato and cucumber seeds used in greenhouses in different regions of Russian Federation can be performed with native strain *Trichoderma harzianum* as ecological safety method.

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