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БАКТЕРИАЛНИ ПЕТНА И БАКТЕРИЙНО СТРУПЯСВАНЕ ПО ДОМАТИТЕ В ОБЛАСТ АЛМАТИ В КАЗАХСТАН
BACTERIAL SPOT AND BACTERIAL SPECK OF TOMATO IN THE ALMATY DISTRICT OF KAZAKHSTAN

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Abstract

Disease surveys were conducted in the Almaty district in the south-west of Kazakhstan (2011/2012). On the basis of pathogenic, morphological and cultural traits studies the phytopathogenic bacterium *Xanthomonas campestris pv.vesicatoria Doidji* was identified as a causal agent for bacterial spot in tomato plants. Another bacterium, *Pseudomonas syringae* pv. *tomato (Окабе*) was identified as an agent for bacterial speck of tomatoes. During these two consecutive years, the spread of the diseases was closely related to the local climatic conditions and the dry weather decreased their values. Laboratory tests revealed that both pathogens could be fully eradicated from inoculated seeds at a temperature of 70°C under an exposure time of 12 to 24 hours. At the same time, that particular regime did not affect negatively the germination energy and the germination rate of the seeds.

Key words: bacterial spot, *Xanthomonas campestris* pv. *vesicatoria*, bacterial speck, *Pseudomonas syringae* pv. *tomato*, thermotherapy.

INTRODUCTION

Bacterial -spot and -speck diseases of tomato (*Lycopersicon esculentum* Mill.) are caused by *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*, respectively. Together, they are considered to be the most important foliar diseases of tomato in most production areas (Yu et al*.,* 1995; Blancard, 1997). Although they are caused by different pathogens, the diseases are commonly found together in mixed infections (Delahaut and Stevenson, 2004), causing symptoms that are so similar and could be often confused, one for the

other (Cuppels et al., 2006). Both pathogens could attack every aboveground part of the tomato plant.

According to Kazenas (1974), 28 diseases of tomatoes have been detected in Kazakhstan. Lopuhina (1966) considers the bacterioses within that number to be the most wide-spread and harmful. She has described 5 types of bacterial diseases encountered in the country. However, until now, many of them have not been isolated as pure cultures and have not undergone the standard bacteriological tests necessary for their identification. With regard to this, during the 2011-2012 period we carried out a research study on the basic bacterial diseases of tomatoes, the characteristics of their progress and harmful effects as well as the on the enhancement of their control and management.

MATERIALS AND METHODS

Surveys were carried out in farmers' fields in the Almaty district of Kazakhstan in the period 2011-2012, to determine the current status of bacterial -speck and -spot diseases in tomato producing areas. The locations were selected on the basis of their history of tomato production, and to ensure the representation of different ecological backgrounds.

The laboratory tests were carried out under laboratory and field conditions within the Fruit and Vegetable Plant Protection Department of the Kazakh Research Institute for Plant Protection and Quarantine. The investigations of the tomato fields, the setting up of the field experiments, collection and analysis of disease-affected sample plants were in compliance with the common methods in phytopathology (Chumakov, 1974; Kiray et al., 1976; Chumaevskaya and Matveeva, 1986; Schaad, 1988). In the course of this study, the type of the disease and its spread in the tomato seedlings grown under plastic cover was first determined. Observations were carried out from the start of germination until planting in the ground.

The studies of the seasonal dynamics of the bacterial diseases of tomatoes were carried out by observations and regular reports on a stationary plot. The estimation of the spread of the disease and the degree of damage was conducted in the fields by means of route surveys starting from germination until the full maturing of the fruit. The counts were done diagonally across the field, on 10 plants at 10 points, 100 plants altogether. The degree of damage was measured by eye and reported applying a 4-grade scale.

In order to assess the effect of the thermal seeds processing both on the bacteria survival and the seeds sowing qualities, two indexes were used – germination energy and laboratory germination rate. Temperature regimes of 65^0, 70^0, 75^0C and exposures of 1, 2, 4, 8, 12, 16, 20, 24 hours were tested for destroying the infection both on the surface and inside the seeds. Tomato seeds, derived from naturally infected plants were let to germinate under wet chamber conditions (petri dishes with wet filter paper) at 25°C, 50 seeds in 4 replications. The germination energy was determined on the 5^{th} day and the laboratory germination rate – on the 10^{th} day by counting the germinated seeds out of the total number.

RESULTS

The research was done on 13 vegetable-growing farms in the Enbekshikazakh, Talgar and Karasaj regions in the Almaty district. The greatest development of the disease was reported on certain farms in the Enbekshikazakh region, for a monoculture was used in that region.

In the course of the studies on the tomato plants, observations were carried out on diseases manifestation and their symptoms as well. Meanwhile, samples of infected plants were collected for laboratory analysis, aiming at reaching a diagnosis.

The bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) produced lesions on all aboveground parts - leaves, petioles, stem, flowers and fruits. Initial leaf symptoms were small, circular-to-irregular, dark lesions, in certain cases surrounded by a yellow halo. The lesions tended to concentrate on the leaf edges and tip and increased in size to a diameter of 3-5 mm. The infected leaves developed a scorched appearance. When the spots were numerous and tended to coalesce, the foliage turned yellow and eventually died, thus leading to defoliation of the lower plant's portion.

Bacterial speck lesions occurred anywhere on the foliage, stems or fruits. The symptoms were very difficult to visually distinguish from the bacterial spot and could be confused with young, early blight lesions. On the leaves the symptoms appeared as black specks, no more than 2 mm in diameter, usually surrounded by a yellow halo. Speck lesions sometimes caused distortion of the leaf, as the infection restricted the expansion of leaf tissue. The lesions were often concentrated near the leaf edges. Severely infected seedlings became stunted.

Performing common isolation techniques, a group of 98 bacterial strains was obtained out of naturally infected tomato samples. The strain identification was done on the basis of cultural (Potato dextrose agar), biochemical (Table 1) and pathogenic characteristics. Based also on the strains' cultural and biochemical characteristics, it was found that they belonged to the *X. campestris* pv. *vesicatoria* (48 strains) and *P. syringae* pv. *tomato* (50 strains) species. Colonies of pathogens first appeared after 36-48 h. *X. campestris* pv. *vesicatoria* on potato agar in Petri dishes formed a colony of a circular shape and yellow colour, the lesions enlarged heavily and had a greasy feel. The isolates of *P.syringae* pv. *tomato* formed white, circular lesions, slightly raised and having a smooth and shiny surface.

Both bacteria were defined as Gram-negative and they were capable to induce HR on *Pelargonium* and tobacco leaves. In the pathogenicity tests on tomato seedlings, typical manifestations of the particular disease were observed on the 4-5th day. When the tomato fruits were inoculated, brown spots appeared after 4 days. Subsequently, the infected areas darkened, the tissue decayed in time and a crack and an exudate appeared on the side of injection.

Table 1

Biochemical characteristics of the bacteria isolated from tomato plants

Test	*X. campestris* pv. *vesicatoria*		*P. syringae* pv. *tomato*	
	This study	Reference data[1]	This study	Reference data[1]
Indole	-[2]	-	+[3]	+
Hydrogen sulfide	+	+	+	+
Nitrates' reduction	-	-	-	-
Gelatin liquefaction	+	+	+	+
Starch hydrolysis	+	+	-	-
Catalase	+	+	+	+
Milk coagulation	+	+	+	+
Arabinose	-	-	+	+
Glucose	+	+	+	+
Saccharose	+	+	+	+
Galactose	+	+	+	+
Fructose	+	+	+	+
Mannose	+	+	-	-
Rhamnose	-	-	+	+
Maltose	+	+	-	-
Xylose	+	+	+	+
Lactose	+	+	-	-

1. Bergey`s *Manual of Systematic Bacteriology* (1980); Schaad, 1988; 2. Negative result; 3. Positive result

The study of the seasonal dynamics of these tomato diseases under investigation pointed out that the amount of rainfall during the vegetative period in 2011, averaged at 201.5 mm, has facilitated the bacterial spot disease by 46% and that of bacterial speck by 32% (Table 2).

Table 2

Seasonal diseases dynamics in the Almaty district (2011/2012)

Period	2011			
	Average temperature (^{0}C)	Rainfall (mm)	Disease spread (%)	
			Bacterial spot	Bacterial speck
May	16.6	108.7	13.0	4.0
June	22.7	18.0	28.0	12.5
July	24.5	12.6	34.5	26.0
August	25.3	62.2	46.0	32
	2012			
May	18.6	68.9	3.0	-
June	21.0	120.0	8.4	3.5
July	25.9	8.6	16.7	8.0
August	24.7	-	20.0	12.0

In comparison with 2011, the vegetation period of 2012 was hot and dry, having an amount of 197.5 mm rainfall. There was lack of rainfall in August which inhibited the growth of the bacteria. In that connection, the disease spread was less intensive and ranged up to 20% for the bacterial spot disease, and up to 12% for the bacterial speck.

The study showed also that the large-scale development of the bacterial diseases of tomatoes during the fruit-bearing period decreased the plant productivity, shortened the vegetative period and deteriorated the fruit quality. It was also found that infected seed proved to be the main source of initial bacterial infection and the thermal processing proved to be the most promising effective method of seed inoculum elimination as it has been stated by Dzhaimurzina (1984). The analysis (Table 3) of the seeds' sowing qualities after the thermotherapy proved that a temperature of 70ºC cannot deteriorate their germination energy and laboratory germination rate at a 12-24-hour exposure. On the other hand, the phytopathological analysis showed that the bacterial infection was fully eliminated under that regime, thus outlining these conditions as most promising for tomato seeds thermotherapy.

Table 3

Influence of the thermal processing on tomato seeds quality and pathogen survival

Subject	Seeds germination energy and rate (%) / Pathogen survival								
	Con-trol (non-treated)	Seeds treatment (Cº)	Exposure (h)						
			1	2	4	8	12	16	20	24
Energy	90	65	90	90	90	90	89	87	86	86
Germination	90		90	90	90	87	86	85	85	85
*Xcv**	+		+	+	+	+	+	+	+	+
*Pst***	+		+	+	+	+	+	+	+	+
Energy	90	70	90	90	90	88	88	86	86	86
Germination	90		90	90	88	86	85	85	85	85
Xcv	+		+	+	+	+	-	-	-	-
Pst	+		+	+	+	-	-	-	-	-
Energy	80	75	75	60	55	43	40	37	35	30
Germination	90		90	86	76	73	70	68	65	60
Xcv	+		-	-	-	-	-	-	-	-
Pst	+		-	-	-	-	-	-	-	-

* *Xcv* - *Xanthomonas campestris* pv. *vesicatoria*; ** *Pst* - *Pseudomonas syringae* pv. *tomato*; (+) or (-) - survival or death of the pathogen

CONCLUSIONS

1. Two main bacterial diseases of tomato were found in the Almaty district in the south-west of Kazakhstan, bacterial spot and bacterial speck caused by *Xanthomonas campestris* pv. *vesicatoria* and *Pseudomonas syringae* pv. *tomato*, respectively.

2. During two consecutive years (2011/2012), the spread of the diseases was closely related to the local climatic conditions and the dry weather decreased their values in August 2012.
3. Both pathogens were fully eradicated from inoculated seeds at a temperature of 70°C under an exposure time of 12 to 24 hours. At the same time, that particular regime did not affect negatively the germination energy and the germination rate of the seeds.

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