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Bacterial Leaf Spot of Araliaceous Plants Caused by *Xanthomonas campestris* pv. *Hederae* in Egypt

Tolba, I. H.
Branch of Plant Pathology- Department of Agricultural-Botany-Faculty of Agriculture-Al Azhar University-Cairo, Egypt.
E-mail address: Ibrahimshahda@yahoo.com.
Tel.: +20 01006496625.

ABSTRACT

A leaf spot symptoms consentient with bacterial leaf spot disease of araliaceous plants were observed on plants of *Schefflera actinophylla* grown in different plots located in Cairo and Giza governorates during 2014. Eight bacterial isolates resemble *Xanthomonas campestris* pv. *hederae* obtained from symptomatic plants were characterized based on physiological and biochemical tests, pathogenicity test and Biolog microbial ID system. Furthermore, the isolates were characterized by comparing them by ERIC PCR which showed highly similar DNA fragment banding patterns between all isolates. Some variance in the isolates virulence were detected when differences in virulence between the isolates were evaluated on the leaves of *Schefflera actinophylla* and *Schefflera elegantissima*. Host range of the isolates within Araliaceae family was determined on 7 araliaceous plants where, all isolates were pathogenic on *Schefflera arboricola, Schefflera elegantissima* and *Hedera helix* in addition to *Schefflera actinophylla* and conversely, none of the isolates were pathogenic on *Aralia nudicaulis*, *Dendropanax trifidus* and *Fatsia japonica*. Incidence and severity of the disease were evaluated on araliaceous plants grown in 10 plots located in Cairo and Giza governorates. The disease incidence values significantly differed within surveyed plots whereas these values ranged from 0 to 56.5 % by overall mean equal to 22.9%. The mean of the disease severity ratings ranged from 38.0 to 77.5 % by overall mean equal to 43.5%.

Keywords: *Xanthomonas campestris* pv. *hederae*, Araliaceae, ERIC PCR, host range, incidence and severity.

INTRODUCTION

Araliaceous plants are commonly grown as ornamental plants for landscaping and indoor decoration foliage or as traditional herb medicines for health foods or the pharmaceutical industry. Economically, the most important members of the family Araliaceae are the species of English ivy, Schefflera, Dizygotheca, Fatsia, Brassaia, Polyscias, and Fatshedera.

The plant pathogenic bacterium *Xanthomonas campestris* pv. *hederae* is the causal agent of the serious diseases that denominated *Xanthomonas*-leaf spot which has been recorded on many araliaceous plants worldwide (Arnaud 1920 and Chase 1984). The pathogen affects entire leaf surfaces and the typical disease symptoms include tiny yellowish lesions which are seen on the lower leaf surface as an initially symptom following stomatal infection. These lesions became raised, irregularly shaped edges with cork-like appearance and may enlarge between leaf veins until entire leaves became chlorotic (Chase 1984).

Several approaches have been followed to differentiate the strains of *X. campestris* including monoclonal antibodies (Alvarez *et al*., 1985), restriction fragment-length polymorphism [(RFLP) (Lazo, and Gabriel, 1987 and Lazo *et al*., 1987)], fatty acid analyses (Norman and Alvarez 1989), DNA homology (Hildebrand *et al*., 1990), genomic fingerprinting (Cooksey and Graham 1989), 16S rRNA characterization (DeParasis and Roth 1990) and repetitive sequence-based polymerase chain reaction (Opgenorth *et al*., 1996).

X. campestris pv. *hederae* was recorded and described for the first time by Arnaud (1920). Since 1920 and prior to 1984, the disease has been recorded worldwide in regions where *H. helix* is grown (Dye 1967, White and McCulloch 1934, Burkholder and Gutterman 1932). In the study conducted by Chase (1984), the host range of this pathogen had expanded to include schefflera (*Brassaia actinophylla*), dwarf schefflera (*Schefflera arboricola*), false aralia (*Dizygotheca elegantissima*), Japanese aralia (*Fatsia japonica*), *Fatshedera sp*., and *Polyscias fruticosa* (Ming aralia).

severity of plant diseases is depending on the susceptibility of the host and definitely on the virulence of the strain. Variation in pathogenicity among strains of *X. campestris* pv. *hederae* was reported by Norman *et al*., 1999.

The majority of the studies on the epidemiological features of plant diseases have concentrated on local disease increase and spread of the pathogen between geographical regions. The occurrence of *Xanthomonas* leaf spot on araliaceous plants through different geographical regions has been previously reported (Dye 1967, White and McCulloch 1934 and Suzuki *et al* 2002). Incidences of *Xanthomonas* leaf spot have been reported as high as 100% for *H. helix* in some greenhouses, and most *Polyscias spp*. plantings in Hawaii were heavily infected by the disease (this was mentioned in Norman *et al*, 1999). Assessment of the disease incidence and severity in different locations provides the estimation of when the disease first established in the locality and the rates of spread of the disease and then, measuring the seriousness of the disease.

This work intended to characterize isolates of *Xanthomonas campestris* pv. *hederae* isolated from the araliaceous plant, *Schefflera actinophylla* and study they virulence as well as they host range. Also, the distribution, and severity of the *Xanthomonas* leaf spot on different araliaceous plants were studied.

MATERIALS AND METHODS

Isolation of the causal pathogen.

Symptomatic leaf samples of *Schefflera actinophylla* (Umbrella Tree) were collected from nurseries, public gardens, building backyards and streets located in Cairo and Giza cities. The causal bacterium was routinely isolated from the collected samples by adopting the standard procedures and plating on yeast-peptone agar and nutrient glucose agar. Small pieces (0.5-1cm2) with typical water soaked lesions were cut from leaf samples

and surface-disinfected with 75 % ethanol for 20 s then rinsed several times in sterile distilled water and then macerated in 10 ml phosphate buffered saline (pH 7) on sterilized glass slide surface. A loopfuls of macerates were streaked on the isolation media then incubated at 28oC and observed daily. Yellow-pigmented, Xanthomonas-like bacterial colonies were purified by streaking three times onto nutrient agar plates. The suspected colonies were streaked on YDC medium and the isolates showed copious, very mucoid and richer yellow on this medium were selected, kept frozen as 30% glycerol stocks at −80°C and has been subjected to the subsequent studies.

Identification of the causal pathogen

Morphological and biochemical tests

Isolates suspected to be *Xanthomonas campestris* pv. *hederae* were tentatively identified according to the colony character on isolation media and YDC medium (Dye, 1962 and Schaad *et al*., 2001). The isolates were tested for Gram stain reaction, cell shape, motility and indo-spore forming. The physiological and biochemical characters of the isolates were examined following the methods described by Dye (1962) and Lelliot and Stead (1987). The type of metabolism (oxidative or fermentative) was determined using oxidative/fermentative (OF) medium supplemented with glucose. Fluorescent pigment production on KB medium was tested. Also, they were tested for: Kovacs' oxidase reaction; starch, gelatin and aesculin hydrolysis; Tween 80 lypolysis; action on litmus milk; reduction of nitrate; catalase activity, levan formation, hydrogen sulfide and indole production; acid formation from arginine, d-arabinose, dulcitol, galactose, d-glucose, maltose, mannose, sorbitol, sucrose and xylose; tolerance to 0.02% triphenyl-tetrazolium chloride and also, growth at 36 and 40 ºC and on 2 % NaCI and hypersensitive reaction on tobacco leaves.

Pathogenicity testing

The pathogenicity testing of the selected isolates was performed on 8 months old apparently healthy plants of *Schefflera actinophylla* (umbrella tree). Inocula were prepared by growing bacteria on NGA medium for 48 h and then suspending the appropriate isolate in 0.85% NaCl and spectrophotometrically adjusted to 0.2 optical density at 590 nm which approximately equivalent to 1 × 107 CFU/ml based on dilution plating assay. Inocula were used within 45 min of preparation. Suspension of the appropriate isolate was sprayed on the abaxial and adaxial side of plant leaves then individually covered with plastic bags for 24 h (Klement 1990). One plant for each isolate was used. Four plants were inoculated by saline solution only and served as negative control. The plants were then transferred to greenhouse in which the temperatures fluctuated between 22 and 33°C with approximately 14 h naturally daylight. The plants were misted twice daily to keep high relative humidity and the symptoms were monitored weekly. Reisolations were performed from the leaf of the inoculated plants after the symptoms had appeared.

Metabolic profile

The metabolic profiles (ability to oxidize carbon sources) of the selected isolates were characterized using Biolog microbial ID system (Verniere *et al*., 1993). The GN microplate system, version 3.5 (Biolog Inc., Hayward, CA, USA) were used and the procedures were performed according to the manufacturer's instructions.

Comparison between the isolates using ERIC-PCR

The ERIC (enterobacterial repetitive intergenic consensus) primer pair targeted the enterobacterial conserved sequences (Versalovic *et al*., 1991) was used to compere the DNA sequences between the isolates. To extracting the total genomic bacterial DNA, each bacterial isolates were grown on YDC agar medium at 28 C for 2 days and single colonies were aseptically transferred to 100µl of sterile deionized water in a 1.5 mL Eppendorf vial and vortexed to became homogenous. The bacterial suspensions were heated for 15 min at 95°C then cooled immediately on ice for 5 min. The resulted suspensions were centrifuged at 14,000 rpm for 5 min and centrifuged and the resulted supernatants were kept at-20 until used (OEPP/EPPO 2010).

Amplification reactions were conducted with primer set ERIC 1R (59-ATGTAAGCTCCTGGGGATTCAC-39) and ERIC 2 (59 AAGTAAGTGACTGGGGTGAGCG-39) as a pair. PCR amplifications was performed in a 50 µl reaction vol. using PCR master mix with 2 µl of DNA template and 0.1 µmol of each primer a 480 DNA thermal cycler (Perkin-Elmer, Norwalk, CN, USA) with the following temperature profiles (OEPP/EPPO 2010): initial denaturation cycle was at 95 °C for 7 min followed by 35 cycles of a three-step PCR program: (94 °C for 1 min, 52°C for 1 min and 65°C for 8 min) followed by a final extension at 65°C for 16 min.

PCR products (10 µl) were separated by electrophoresis in 2% (w/v) agarose gels in 0.5× Tris-borate-EDTA buffer for 2.5 h at 90 V constant voltage (this corresponds to 6 V cm-1, measured at the distance between the electrodes) (OEPP/EPPO 2010). Gel was stained for 30 min in an ethidium bromide solution of 0.6 mg mL-1 and destained for 30 min in distilled water. The patterns of the DNA migration were analyzed visually. The band patterns were determined comparatively to the 1 kb plus DNA ladder (GIBCO BRL, Rockville, MD, USA).

Isolates virulence

The virulence of the identified isolates was evaluated on leaves of *Schefflera actinophylla* and *Schefflera elegantissima* after artificial inoculation using spraying methods Norman *et al* (1999). Inoculum of each bacterial isolates was prepared as mentioned above. From each tested plant, 5 leaves with intermediate arrangement on plant stem were sprayed to runoff with 100 ml from bacterial suspension of appropriate isolate and loosely enclosed in a plastic bag for 24 h. The treated plants were kept in greenhouse which the temperature average from 22°C to 32°C. Three plants were used for each isolates and the assays were repeated three times and the results of all tests were averaged and reported. The reactions were evaluated 4 weeks after inoculation according to the criteria described by Norman *et al* (1999) whereas the reactions were rated with the following index: 1 = no symptoms, 2 = slight symptoms (1 to 10 lesions <1 mm in diameter), 3 = moderate symptoms (lesions >1 mm in diameter on >25% of the leaves), and 4 = severe symptoms (lesions coalescing and causing leaf abscission or covering >50% of the leaf surface).

Determining the host range within araliaceous plants

The host range of identified isolates was determined on several araliaceous plants using spray inoculation methods. The tested plants include *Schefflera arboricola, Schefflera elegantissima*, *Aralia nudicaulisn Dendropanax trifidus, Fatsia japonica* and *Hedera helix.* Inocula preparation and inoculation procedures were performed as described in pathogenicity test. The inoculated plants were kept in net house. The average of temperature degrees in the duration of the experiments fluctuated between 23 to 34 °C. The experimental plants were monitored daily during 1 month from inoculation date and resulted responses were recorded.

Disease incidence and severity

Araliaceous plants of different genera or species belongs to family araliaceae in 10 plots located in Cairo and Giza governorates were surveyed for the incidence and severity of the *Xanthomonas* leaf spot disease during the period from April to November 2016. The plants within these plots were visually inspected and the number of the symptomatic plans was estimated. The incidence of the disease was expressed as number of symptomatic plants per number of investigated plants as a whole. The disease severity was assessed on 10 symptomatic plants in plots that contain 10 or more symptomatic plants. The assessed plants were randomly selected and visually investigated Disease severity was calculated according to Norman *et al* (1999) as described above.

Statistical analysis

The experiments were performed using randomized complete block design. The mean values were compared by the least significant difference (LSD) testing at p = 0.05. Overall means were compared using Duncan's multiple Range test at p = 0.05. The analyses were achieved using the statistical computer software SPSS (version 11).

RESULTS

Isolation of the causal pathogen

Isolation from symptomatic leaves of *Schefflera actinophylla* (Umbrella tree) on yeast peptone agar and nutrient glucose agar consistently yielded Xanthomonas-like bacterial colonies on both isolation media (Fig 1, A and B). After 3 days' incubation, suspected colonies were small (2 – 3 mm) Yellow-pigmented, round, convex, smooth and shiny. Based on characteristic growth (copious growth, very mucoid and richer yellow) on YDC medium (Fig 1, C), 8 isolates were selected, and then designated XCH1, XCH2, XCH3, XCH4, XCH5, XCH6, XCH7 and XCH8.

Fig. 1. The colony morphology of *Xanthomonas campestris* pv. *hederae* on yeast peptone agar (A, arrows) and nutrient glucose agar (B) and YDC (C) media.

Identification of the causal pathogen

Morphological and biochemical tests

The morphological and biochemical characteristics of the selected isolates closely matched the characteristics of *Xanthomonas sp* (Table 1). The isolates were gram-negative, rod-shaped, motile and non-spore forming. None of isolates produced fluorescent pigment in KB medium. They caused hypersensitive reaction on tobacco leaves. They metabolized glucose oxidatively and never fermentatively. They hydrolyzed esculin, starch, casein, gelatin and lipolysis Tween 80. They reacted positively for production of levan, H2S, urease and alkali in litmus milk. They reacted negatively for oxidase, nitrate reduction, L-tartrate utilization and indole production. Acid was produced from arginine, d-arabinose, dulcitol, galactose, d glucose, maltose, mannose, sorbitol, sucrose and xylose. All isolates grew in the presence NaCl at a concentration of 2 %, did not grew at 0.02% triphenyl tetrazolium chloride and the maximum growth temperature was 36°C. The results of all performed testes were identical among the isolates which is consistent with their identity as *X. campestris* pv. *hederae*.

Table 1. Morphological, physiological and biochemical characteristics of eight *Xanthomonas campestris* pv. *hederae* isolates obtained from symptomatic leaves of *Schefflera actinophylla*.

Characteristic	Reaction
Growth on YDC medium	Mucoid
Cell shape	straight rod
Gram test	+
Indo spore forming	-
Tobacco HR	+
Fluorescent pigmentation	-
Oxidase reaction	-
Nitrate- reductase activity	-
Catalase test	+
Hydrolysis of:	
Casein	+
Gelatin	+
Potato starch	+
Tween 80	+
Glucose metabolism:	
oxidatively	+
Fermentatively	-
Action on litmus milk	Alkaline
H_2S from L-cysteine	+
Acid production from:	
d-arabinose	+
arginine	+
dulcitol	+
galactose	+
d glucose	+
maltose	+
mannose	+
sorbitol	+
sucrose	+
xylose	+
Growth at	
36 ^{o}C	+
40^{o}C	-
2 % (w/v) NaCl	-
0.02% TTC	-

+ = Positive reaction - = Negative reaction

Pathogenicity testing

All the tested isolates were pathogenic on *Schefflera actinophylla* when leaves were sprayed with 1×10^7 CFU/ml of bacterial suspension. As in naturally diseased plants, similar leaf spot symptoms were observed evidently on the inoculated leaves. Within 8 to

12 days after inoculation, small water-soaked lesions were developed on the lower surfaces of inoculated leaves (Fig 2A). These lesions coalesced and gradually turned chlorotic and the centers of lesions became brown and surrounded by greenish brown water-soaked, irregular margins (Fig 2B). At last, the margins were raised, dried out then became corky (Fig. 2C). The leaves of control plants remained healthy. The bacterium was readily reisolated from symptomatic leaves.

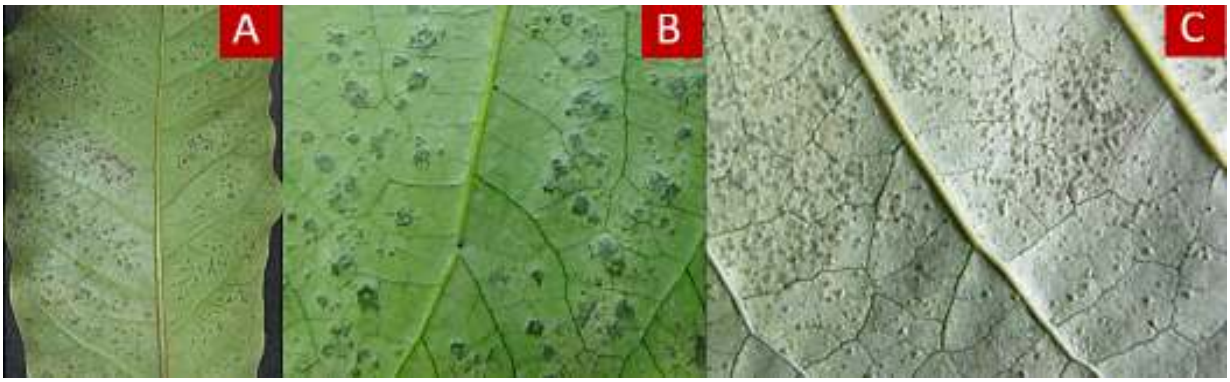

Fig. 2. Symptoms progress on artificially inoculated *Schefflera actinophylla* leaves. A: small water-soaked lesions developed on the lower surfaces of inoculated leaves 10 days after inoculation; B: coalesced with irregular margins water-soaked lesions, 15 days after inoculation; C: corky lesions with raised dried margins, 25 days after inoculation.

Metabolic profile

Capability of the selected isolates to oxidize carbon sources were tested using GN microplate system. Using the option of Biolog dendrogram cluster analysis (data not shown), all isolates were identified to the genus species level as *Xanthomonas campestris*. At the pathovar level, two isolates (XCH 5 and XCH 8) had similarity indexes less than 0.5 which identified to species level and were closer to 12 pathovars other than pv *hederae* while the other isolates were identified as *X. campestris* pv. *hederae* with similarity indexes ranged from 0.869 to 0.890 (Table 2).

Table 2. Biolog identity of eight isolates of *X. campestris* pv. *hederae*, isolated from symptomatic leaves of *Schefflera actinophylla*.

Isolate	Identity	Similarity value
XCH 1	*Xanthomonas campestris* pv. *hederae*	0.886
XCH 2	*Xanthomonas campestris* pv. *hederae*	0.888
XCH 3	*Xanthomonas campestris* pv. *hederae*	0.869
XCH 4	*Xanthomonas campestris* pv. *hederae*	0.890
XCH 5	*Xanthomonas campestris*	0.492
XCH 6	*Xanthomonas campestris* pv. *hederae*	0.898
XCH 7	*Xanthomonas campestris* pv. *hederae*	0.890
XCH 8	*Xanthomonas campestris*	0.448

Comparison between the isolates using ERIC PCR

ERIC PCR was conducted using the primer set ERIC 1R / ERIC 2 to comparing the amplification products of the isolates. Based on the ERIC fingerprint pattern, all isolates showed similar patterns and definitive discrimination of the tested isolates depending on visually screening was not possible (Fig. 3). Several diagnostic and few polymorphic bands varying from 130 to 2000 bp were observed amongst the tested isolates. Major conserved products from all isolates were co-migrated near the DNA markers at 300, 500, 750 and 2000 bp.

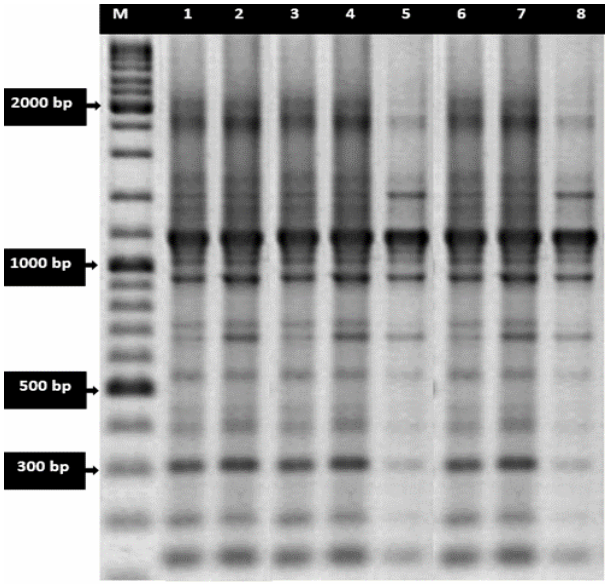

Fig. 3. ERIC-PCR of eight *Xanthomonas campestris* pv. *hederae* isolates obtained from *Schefflera actinophylla*. Lane M: marker (GeneRuler DNA Ladder mix SM033); lanes 1-8: XCH 1, XCH2, XCH3, XCH4, XCH5, XCH6, XCH7 and XCH 8 respectively.

Virulence of the isolates

Differences in virulence between the isolates were evaluated on the leaves of *Schefflera actinophylla* and *Schefflera elegantissima*. Generally, some variances in the virulence were detected between the isolates on both tested plants. Also, the rating values of virulence of each isolate markedly correlated within both tested plants. The virulence levels produced by isolates designated XCH 5 and XCH 6 were significantly the lowest. Other isolates produced converging levels of virulence whereas rating scale get around 3.5 of virulence level.

Determining the host range within araliaceous plants

The host range of the isolates was determined on six araliaceous plants in addition to the *Schefflera actinophylla*

(the original host species that has been the source of isolates and used in testing pathogenicity) using spray inoculation method. In general, the isolates did not vary in their pathogenicity to the tested plants. All isolates were pathogenic on *Schefflera arboricola, Schefflera elegantissima* and *Hedera helix*. Conversely, none of the isolates were pathogenic to *Aralia nudicaulis*, *Dendropanax trifidus* and *Fatsia japonica* (Table 3).

Table 3. Virulence of eight *X. campestris* pv. *hederae* isolates on the araliaceous plants, *Schefflera actinophylla* and *Schefflera elegantissima*.

Isolate	Virulence rating*			
	S. actinophylla	%	*S. elegantissima*	%
XCH 1	3.4	85	3.5	87.5
XCH 2	3.3	82.5	3.4	85
XCH 3	3.4	85	3.4	85
XCH 4	3.7	92.5	3.5	87.5
XCH 5	2.5	62.5	2.4	60
XCH 6	2.4	60	2.3	57.5
XCH 7	3.3	82.5	3.5	87.5
XCH 8	3.5	87.5	3.4	85
LSD	0.6		0.6	

***: The reactions were rated according to Norman *et al* (1999) as: 1 = no symptoms 2 = slight symptoms (1 to 10 lesions <1 mm in diameter) 3 = moderate symptoms (lesions >1 mm in diameter on >25 % of the leaves) 4 = severe symptoms (lesions coalescing and causing leaf abscission or covering >50% of the leaf surface).. Virulence values were averaged from 3 separate experiments.**

Symptoms on *Schefflera arboricola* developed as tiny sunken, water- soaked lesions less than 1-mm diameter within 10 to 12 days after inoculation (Fig 4, A). These lesions turned tan and later became corky (Fig 4, B).

On *Hedera helix*, Symptoms developed as small water-soaked lesions or water- soaked areas within 8 to 10 days after inoculation (Fig 5, A & B). Later, this lesion coalesced, gradually turned chlorotic and then necrotic and finally leaves became deformed or even completely collapsed (Fig 5, C).

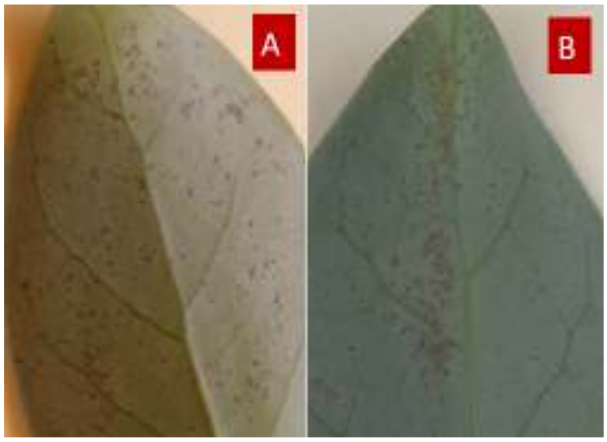

Fig. 4. Symptoms on *Schefflera arboricola*; tiny sunken, water- soaked lesions developed within 10 to 12 days after inoculation (A); these lesions turned tan and later became corky (B).

Table 4. Host range of eight *Xanthomonas campestris* pv. *hederae* isolates on different araliaceous plants.

Tested plant		Isolate reaction							
Scientific name	Common name	XCH1	XCH2	XCH3	XCH4	XCH5	XCH6	XCH7	XCH8
Aralia nudicaulis	Wild sarsaparilla	-	-	-	-	-	-	-	-
Dendropanax trifidus	Kakuremino	-	-	-	-	-	-	-	-
Fatsia japonica	Fatsia	-	-	-	-	-	-	-	-
Hedera helix	English ivy	+	+	+	+	+	+	+	+
Schefflera actinophylla *	Umbrella tree	+	+	+	+	+	+	+	+
Schefflera arboricola	Dwarf umbrella tree	+	+	+	+	+	+	+	+
Schefflera elegantissima	False Aralia	+	+	+	+	+	+	+	+

***: The plant that has been the source of isolates and used in testing pathogenicity.**
+: pathogenic. -: not pathogenic.

Fig. 5. Symptoms on *Hedera helix*; small water-soaked lesions (A) or water- soaked areas (B) developed within 8 to 10 days after inoculation. Later, leaves became deformed or even completely collapsed (C).

On *Schefflera elegantissima* were similar to those on *Schefflera arboricola* but more frequently, coalesced, enlarged and sometimes developed water- soaked areas more than 1 cm in diameter (Fig 6). In all cases, leaf abscission was common.

Disease incidence and severity

A total of 1371 Araliaceous plants with different genera or species grown in 10 plots located in Cairo and Giza governorates were surveyed in order to evaluate the incidence percentage as well as the severity of the *Xanthomonas* leaf spot disease on these plants.

The total number of the plants that showed symptoms within all surveyed plants was 314 plants corresponding to 22.9 % of disease incidence (Table 5). The incidence values significantly differed within surveyed plots whereas these values ranged from 0 to 56.5 % by overall mean equal to 22.9%. All of symptomatic plants that were recorded were constricted only in *S. actinophylla*, *S elegantissima* and *S. arboricola*. Interestingly, no symptoms coincided with bacterial leaf spot were observed on *H helix* although it was susceptible in artificially inoculation.

Disease severity assessment on 10 symptomatic plants represented each diseased plot revealed that, the mean of the severity ratings ranged from 38.0 to 77.5 % by overall mean equal to 43.5 % (Table 5). Excepting the plots number 7 and 8, most of severity values were considerably close.

Table 5. Incidence and severity of *Xanthomonas* leaf spot disease on araliaceous plants grown in 10 plots located in Cairo and Giza governorates.

Governorate	Plot location	Plot	Plants type	Disease incidence*					Disease severity**	
				Number of surveyed plants		Symptomatic plants			Rating (0-4)	%
				Per type	Total	Per type	Total	%		
Cairo	Fifth settlement	1	*S. actinophylla*	105	157	53	67	42.6	2.3	57.5
			S. arboricola	43		14				
			H. helix	9		0				
		2	*S. actinophylla*	68	92	41	52	56.5	2.4	60.0
			S elegantissima	24		11				
		3	*A. nudicaulis*	45	140	0	45	32.1	2.5	62.5
			H. helix	33		0				
			S. actinophylla	62		45				
	Nasr city	4	*A. nudicaulis*	88	217	0	53	24.4	2.1	52.5
			H. helix	41		0				
			S. actinophylla	78		53				
		5	*A. nudicaulis*	57	87	0	0	0	NA	
			D. trifidus	30		0				
Giza	El Docky	6	*A. nudicaulis*	51	112	0	26	23.2	2.1	52.5
			F. japonica	20		0				
			S. actinophylla	41		26				
		7	*A. nudicaulis*	63	122	0	38	31.1	2.9	38.0
			F. japonica	12		0				
			S. actinophylla	47		38				
	Al Agouza	8	*A. nudicaulis*	74	168	0	33	19.6	3.1	77.5
			D. trifidus	40		0				
			S. actinophylla	54		33				
	Al Monibe	9	*A. nudicaulis*	104	140	0	0	0	NA	
			H. helix	36		0				
		10	*A. nudicaulis*	113	136	0	0	0	NA	
			H. helix	23		0				
Overall				1371		314		22.9	1.74	43.5
LSD								8.7		14.4

NA: not assessed. *: Number of symptomatic plants per whole number of investigated plants.
****: Calculated from 10 symptomatic plants per each plot.**

Fig. 6. Symptoms on *Schefflera elegantissima;* tiny sunken, water- soaked lesions and enlarged water- soaked areas (A); and corky lesions with raised dried margins (B).

DISCUSSION

Araliaceous plants are one of the ornamental, landscaping and hedgerow plantings as well as also commonly grown as indoor foliage with widespread cultivation in Egypt, especially in the streets of the cities, public parks, governmental institutions and houses gardens. Although the importance of these plants in Egypt, it did not receive sufficient attention to care of it, especially in terms of diseases that affect these plants. Therefore, this work draws attention to the seriousness of one of these diseases and the losses that may be incurred by these plants as a result of that disease infection. Symptoms similar to bacterial leaf spot were observed on plants of *Schefflera actinophylla* grown in different plots located in Cairo and Giza governorates during 2014. The beginning was from here, where symptomatic leaf samples were collected from these plants in preparation for study of the causes and then study some aspects related to this disease.

Xanthomond-like bacterial colonies (yellow, circular, translucent and raised) were constantly isolated from the symptomatic leaf samples. By streaking these suspected colonies on YDC medium, typical growth of *Xanthomonas* (copious growth, very mucoid and richer yellow) resulted in this medium and accordingly, 8 isolates were selected to carry out the other tests. The characteristic yellow color of xanthomonads is originating from the water insoluble yellow pigments named xanthomonadins (Schaad, 1988). The YDC agar medium was reported to be very useful for the colony characterization of xanthomonads (Dye, 1962)

The phenotypic characteristics of the selected isolates were uniform and fully compatible with the characteristics of *Xanthomonas campestris* pv. *hederae*. These characteristics represented in being all isolates were gram-negative, rod-shaped, motile and non-spore forming; did not produce fluorescent pigment in KB medium; caused a hypersensitive reaction on tobacco leaves; oxidatively metabolized glucose; hydrolyzed esculin,

starch, casein, gelatin and lipolysis Tween 80; produced levan, H2S, urease and alkali in litmus milk; reacted Negatively in tests for oxidase, nitrate reduction, L-tartrate utilization and indole production; produced acid from arginine, d-arabinose, dulcitol, galactose, d glucose, sorbitol, maltose, mannose, sucrose and xylose; grew at 2 % NaC and at 36°C. these characteristics are concord with physiological and biochemical characteristics of *Xanthomonas campestris* pv. *hederae* that were described before (Schaad and Alvarez, 1993; Swings *et al*., 1993; Alvarez *et al*., 1994; Suzuki *et al*., 2002; Popovic *et al*., 2013). The results of physiological and biochemical characteristics of the tested isolates suggest that they are very homogeneous. Physiological and biochemical tests proved to be meaningful for identification of bacterial plant pathogens particularly to the genus and species. These testes are still used to study and characterize the phenotypic variation among *Xanthomonas* species isolated from different host plants (Schaad *et al*., 2001).

Checking the pathogenicity of these isolates is the step to be taken following the isolation and physiological and biochemical identification. Accordingly, pathogenicity test was carried out on leaves of *Schefflera actinophylla* (the plant from which the bacteria have been isolated). All isolates caused typical disease symptoms on *Schefflera actinophylla* leaves after artificially inoculated by bacterial suspensions. Fulfillment of the Koch's postulates, the bacterium was readily reisolated from symptomatic leaves which prove that, these isolates are responsible for causing these symptoms.

Biolog system has been followed for the identification of the putative isolates. Using this system, all isolates were identified up to the genus and species levels as *Xanthomonas campestris*; but, at the pathovar level, two isolates (XCH 5 and XCH 8) had similarity indexes less than 0.5 which identified to species level while the other isolates were identified as *X. campestris* pv. *hederae* with similarity indexes ranged from 0.869 to 0.890. Vauterin *et al*. (1995) reported that, the Biolog system could distinguish the *hortorum* group from other *Xanthomonas* species, using 16 positive and 27 negative tests. But Norman *et al*. (1999) tested 59 strains of *Xanthomonas campestris* pv. *hederae* and they found the metabolic fingerprints of the tested strains were far more complex than reported by Vauterin *et al*. (1995). Although, it is being considered as complementary test, the Biolog system has manifested to be very adequate to identification of different bacteria including xanthomonads (Schaad *et al*., 2005 and Verniere *et al*., 1998). This automated identification system is based on the diversity among microorganisms for their ability to utilization of carbon source which has been successfully used for providing the full metabolic profiles of the bacterial spot's xanthomonads (Mariya Stoyanova *et al*., 2014).

ERIC PCR was used to compere the DNA sequences between the isolates. The ERIC fingerprint patterns of the tested isolates were similar and definitive discrimination between them based in ERIC PCR was not possible. This result can be epidemiologically valuable because it shows that, the diversity of the causative agent has not yet been present, and therefore the adopted procedures to disease management will be standardized. The ERIC (enterobacterial repetitive intergenic consensus) targeted the enterobacterial conserved sequences (Versalovic *et al*., 1991) is a part from the rep-PCR (repetitive element sequence-based PCR) genomic fingerprinting which consisted of three families of repetitive sequences including the 35–40 bp repetitive extragenic palindromic (REP) sequences, the 124 127 bp enterobacterial repetitive intergenic consensus (ERIC) sequence, and the 154 bp BOX element (Versalovic *et al*., 1994). This genomic fingerprinting makes use of DNA primers complementary to naturally occurring, highly conserved, repetitive DNA sequences, present in multiple copies in the genomes of most Gram-negative and several Gram-positive bacteria (Rademaker *et al*., 1997). Many previous studies have stated the variation in the ability of rep-PCR primers to discover the diversity in *Xanthomonas* spp (Trindade *et al*., 2005; Lopez *et al*., 2006; Jensen *et al*., 2010; Lema *et al*., 2012).

Differences in virulence between the isolates were evaluated on the leaves of *Schefflera actinophylla* and *Schefflera elegantissima*. Generally, some variance in the isolates virulence were detected in both tested plants. Also, the rating values of virulence of each isolate markedly correlated within both tested plants. The virulence levels produced by isolates designated XCH 5 and XCH 6 were significantly the lowest. Other isolates produced converging levels of virulence whereas rating scale get around 3.5 of virulence level. The heterogenic diversity of *X. campestris* pv. *hederae* strains were reported and the severity of symptoms usually was more severe on the host of origin (Norman *et al*., 1999). Norman *et al*. (1999) suggested that the group of *hederae* can be divided into two pathovars. It is necessary before this can be proposed, several additional research such as DNA-DNA hybridization and extensive host range studies should be cured out.

The host range of the isolates was confined in four of the total seven-tested araliaceous plants. Except for *Hedera helix*, all susceptible plant spices belong to genus *Schefflera* where the isolates were pathogenic on *Schefflera arboricola, Schefflera elegantissima* and *Hedera helix* but not on *Aralia nudicaulis*, *Dendropanax trifidus* and *Fatsia japonica.* These results are partially concurrent with those obtained by Chase (1984) and Suzuki *et al* (2002) whose reported infection of *X. c.* pv. *hederae* on *Araliaceous* plants, including *Hedera helix* and *schefflera spp.* but contradict in the infection on *Fatsia japonica*. Since 1920, the time which *X. campestris* pv. *hederae* was described for the first time (Arnaud, 1920), the disease has been reported throughout the world but only on *H. helix* (Dye 1967, White and McCulloch 1934, Burkholder and Gutterman 1932.,). By 1984 (Chase 1984), the list of plants infected by *X. campestris* pv. *hederae* had expanded to include *Brassaia actinophylla* (schefflera), *Dizygotheca elegantissima* (false aralia), *Fatsia japonica* (Japanese aralia), *Fatshedera* sp.*, Polyscias fruticosa* (ming aralia), and *Schefflera arboricola* (dwarf schefflera).

The total number of the plants that showed symptoms within 1371 surveyed plants was 314 plants corresponding to 22.9 % of disease incidence. The incidence values significantly differed within surveyed plots whereas these values ranged from 0 to 56.5 % by

overall mean equal to 22.9%. All of symptomatic plants that were recorded were constricted only in *S. actinophylla*, *S elegantissima* and *S. arboricola*. Interestingly, no symptoms coincided with bacterial leaf spot were observed on *H helix* although it was susceptible in artificially inoculation. This bewildering fact can be roughly interpreted by that, one factors or more that required for causing the disease on this plant under natural conditions unconducive or not present completely. Assessment of the disease incidence is important to quantifying the seriousness of the disease and be considered easily method to determine the disease development than assessing the severity of the disease on individual plants or groups of plants (Danos *et al*., 1981). A strong correlation between incidence and severity has been reported in citrus canker (a bacterial disease caused by *Xanthomonas citri* subsp. *citri*) by Agostini *et al*. (1985). This suggesting that, the disease progress could be usefully and more simply followed by assessment of disease incidence.

Disease severity assessment on 10 symptomatic plants represented each diseased plot revealed that, the mean of the severity ratings ranged from 38.0 to 77.5% by overall mean equal to 43.5%. Most of severity values were considerably close. Evaluation of the disease severity is one of the most important measurements practiced in epidemiological studies such as comparisons of the control measures (Das and Singh, 2001) and also genetic resistance for bacterial diseases (Agostini *et al*, 1985 and Das and Singh, 2001).

This work confirmed the presence of *Xanthomonas* leaf spot on araliaceous plants in Egypt. Thus, it is necessary to take the sanitary precautions and follow the correct cultivation practices to minimize the seriousness of the disease on araliaceous plants.

REFERENCES

Agostini, J. P., Graham, J. H., and Timmer, L. W. 1985. Relationship between development of citrus canker and rootstock cultivar for young 'Valencia' orange trees in Misiones, Argentina. Proc. Fla. State Hortic Soc., 98:19-22.

Alvarez A., Benedict A.A., Mizumoto C.Y., Hunter J.E., Gabriel, D.W., 1994. Serological, pathological, and genetic diversity among strains of *Xanthomonas campestris* infecting crucifers. *Phytopathology* 81: 1449-1457.

Alvarez, A. M., Benedict, A. A., and Mizumoto, C. Y. 1985 Identification of xanthomonads and grouping of strains of *Xanthomonas campestris* pv. *campestris* with monoclonal antibodies. Phytopathology 75:722-728.

Arnaud, G. 1920. Une maladie bacterienne du lierra (*Hedera helix* L.) C.R. Hebd. Seances Acad. Sci. Ser. D 171:121-122.

Burkholder, W. H., and Gutterman, C. E. F. 1932. Synergism in a bacterial disease of *Hedera helix*. Phytopathology 22:781-784.

Chase, A. R. 1984. *Xanthomonas campestris* pv. *hederae* causes a leaf spot of five species of Araliaceae. Plant Pathol. 33:439-440.

Cooksey, D. A., and Graham, J. H. 1989. Genomic fingerprinting of two pathovars of phytopathogenic bacteria by rare-cutting restriction enzymes and field inversion gel electrophoresis. Phytopathology 79:745-750.

Danos, E., Bonazzola, R., Berger, R. D., Stall, R. B., and Miller, J. W. 1981. Progress of citrus canker on some species and combinations in Argentina. Proc. Fla. State Hortic. Soc. 94:15-18.

Das, A. K. and Shyam Singh, 2001. Managing citrus bacterial diseases in the state of Maharashtra. Indian Hort., 46(2): 11-13.

DeParasis, J., and Roth, D. A. 1990. Nucleic acid probes for identification of phytobacteria: Identification of genus-specific 16S RNA sequences. Phytopathology 80:618-621.

Dye, D. W. 1967. Bacterial spot of ivy caused by *Xanthomonas hederae* (Arnaud, 1920) Dowson, 1939, in New Zealand. N.Z. J. Sci. 10: 481-485.

Hildebrand, D. C., Palleroni, N. J., and Schroth, M. N. 1990. Deoxyribonucleic acid relatedness of 24 Xanthomonad strains representing 23 *Xanthomonas campestris* pathovars and *Xanthomonas fragariae*. J. Appl. Bacteriol. 68:263-269.

Jensen B.D., Vicente J.G., Manandhar H.K., Roberts S.J., 2010. Occurrence and diversity of *Xanthomonas campestris* pv. *campestris* in vegetable *Brassica* fields in Nepal. *Plant Disease* 94: 298-305.

Klement Z., 1990. Inoculation of Plant Tissues. In: Methods in Phytobacteriology. Akadémiai Kiadó, Budapest.

Lazo, G. R., and Gabriel, D. W. 1987. Conservation of plasmid DNA sequences and pathovar identification of strains of *Xanthomonas campestris*. Phytopathology 77:448-453.

Lazo, G. R., Roffey, R., and Gabriel, D. W. 1987. Pathovars of *Xanthomonas campestris* are distinguishable by restriction fragment-length polymorphism. Int. J. Syst. Bacteriol. 37:214-221.

Lelliott R.A., Stead D.E., 1987. Methods for the Diagnosis of Bacterial Diseases of Plants. Blackwell Scientific Publications, Oxford, UK.

Lema M., Cartea M.E., Sotelo T., Velasco P., Soengas P., 2012. Discrimination of *Xanthomonas campestris* pv. *campestris* races among strains from northwestern Spain by *Brassica* spp. genotypes and rep-PCR. *European Journal of Plant Pathology* 133: 159-169.

Lopez R., Asensio C., Gilbertson R.L., 2006. Phenotypic and genetic diversity in strains of common blight bacteria (*Xanthomonas campestris* pv. *phaseoli* and *X. campestris* pv. *phaseoli* var. *fuscans*) in a secondary center of diversity of the common bean host suggests multiple introduction events. *Phytopathology* 96: 1204-1213.

Mariya Stoyanova, Taca Vancheva, Penka Moncheva, and Nevena Bogatzevska, 2014. Differentiation of *Xanthomonas spp*. Causing Bacterial Spot in Bulgaria Based on Biolog System. International Journal of Microbiology, vol. 2014, Article ID 495476, 7 pages, 2014. doi:10.1155/2014/495476.

Norman, D. J., Chase, A. R., Stall, R. E., and Jones, J. B. 1999. Heterogeneity of *Xanthomonas campestris* pv. *hederae* strains from araliaceous hosts. Phytopathology 89:646-652.

Norman, D., and Alvarez, A. 1989. A rapid method for presumptive identification of *Xanthomonas campestris* pv. *dieffenbachiae* and other xanthomonads. Plant Dis. 73:654-658.

OEPP/EPPO (2010). EPPO Standard PM 7/100(1) Rep-PCR tests for identification of bacteria. Bulletin OEPP/EPP Bulletin 40, 365–368.

Opgenorth, D. C., Smart, C. D., Louws, F. J., de Bruijn, F. J., and Kirkpatrick, B. C. 1996. Identification of *Xanthomonas fragariae* field isolates by rep-PCR genomic fingerprinting. Plant Dis. 80:868-873.

Popovic´ T., Jošic´ D., Starovic´ M., Milovanovic´ P., Dolovac N., Poštic´ D., Stankovic´ S., 2013. Phenotypic and genotypic characterization of *Xanthomonas campestris* strains isolated from cabbage, kale and broccoli. *Archives of Biological Science* 65: 585-593.

Rademaker, J. L. W., Louws, F. J., Schultz, M. H., Rossbach, U., Vauterin, L., Swings, J., and de Bruijn, F. J. 1997. Molecular systematics of xanthomonads by rep-PCR genomic fingerprinting and computer-assisted pattern analysis. (Abstr.) Phytopathology 87(suppl.): S81.

Schaad N.W., 1988. Laboratory Guide for Identification of plant pathogenic bacteria. APS Press, St. Paul, MN, USA.

Schaad N.W., Alvarez A., 1993. *Xanthomonas campestris* pv. *campestris*: cause of black rot of crucifers. In: Swings J.S., Civerolo, E.L. (eds). *Xanthomonas*, pp. 51-56. Chapman and Hall, London, UK.

Schaad, N. W., Jones, J. B., and Lacy, G. H. 2001. *Xanthomonas.* In: Laboratory Guide for Identification of Plant-Pathogenic Bacteria, 3rd ed. N. W. Schaad, J. B. Jones, and W. Chun, eds. American Phytopathological

Suzuk A., Kusumoto S., Horie H. And Takikawa Y. 2002. Bacterial Leaf Spot of Ivy Caused by Xanthomonas campestris pv. hederae. J. Gen. Plant Pathol. 68: 398-400.

Swings J., Vauterin L., Kersters K., 1993. The bacterium *Xanthomonas.* In: Swings J.S., Civerolo E.L. (eds). *Xanthomonas*, pp. 121-156. Chapman and Hall, London, UK.

Trindade L. C., Lima M. F., Ferreira M. A. S. V., 2005. Molecular characterization of Brazilian strains of X*anthomonas campestris* pv. *viticola* by rep-PCR fingerprinting. Fitopatologia Brasileira 30: 46-54.

Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.

Verniere C., Pruvost O., Civerolo E. L., Gambin O., Jacquemoud-Collet J. P., Luisetti J. 1993. Evaluation of the Biolog substrate utilization system to identify and assess metabolic variation among strains of *Xanthomonas campestris* pv. *citri*. Appl-Environ-Microbiol. 59 (1) p. 243-249.

Versalovic J., Koeuth T. and Lupski J.R. 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. 9: 6823–6831.

Versalovic J., Schneid M., de Brujin F.J. and Lupski J.R. 1994. Genomic fingerprinting of bacteria using repetitive sequence based polymerase chain reaction. Methods Mol. Cell. Biol. 5: 25–40.

White, R. P., and McCulloch, L. 1934. A bacterial disease of *Hedera helix*. J. Agric. Res. 48:807-815.

مرض تبقع الأوراق البكتيرى على نباتات العائلة الأرالية المتسبب عن البكتريوم *Xanthomonas campestris* pv. *hederae* فى مصر

إبراهيم حسن محمد طلبة

فرع أمراض النبات – قسم النبات الزراعى – كلية الزراعة – جامعة الأزهر – القاهرة

لوحظ تواجد أعراض مرضية مشابهة لأعراض مرض تبقع الأوراق البكتيرى على نباتات من الشوفريلا أو ما يسمى بشجرة المظلة *Schefflera actinophylla*)) فى العديد من الأماكن داخل محافظتى القاهرة و الجيزة و بالتالى أجريت هذه الدراسة للتعرف على المتسبب فى إحداث هذه الأعراض و دراسة أهم خصائصه كمسبب مرضى ثم دراسة مدى إنتشار هذا المرض على نباتات العائلة الأرالية و كم الضرر المتسبب عنه لهذه النباتات.؛ هذا" و كانت خطوات الدراسة و أهم نتائجها كالتالى: بإجراء عملية العزل من العينات المشتبه فى إصابتها بالمرض تم التحصل على ثمان عزلات مشابهة فى خواصها المزرعية للجنس *Xanthomonas*، و بدراسة الخصائص الميكروسكوبية و الفسيولوجية و البيوكيماوية لهذه العزلات، كانت النتائج متطابقة مع الخصائص المسجلة للمسبب المرضى: *Xanthomonas campestris* pv. *hederae*و لتأكيد التعريف لهذه العزلات تم إخضاعها لنظام التعريف المبرمج (Biolog GN microplate system) حيث كانت التعريفات لست منها متطابقة مع البكتريوم *Xanthomonas campestris* pv. *hederae* و كانت تعريفات العزلاتان الباقيتان متطابقة على مستوى الجنس و النوع فقط حيث كانت *Xanthomonas campestris*، ثم بإجراء إختبار القدرة المرضية لهذه العزلات على أوراق نباتات من نفس النوع النباتى الذى تم عزل هذه العزلات منه، كانت جميع العزلات لديها المقدرة على إحداث أعرض متطابقة مع الأعراض التى تظهر على النباتات المصابة طبيعياً.؛ بعد ذلك تمت المقارنة بين العزلات على المستوي الجزيئي بإستخدام طريقة ERIC PCR و ذلك بهدف الوقوف على مدى التباينات الوراثية فيما بينها و قد أوضحت النتائج التشابه لحد كبير بين هذه العزلات.؛ و للوقوف على مدى التباين فيما بين هذه العزلات فى نسبة الشراسة المرضية تمت العدوى المصتنعة على نباتات من النوعين النباتيين *Schefflera actinophylla* و *Schefflera elegantissima*و بقياس رد الفعل المرضى لهذه العزلات على كلا الوعين تبين وجود بعض التباين فى الشراسة المرضية فيما بينها.؛أيضاً تم دراسة المدى العوائلى لهذه العزلات على سبعة أنواع مختلفة من نباتات العائلة الأرالية حيث تبين قدرتها جميعاً على إحداث الإصابة لأربعة أنواع هى: *Schefflera actinophylla* و *Schefflera arboricola* و *Schefflera elegantissima* و *Hedera helix* بينما لم تكن لها القدرة على إحداث الإصابة لثلاثة أنواع وهى: *Aralia nudicaulis* و *Dendropanax trifidus* و *Fatsia japonica*.؛ بدراسة نسبة حدوث الإصابة بالمرض و ذلك بإحصاء نسبة النباتات المصابة إلى السليمة فى عشرة مواقع داخل مدينتى القاهرة و الجيزة كانت نسبة الحدوث متأرجحة ما بين 0.0 % إلى 56.5 % بمتوسط عام بلغ 22.9 %.؛ و أخيراً تم تقدير شدة الإصابة بالمرض فى المواقع التى تتواجد بها نباتات مصابة تساوى عشرة نباتات أو تزيد حيث تم الإختيارالعشوائى لعشرة نباتات مصابة تمثل كل موقع من المواقع المصابة وتقدير شدة الأصابة لكل نبات على حدة ثم حساب متوسط شدة الإصابة لكل موقع و من ثم تقدير المتوسط العام لشدة الإصابة على جميع النباتات المفحوصة حيث تراوح متوسط شدة الإصابة بين المواقع المختلفة ما بين 38.0 % إلى 77.5 % بمتوسط عام لشدة الإصابة يساوى 43.5 %. نتائج هذه الدراسة أثبتت بشكل قاطع أن الأعراض المتواجدة على نباتات العائلة الآرالية مسببها هو البكتريوم *Xanthomonas campestris* pv. *hederae* و كذلك أوضحت مقدار الضرر الذى يحدثه هذا المرض على هذه النباتات؛ الأمر الذى يستوجب إتباع الإجراءات و تطبيق العمليات التى يكون مؤداها هو الحد من ضرر و تقليل إنتشار هذا المرض فى مصر.