RESEARCH ARTICLE

Impact of Bioecological Factors on the Development of Tomato Pathogens in Greenhouse with Biological Control Measures

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Study Area: Azerbaijan, Baku

Coordinates: 40°23'43"N, 49°52'56"E.
40.3°N 47.7°E

Key words: Solanaceae, *Alternaria solani*, *Fusarium solani*, *Echinacea purpurea*, *Trichoderma*

Abstract

The biological characteristics of tomato pathogens in a greenhouse shown that growing tomatoes for 3-4 years in one place forms a specialization of pathogens from the genera *Alternaria*, *Fusarium*, *Verticillium*. The dynamics of the development of pathogens in the nutrient medium was also studied. The optimum level of temperature, humidity, pH of the substrate for the development of pathogens was determined. The ability of *Alternaria solani*, *Fusarium solani*, *Phytophthora infestans* fungi, to infect potatoes, peppers from the Solanaceae family was clarified and plants were vaccinated. Factors and elements of substrates on the development of pathogens at different temperatures were studied. Agar medium with the addition of tomato juice activates the growth of pathogens from the genus *Fusarium* and *Verticillium*. The level of toxic effects of pathogens from the genera of seeds of *Alternaria* and *Fusarium* on germination of tomato seedlings has been clarified. An extract of *Echinacea purpurea* was used for the biological control of pathogens. Positive results was obtained against *Alternaria solani* and *Fusarium solani* pathogens.

Introduction:

The rich biochemical composition of crops belonging to the Solanaceae family enables various microorganisms to be supplied with them. The optimal conditions on tomato plant tissues for the feeding and development of Chromista, fungi, bacteria, mycoplasma and viruses end with the formation of different types of infectious diseases. Random sowing in agroecosystems is one of the reasons for the specialization of pathogens on plants. One of the important bio-ecological features of pathogens is the study of its pathogenic features.

Alternaria solani Sorauer, 1896, *Fusarium solani* (Mart.) Sacc., 1881 and *Phytophthora infestans* (Mont.) de Bary fungus are infection of tomato, they were separated by us from damaged tomato in 1998 and brought up to the pure culture of pathogen. The fungus has originated from Saprotroph and parasitize plants in suitable conditions.

Observations over the past years showed that *Alternaria solani* has already specialized in Solanales plants, parasitizing them in Absheron.

During pathological processes, fungi excrete different ferments, toxins, antibiotic compounds, substances possessing toxic impacts. They break down complex

polymers of plant tissues and organic substances (Monasirskiy & Svirelis, 2008)

By many factors, *Fusarium* and *Alternaria* fungi excrete different toxic substances, such as mycomarazm in, *Fusarium* acids, Yavanizin, Martizin, Enniatin etc. (Bilay, 1978; Ashmarina, 2007; Lu Zhen *et al.*, 2008). Because of these substances cell membranes of plants are weakened and then they penetrate endoderm. Afterwards, storage and transportation of water in transportation processes are weakened by pathogenesis processes occurred in plant tissues. Therefore the total water balance is broken.

The activity levels of impacts of fungal toxins on oxidation process of plant cells provided by enzymes are reduced, the structure of the nucleus is changed, and the activity of mitochondria is diminished (Alekseyeva, 2010). Due to the high prevalence of toxic effects, the use of biological control agents and natural antibiotics, such as antagonists, hyperparasites, phytohormones, phytoncides, against the poisoned disease derivations of plants, is propagated (Borovkov & Beresteskiy, 1983; Malevannaya & Alekseyeva, 2006). Zirkon obtained from *Echinacea purpurea* L preparations is used to increase anti-stress resistance of plants, as a regulator of the growth and development, flowering, fruiting of plants and to increase

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anti-diseases resistance. Zirkon is used as a stimulator in the regulation of the immune system in different plants.

Materials and methods:

The pathogenicity of *Alternaria solani*, *Fusarium solani* and *Phytophthora infestans* fungi on potato, tomato, eggplant and pepper of Solanales group were studied in four versions and the experiment carried out in 4 repeats.

Fungal contamination was carried out with the plants, each numbering 70. They were infected with the intersection method by artificial inoculation (Mirinchik, 1970). The cultural solution of pathogens derived from tomato surface artificially infected other plants by sprinkling over and rubbing on them. After that records were taken on a spread of this pathogen over these plants.

Definition of the optimal substrate is significant for the optimal development of pathogens. By experimental mycological methods, the samples have been taken from leaves, fruit and root collar in clean medium (Kirilenko, 1982). The experiment with agar wort, potato agar has been carried out in order to study the biology of *Fusarium solani*, *Foxysporum* f.sp. *radicis lycopersici* and *Alternaria solani* fungi in laboratory conditions but development extract of leaves and branches of tomato in agar conditions was studied in Petri dishes.

Agar wort is a standard substrate and obtained results are compared with this version. Pathogens have been put into thermostat at 25°C. Daily development of pathogens was recorded and observation of development dynamics continued for 15 days. In order to study the development of *Fusarium solani*, *Alternaria solani* and *Verticillium* sp. fungi at various temperatures, the development of a pure culture of pathogens was studied at different temperatures (in poly thermostat) from 0°C to 40°C during 15 days (Lilly & Barnett, 1953; Pescova, 1973).

The methods to research the phytotoxic abilities of *Solanum tuberosum*, *Solanum lycopersicum*, *Capsicum*, *Solanum melongena* species leaf spots in laboratory condition withering induced by *Alternaria* sp., *Fusarium solani* and *Foxysporum* f.sp. *lycopersici* fungi were studied by Berestetskiy (1978), and Bilay (1978).

Phytotoxic abilities determined by measuring the growth and development of *Zea mays* and *Solanum lycopersicum* seeds after moisten by pathogenic cultural decoction.

Results calculated by the formula:

$$Af = \frac{100 (Dx \times Dn)}{(Dk \times Dn)} \times 100 \quad (\text{Eg.1})$$

Therein,

Af – phytotoxic activity, %;

Dx – length of shoots after 24, 48 and 72 hours later, in mm;

Dk – length of shoots in control version, in mm;

Dn – initial length of shoots, in mm.

Zirkon is used in a closed area on the Amiry F1 hybrid

of *Solanum lycopersicum* species for increasing the resistance against diseases.

Experiments were conducted in 5 variants: 1. Zirkon 10 ml/ha; 2. Zirkon 12,5 ml/ha; 3. Zirkon 15 ml/ha; 4. Standard: Megafol 0,1 l/ha; 5. Control: no acts proceeded.

Experiments have proceeded in 4 repeats. The spread of diseases in plants was carried out on the basis of records of the 5-point scale of evaluation of the effect of Zirkon on resistance of plants against diseases.

Results and Discussion:

The results on pathogenicity study show that in relation to eggplant, pepper and potato *Alternaria solani*, *Fusarium solani* and *Phytophthora infestans* fungi have high pathogenic features. So, *A.solani* fungi, derived from damaged organs of tomato infected eggplant 65,7%, pepper 31,4%, potato 88,5% by artificial inoculation (Table-1).

Artificial inoculation with *Fusarium solani* fungi infected eggplant 41,4%, pepper 22,8%, potato 44,2%. *Ph.infestans* infected all plants seriously and outstripped other infections by making serious damages. Infection per cent was 100% in tomato and potato, in eggplant it was 64,2%, in pepper it was 67,1%.

Table-1: Pathogenicity of *Alternaria solani*, *Fusarium solani* and *Phytophthora infestans* in relation to plants of Solanaceae family

Plant	Replicates	Infected plants					
		<i>A.solani</i>		<i>F.solani</i>		<i>P.infestans</i>	
		Num	%	Num	%	Num.	%
Tomato	70	70	100	70	100	70	100
Eggplant	70	46	65.7	29	41.4	45	64.2
Sweet pepper	70	22	31.4	16	22.8	47	67.1
Potato	70	62	88.5	31	44.2	70	100

Alternaria solani, *Fusarium solani* and *Phytophthora infestans* fungi specialized on potato, tomato and pepper plants and showed high pathogenicity in relation to those plants. Therefore, during the successive sowing system placing of one of these plants before or after tomato is inadmissible. The planting of these vegetables next to sowing areas also is not advisable.

In substrates of agar wort, potato agar and tomato in agar condition the development dynamics of *Fusarium solani*, *Foxysporum* f.sp. *lycopersici* and *Alternaria solani* fungi, derived from the damaged organ of tomato and *Pseudomonas corrugata* was as following. At three nutritional environments, the development of pathogens obtained from samples of tomato leaves, fruit and root collar was high.

However, on 15th day, development of all pathogens was maximal and it shows that tomato plant is attractive for these pathogens. According to the results of the development of pathogens in potato agar condition, it might be said that the pathogens are specialized freely on potato plant and they can widespread in sowing areas if they have resources and damage them intensively.

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Pathogens widespread in tomato in agar condition on 8th day, in *Alternaria* it occurred on 10th day, *Ps.corruqata* on 15th day. Total development of investigated pathogens in tomato in agar condition shows that they have already specialized on tomato (Table-2).

The optimal level of temperature has significance for the normal development and spread of the pathogen. The main condition of organizing of pathogen control during the process is to keep in hand the development of the pathogen. Most of the scientists had carried out various researches in this field. The optimal temperature of development of a pure culture of fungi coincides with the requested temperature for their formation in pure culture.

Table-2: Development of pathogens in various substrates

Pathogens	Development of pathogens in petri dishes, mm					
	2 nd day	4 th day	6 th day	8 th day	10 th day	15 th day
In potato agar						
<i>F.solani</i>	22x29	45x50	76x85	90x90	90x90	90x90
<i>F.oxysporum....</i>	20x30x	50x58	80x85	90x90	90x90	90x90
<i>A.solani</i>	12x18	22x27	46x60	65x82	83x90	90x90
<i>P.corruqata</i>	-	8x14	32x55	67x72	90x90	90x90
In tomato in agar condition						
<i>F.solani</i>	18x16	30x30	65x70	90x90	90x90	90x90
<i>F.oxysporum....</i>	20x20	30x30	60x66	90x90	90x90	90x90
<i>A.solani</i>	9x15	26x28	42x53	76x82	90x90	90x90
<i>P.corruqata</i>	14x19	21x30	34x57	72x77	87x90	90x90
In agar wort						
<i>F.solani</i>	44x57	85x85	90x90	90x90	90x90	90x90
<i>F.oxysporum....</i>	45x65	87x90	90x90	90x90	90x90	90x90
<i>A.solani</i>	43x46	78x84	90x90	90x90	90x90	90x90
<i>P.corruqata</i>	23x31	34x46	56x67	78x85	90x90	90x90

The pure culture of *Fusarium solani* fungi grown at 28°C has the most toxic ability for tomato. The development of tomato pathogens such as *Fusarium solani*, *Alternaria solani* and *Verticillium* sp. was specified in various temperature. Development of *Fusarium*, *Alternaria*, *Verticillium* fungi was studied in various temperature modes (0, 5, 15, 20, 25, 30, 40°C) on 5th, 10th, 15th days.

At 5°C the spores of *Fusarium* and *Verticillium* fungi did not finish developing. Therefore, *Alternaria* fungi did not develop at all. The results from researches showed that at 25°C all of three fungi shown maximum progress on 5th day. On 5th day in Petri dishes the culture area was covered totally 90 mm. at 40°C fungi did not develop. *Alternaria* fungi begin to develop and in pure culture, it grew up 26 mm during 15 days, then its development stopped (Table-3).

According to the results, the minimum development temperature of *Fusarium solani* fungi is 5°C, the optimal temperature was 20-30°C, and the maximum temperature was 40°C. The minimum development temperature of *Alternaria* sp. fungi was 5-7°C, the optimal temperature was 20-30°C, and the maximum temperature was more than 40°C. The minimum development temperature of *Verticillium* sp. Fungi was 5°C, the optimal temperature is 20-30°C, and the maximum temperature was 40°C. Obtained

results can be used in the preparation of short-term projection of recorded diseases and for the efficient organization of protecting and preventive measures for the pathogen in suitable weather temperature periods.

Solanum lycopersicum seed shoots were moistened in cultural decoction by artificial infection of pathogens. After 72 hours size of shoots in control was 26 mm, moistened in *Alternaria solani* by CD, and the size of shoots was 8 mm, moistened in *Fusarium solani* fungus (Table-4).

Table-3: Development of pathogens in various temperatures (in agar wort condition)

T°C	Diameter of colonies on recorded days, mm								
	<i>Fusarium solani</i>			<i>Alternaria solani</i>			<i>Verticillium</i> sp.		
	5 th day	10 th day	15 th day	5 th day	10 th day	15 th day	5 th day	10 th day	15 th day
0	0	0	0	0	0	0	0	0	0
5	6	11	13	0	0	0	0	5	6
15	41	77	90	20	55	80	10	55	70
20	75	90	90	54	72	90	42	70	90
25	90	90	90	90	90	90	90	90	90
30	80	90	90	65	90	90	56	80	90
40	0	0	0	8	20	26	0	0	0

Table 4. Toxic impacts and growth indications of *Solanum lycopersicum* seeds shoots pathogens

Variants	IS*	Length of shoots (mm)				Before growth (%)			
		24 h	36 h	48 h	72 h	24 h	36 h	48 h	72 h
<i>A.solani</i>	2	5	7	8	8	37.5	46.1	55.5	69.2
<i>F.solani</i>	2	6	9	2	3	25	30.7	33.3	50
<i>F.oxysporum</i>	2	7	0	1	2	2.5	23.1	38.9	53.8
<i>f.sp.lycopersici</i>									
Control**	2	8	3	8	6	-	-	-	-

*Initial shoot (mm) **moistened in distilled water

Table-5: Increase of resistance effects of *Zirkonon solanum lycopersicum* seeds shoots pathogens Amiry Fl. Station: in Buzovna private greenhouse

Experiment versions	<i>A.solani</i> in %			<i>F.solani</i> in %		
	a	b	c	a	b	c
Zirkon 10 ml/ha	17.8	6.2	48.5	28.5	14.2	33.1
Zirkon 12.5 ml/ha	14.2	5.7	58.9	24.0	10.6	43.6
Zirkon 15 ml/ha	12.5	4.2	63.9	16.2	4.0	-
Standart Megafol 1 l/ha	20.9	10.6	39.5	30.4	15.0	28.6
Control:	34.6	18.9	-	42.6	21.8	-

a- Distribution; b- Intensity; c- Biology benefits;

The stagnation occurred in the growth and development of seeds by impacts of pathogen toxins nevertheless the sharp difference in control compare under the same sprout condition belonging to the same seeds exposures. Microscope observation of width of shoots under experiments showed that *Fusarium solani* and *F.oxysporum* fibres were spread in xylem tubes. *Alternaria solani* fungus in candidies and fruit particles was clearly seen in the grown shoots under microscope observations. Zirkon obtained from the terrestrial space of *Echinacea*

purpurea L. and 3 organic acids combination against withering diseases the *Alternaria* and *Fusarium* were tested as a biological struggle against propagation of pathogens. It is profitable to eliminate influences of diseases and their toxic impacts during maturity periods of products with the application of biological drugs.

Obtained results show that in control version the distribution of *Alternaria* was 34.6%, in standard version 20.9%, in control version of *Fusarium* against withering disease was 42.6%, by use of Zirkon in normal version to 15 ml/ha it was 16.2%, the intensity was in 4%. The results obtained from the use of Zirkon were more in comparison with other versions. Percent of biology benefits of the version was high - 63.9% (Table-5).

Conclusively, *Alternaria solani*, *Fusarium solani* and *Ph.infenstans* Solanales fungi specialized on potato, tomato and pepper plants and showed high pathogenicity in relation to those plants. Therefore, during the successive sowing system placing of one of these plants before or after tomato is inadmissible. The planting of these vegetables next to sowing areas also is not advisable.

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