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Occurrence of *Pseudomonas syringae* pv. *lachrymans* [(Smith and Bryan) Young, Dye and Wilkie] at Bafra Province Greenhouses

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Abstract: A survey was conducted to determine the severity of disease caused by *Pseudomonas syringae* pv. *lachrymans* on greenhouse raised cucumber plant (*Cucumis sativus*). In this survey, isolations were made from the samples which displays typical symptoms of the disease and six isolates of *P. syringae* pv. *lachrymans* were found. Pathogenicity, tobacco hypersensitivity and biochemical tests were applied to these isolates to recognise the disease.

Key words: *Pseudomonas syringae* pv. *lachrymans*, cucumber, *Cucumis sativus*

INTRODUCTION

Cucumber as being one of the foremost vegetables raised in greenhouse, belongs to the Cucurbitaceae family. There are 90 genus and 750 species. It is consumed either in fresh form or as pickle in food industry or in cosmetics industry[1]. Annual production of cucumber in Turkey is 600 thousand tons in 40 thousand hectare area. 30-40% of the production is produced in the greenhouse. The production rate in Bafra province is 9000 tons in 300 hectare area[2]. *P. syringae* pv. *lachrymans* as the agent of angular leaf spot caused is among the factors which reduce the production rate of cucumber. The disease causes considerable loses in the cucumber production areas. Early harvesting is impossible in the infected areas[3]. In this study, the severity of the disease in greenhouses is determined and pathogenicity, tobacco hypersensitivity reaction and biochemical test methods were applied to determine the disease in isolates.

MATERIALS AND METHODS

Survey: The main material of this survey is the cucumber greenhouses of Bafra province. In the survey, 50 randomly selected plants in each greenhouse were observed for determining the severity of disease. 0-4 scale is used to determine the rate of disease.

- Healthy
- Chlorotic and necrotic area on the leaves is less than 20%
- Chlorotic and necrotic area on the leaves is between 20 and 30%
- Chlorotic and necrotic area on the leaves is between 20 and 50%
- Necrotic area on the leave is more than 50% plus death of the plant

Towsend-Heuberger formula was applied to this scale:

$$D = \frac{\Sigma(n \cdot V)}{Z \cdot N}$$

D : The rate of disease,
n : Number of plants,
V : Scale value,
Z : Maximum scale value,
N : Total number of plants.

Isolation: The agent of the disease is isolated from the diseased leaves brought to the laboratory in ice box. The diseased leaves were gently washed three times in sterile water, then crushed in sterile petri dish containing 2 ml^{-1} sterile water and kept for 30 min the obtained suspension was streaked on King's Medium B[4] and incubated at 24-26°C for 24-48 h.

Identification of bacteria: The isolates were developed in nutrient glucose agar for 24-48 h then Gram stained according to methods of Schaad[5] and described colony morphology.

Flourescent pigmentation test: Isolates were developed in KBA at 24-26°C for 24-48 h then inspected under 366 nm wavelength ultraviolet light in dark room for the existence of flourescent effect. The isolates which exhibit flourescent development were recorded as positive[6].

KOH test: 1-2 drops of 3% KOH solution was placed on the microscope slides, bacterial isolates developed in NGA for 24 h was slowly stirred in the KOH solution by means of a loopfull and then the loopfull was slowly raised from the solution. If the solution is mucoid and it was stretched in the form of fibers it was recorded as positive. If the solution was watery, not mucoid and fibers formation was not occurred while the loopfull was raised, it was recorded as negative[7].

Catalase test: This test was performed to all isolates to check their liveliness. Standard cultures are used for control purpose. One milliliter of 3% hydrogen peroxide was placed on the microscope slides, bacterial isolates developed in NGA for 24 h were added by a loopfull. Bubbles arising from the solution was recorded as positive reaction[6].

Gelatin liquefaction: By using loopfull, isolates were grafted in gelatin in the test tubes. Incubated 7-14 days at 20°C. Then tubes were put in a refrigerator for 30 min at +4°C. If the fluid in tubes flows easily it is recorded as positive reaction. If it remained as it was before grafting, it was recorded as negative reaction[8].

Utilization of glucose (O/F) test: Different mediums are utilized for the oxidative or fermentive use of carbohydrates. This test was conducted according to Ayers *et al.*[9]. Two tubes were used for each bacterial isolates. The same bacterial isolate was placed in two tubes by loopfull. The isolate in one of these two tubes was covered by 3% agar. All isolates incubated in 24-26°C. The change of colour was recorded in 7-14 days.

Levan production test: Nutrient agar containing 5% sucrose were used for levan production. Large, white, domed, mucoid growth was recorded as positive.

Oxidase test: Kovacs[10] method was used. The development of distinct purple colour in 10 sec was recorded as positive.

Potato soft rot: Whole potatoes were dipped in ethyl alcohol and flamed twice for surface sterilization. The potato was cut into about 1 cm thick slices as aseptical and placed in petri dishes. The bottom of the petri dish was covered with sterile water to keep the potato moist. A loopfull of the culture was placed in the center of the potato slice and was incubated at 24-26°C for 48-72 h. The condition of the potatoes was examined by inserting toothpicks if they are rotten or not[8].

Arginine dihydrolase: Formation of alkali in arginine medium, in sealed tubes was recorded as positive[11].

Table 1: The severity of angular leaf spot disease in villages

Village name	The severity of disease (%)
Bafra-Adakoy	15.2
Bafra-Albasa	27.4
Bafra-Emenli	6.8
Bafra-Kalayci	21.9
Bafra-Kaygusuz	18.3
Bafra-Kuscular	23.4

Tobacco hypersensitivity reaction: Tobacco plants (*Nicotiana glutinosa*) were used in this study. Isolates developed in NGA for 24-48 h were added sterile water to obtain a suspension of 10^8 cells ml^{-1}. The suspension was injected into the intercellular space of the tobacco leaves by using hypodermic needle. Plants were incubated at 24-26°C for 48-96 h. Necrotic lesion on the leaves were recorded as positive[12].

Production of acids from carbohydrate: Nutrient agar of Ayers *et al.*[9] was used for this test. D-tartrate, L-lactate, L-tartrate, mannitol, sorbitol and sucrose were used as carbohydrate. All carbon sources were sterilised separately and added to the medium to give 1% concentration. Acid production was recorded for 7-14 days.

Pathogenicity tests: Isolates developed in NGA for 24-48 h were added sterile water to obtain 10^6-10^7 cells ml^{-1} suspension. The suspension was sprayed onto the leaves of cucumber plants with 3-4 leaves raised in pots by using hand pulverisator. Inoculated plants were kept in a dark room having 80-90% humidity for 24 h. Then incubated at 24-26°C for 7-14 days. The experiment had 4 replicates and was repeated twice[5,13].

RESULTS AND DISCUSSION

In this survey, the severity of disease of angular leaf spot on cucumber plants raised in plastic greenhouses in Bafra province was determined. Forty seven percent of 1381.5 da greenhouse area in Bafra province[14] which includes Adakoy, Albasa, Emenli, Kalayci, Kaygusuz, Kuscular villages was investigated.

The rate of disease is the highest in Albasa by 27.4% (Table 1). The disease was encountered in all villages. The reason for this condition is decided as continuous raising, suitable environmental condition for the disease, choosing the sensitive types.

Six isolates of *P. syringae* pv. *lachrymans* were identified as the result of tests. The isolates which do not meet the identification criteria were eliminated (Table 2).

Colonies of all the isolates took domed shape, developed as whitish and mucoid form in NGA. All isolates were Gram negative rods; recorded as positive in KOH, catalase, flourescent pigmentation, gelatin liquefied

Table 2: Identification test of *Pseudomonas syringae* pv. *lachrymans*

												Production acids from carbohydrate					
Isolate No.	GS*	KOH	GL	O/F	C	F	L	O	P	A	T	Dt	Ll	Lt	Ma	So	Su
Bad28	-	+	+	Ox	+	+	+	-	-	-	+	-	-	+	+	+	+
BAl7	-	+	+	Ox	+	+	+	-	-	-	+	-	-	+	+	+	+
BEm9	-	+	+	Ox	+	+	+	-	-	-	+	-	-	+	+	+	+
BKl12	-	+	+	Ox	+	+	+	-	-	-	+	-	-	+	+	+	+
BKy11	-	+	+	Ox	+	+	+	-	-	-	+	-	-	+	+	+	+
BKu19	-	+	+	Ox	+	+	+	-	-	-	+	-	-	+	+	+	+

*GS: Gram Stain; GL: Gelatin liquefaction; C: Catalase; F: Flouresens; L: Levan; O: Oxidase; P: Potato soft-rot; A: Arginine dihyrolase; T: Tobacco hypersensitivity reaction; Dt: D-tartrate; Ll: L-lactate; Lt: L-tartrate; Ma: Mannitol; So: Sorbitol; Su: Sucrose; Ox: Oxidative; +: positive, -: negative

and tobacco hypersensitive. All isolates were recorded as negative in oxidase, arginine dihyrolase and potato soft-rot tests. They are positive in production acids from L-tartrate, mannitol, sorbitol and sucrose. They gave negative reaction in D-tartrate and L-lactate. Identification test criteria for *P. syringae* pv. *lachrymans* was determined according to Lelliot and Stead[8] and Kagiwata[15].

The reasons for the widespread occurrence of angular leaf spot across Bafra district were the use of susceptible varieties and adverse environmental conditions. This situation is similar to other studies[16-18]. For these reasons, locally adapted, certified resistant varieties and copper or mixture of copper plus mancozeb should be used to prevent development of disease.

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