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Xanthomonas euvesicatoria Associated with Bacterial Spot on Pepper Fruits in Egypt

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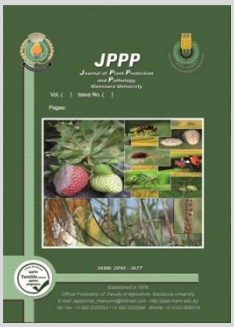

ABSTRACT

Necrotic spots on pepper fruits consider a typical symptom of bacterial spots disease.*Xanthomonas euvesicatoria* is the causative agent for this bacterial disease on pepper. Colonies isolated from symptomatic pepper fruits were exhibited typical growth on nutrient agar(NA), yeast dextrose calcium carbonate(YDC) and mTMB media. Colonies were convex, mucoid, glistening, wet and circular with an entire edge with yellow pigment.The bacterial isolates were matched with *Xanthomonas* bacteria in microscopical and biochemical tests. The isolates showed weakness in the hydrolysis of starch and negative in degrade pectate. Leaves of pepper plants exhibited water-soaked as a first reaction in the pathogenicity test. The spots turned to dark necrotic lesions and may fall and the leaves became perforated. On the stem, necrotic lesions were appeared then turned to dark color also the spots on surface fruits turned to dark color. Confirmation of the identity of the pathogenic isolate was performed by BSXeF/BSXeR as specific primers for *X. euvesicatoria* which was showed amplification of a 173bp fragment. BLAST in the NCBI GenBank database with the sequence of 16S rDNA indicated that the nucleotide sequence of a bacterial isolate obtained from the bacterial spot of pepper fruits in Egypt was 96.49% homologs to *X. euvesicatoria*. The nucleotide sequence of the tested isolate was deposited in NCBI GenBank with accession number MZ540733. *X. euvesicatoria* survived in pepper plant debris for one month to nine months while cannot survive in seed for the same time. *X. euvesicatoria* induced the symptoms on tomato, ground cherry, and London rocket plants.

***Keywords**: Xanthomonas euvesicatoria*, pepper, tomato, ground cherry, bacterial spot.

INTRODUCTION

Pepper is one member of the Family Solanaceae and was considered one of the first Genera cultivated early in the new world (Heiser, 1973). Pepper fruits became an economic vegetable crop in Egypt. Losses of pepper fruits yield were ranged between 23% to 44% as direct losses at artificially infected with pepper bacterial spot by *Xanthomonas campestris* pv. *vesicatoria*, while indirect losses of up to 95% of pepper fruits cause lost commercial value (Bashan *et al*., 1989). Bacterial spot disease was first time recorded on tomato plants in South Africa and the United States (Doidge, 1921; Gardner and Kendrick, 1921), and then was described on sweet pepper in Florida (Gardner and Kendrick, 1923). In warm and moist areas, the bacterial spot disease appeared on pepper and tomato plants as lesions on the leaves, stems, and fruits (Jones *et al*., 2000; Stall *et al*., 1994). Abdalla (2000) reported that *Xanthomonas campestris* pv. *vesicatoria* were detected and isolated from imported tomato seed lots of different cultivars as a first report of the occurrence of these important pathogen on tomato seeds in Egypt. *Xanthomonas euvesicatoria*, *X. vesicatoria*, and *X. perforans* were caused by a bacterial spot disease of tomato and pepper in Taiwan. *Xanthomonas* species were identified based on the use of carbon sources with the Biolog GN2 microplate (Lue *et al*., 2010). Bacterial spot on pepper plants in Korea was caused by *Xanthomonas euvesicatoria* according to reidentified the pathogen based on the phenotypic and genotypic characteristics (Kyeon *et al*., 2016). The causal agents of bacterial leaf spot disease on tomato, pepper, and chili plants in eastern Australia were determined and identified by phylogenetic and biochemical analyses as *X. euvesicatoria*, *X. perforans*, and *X. vesicatoria* with the most frequently recovered pathogenic species (Roach *et al*., 2018). Song *et al*, (2019) reported that bacterial colonies with pale-yellow, mucoid, convex, and circular were repeatedly isolated from infected ground cherry leaves. These bacterial isolates were identified as *Xanthomonas euvesicatoria* pv. *euvesicatoria* based on biochemical tests and genetic features of *hrpB* and sequenced four housekeeping genes (*lepA-gyrB-gapA-gltA*). Many studies were demonstrated diversity between pathogenic strains of *Xanthomonas euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* at the genome sequences level, which led to identifying specific genes to pepper pathogens and other specific genes with other strains. This showed differences in host susceptibility to infection, aggressiveness, and virulence (Potnis *et al*., 2011). Many specific primers for designated for bacterial causal agents such as *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* which caused bacterial spot disease in Solanaceae plants were described by Koenraadt *et al*. (2009) which showed the differentiation between the species and pathovars. Pepper and tomato plants behaved as main hosts for *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* while a little of the weeds behaved as alternative hosts with those bacteria in the grown areas (EPPO 2013). The weeds were considered hosts for the pathogenic causal agents and causes damage to commercial crops (Mileo *et al*., 2006). Weeds were played a great step in infection by *Xanthomonas*

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sps. and continuation the disease in growing areas (Jones *et al*., 1986; Ignatov *et al*., 2007; Araújo *et al*., 2015). The present study aimed to isolation and characterization of *Xanthomonas euvesicatoria* from infected fruits of pepper plant as the causal agent of pepper bacterial spots disease under Egyptian conditions with the evaluation of its ability to survive in pepper debris and seeds taken from infected fruits and testing its the pathogenicity on different plants, including some of the solanaceous plants and other families.

MATERIALS AND METHODS

Pathogen isolation

Samples of sweet pepper (*Capsicum annum* L.) showing typical bacterial spot lesions on the fruits were collected from Egypt during 2019. For the pathogen isolation, the necrotic lesions on fruits with surrounding healthy tissues were surface sterilized for 10 seconds in 70% ethanol and rinsed several times in sterile distilled water and then tissues were cut into small pieces with the help of a sharp sterilized scalpel (Song *et al*., 2019). The sterilized small pieces were crushed in 5 ml of sterilized distilled water with the help of a sterilized glass rod in sterilized Petri plates to form the suspension. The suspension was kept for 30 min to allow the bacteria to diffuse into the suspension. The suspension was streaked on a nutrient agar (NA) medium and incubated at 27ºC for 3-5 days. Colonies exhibited typical growth of *Xanthomonas* on NA medium were transferred to potato dextrose agar (PDA) slants. After incubation at 28 °C for 2 days, the slant cultures were stored at 4°C for further studies.

Identification of the pathogen

Morphological and microscopical characteristics

The isolates suspected to *Xanthomonas*, streaked on yeast dextrose calcium carbonate (YDC) agar medium contain per liter on 20g Dextrose,10g Yeast extract, 20g $CaCO_3$, and 15g agar (Schaad, 1988) and on mTMB agar medium or modified Tween B medium contain per liter on; 10g bacto peptone, 0.1g H_3BO_3 (Boric acid), 10g KBr, 0.25g $CaCl_2$ anhydrous, 15g Bacto agar. After autoclaving, add 10ml sterilize Tween 80, 65 mg of cephalexin, 12mg 5-fluorouracil, 0.2mg tobramycin sulphate, 35mg nystatin (McGuire *et al*., 1986). Cultures incubated at 27ºC for 3-5 days with YDC medium and 3-7 days with mTMB agar medium. The bacterial isolates were identified based on culture and colony features and microscopical characteristics including Gram staining, cell shape, and sporulation according to Schaad *et al*., 2001.

Biochemical tests

Biochemical tests were performed on bacterial cultures 48 h old including oxidase, catalase production, and potassium hydroxide 3% (KOH) tests as described by Schaad and Stall, 1989; Schaad *et al*., 2001.

Amylolytic and pectolytic assays

The amylolytic and pectolytic assays were performed according to Stall *et al*. (1994). Nutrient agar (NA) medium was supplemented with 1.5% soluble starch to test the ability of the bacterial isolates to hydrolysis starch. The bacterial isolates were streaked on medium and incubated at 27ºC for 72 h. The cultures were flooded with aqueous iodine (iodine 1.0 g, potassium iodine 2.0 g, and distilled water 100 ml). The positive strain was detected when found clearing haloes around its colonies (Lue *et al*., 2010). The pectolytic assay was performed by potato slices (Lelliott and Stead 1987; Schaad *et al*., 2001). The pectolytic activity of bacterial isolates was assessed according to the severity of rotting on the potato slices (Osdaghi *et al*., 2017).

Hypersensitive reaction

The hypersensitive reaction was carried out to assess the pathogenicity of the isolates according to Element *et al*., 1964. Leaves of *Nicotiana glutinosa* L. plants and pepper plants (*Capsicum annum* L.) 6 weeks old were infiltrated with a bacterial suspension of the isolates. An inoculum was infiltrated into the leaf by a 1 ml hypodermic syringe. Sterilized water served as the negative control. Two to three plants were used for each infiltration experiment and two to three leaves per plant were infiltrated with each sample. The plants were maintained at 28 ± 2°C under a 16 h photoperiod for 7 days.

Pathogenicity test

A pathogenicity test was performed according to EPPO (2013). Pathogenicity test carried out on young and fruiting pepper plants (*Capsicum annum* and *Capsicum frutescens*). Plants were grown in 25 cm diameter pots containing sandy and clay mixed (1:2) with normal watering and fertilization.

The bacterial isolates were cultured on an YDC agar medium at 28°C for 2 days. The bacterial suspensions were prepared in sterile distilled water. The bacterial concentration was estimated by measuring on a spectrophotometer and adjusted to OD600 ($0.2x10^8$ CFU/mL) with sterilized water (Roach *et al*., 2018).

Leaves were inoculated by using a cotton swab saturated with inoculum with added carborundum for scratching. Inoculated plants were covered with clear plastic bags to preserve the humidity at a high level for 4 days after inoculation. Negative control was inoculated by sterilized distilled water only without bacteria and maintained under the same conditions. The plants were checked weekly for three weeks.

Water-soaked spots or hypersensitive responses were considered a positive reaction. The plants which appeared positive reaction were re-isolated from symptomatic tissues.

Molecule characterization

The bacterial isolate (designated 1X) used in molecular studies was isolated from symptomatic plants and exhibited typical symptoms on pepper plants with artificial infection.

Bacterial DNA extraction

For DNA extraction, the bacterial isolate was grown on an YDC medium for 48h at 28°C. Single colony was taken by sterilizing a toothpick and suspended in 100µl sterilized distilled water (Lue *et al*., 2010). DNA extraction was performed according to EPPO (2016), the bacterial suspension was heated at 95°C for 10 min in a water bath following cooled in ice for 5 min. The bacterial suspension was centrifuged at12000 rpm for 2 min. the supernatant containing DNA was preserved at -20° C for use in gene amplification and sequencing.

Species and specific primers

Xanthomonas euvesicatoria and *X. perforans* were associated with the bacterial spot disease of Pepper according to Roach *et al*., 2018. Specific primers were used for the identification of *Xanthomonas* species according to Koenraadt *et al*., 2009. For *Xanthomonas euvesicatoria*, Bs-XeF (5'-CATGAAGAACTCGGCGTATCG-3') and Bs-XeR (5'-GTCGGACATAGTGGACACATAC-3') and for *Xanthomonas perforans*, BS-XpF (5'-

GTCGTGTTGATGGAGCGTTC-3') and Bs-XpR (5′-GTG CGA GTC AAT TAT CAG AAT GTG G-3′). PCR cocktail, Taq DNA Polymerase master mix was used according to the manufacturer's recommendations (GATC Biotech Company in Konstanz, Germany, https://www.eurofinsgenomics.eu/) mixed with 50ng total DNA and 1µl of each primer. The annealing temperatures were 64ºC for both of them. The bands appeared on 1.5 % agarose gel after electrophoresis.

Sequencing by 16S rDNA

Bacterial isolates identification was dependent on the nucleotide sequences and phylogenetic analysis of bacterial DNA. The 16S rDNA was amplified with primers, F (5'-AGAGTTTGATCMTGGCTCAG-3') and R (5'-GGTTACCTTGTTACGACTT-3') Kyeon *et al*. (2016). PCR reaction was performed by 25 µl Taq Red Mix, 1 µl (20 Pico mol) Forward Primer, 8 µl DNA Template, 1 µl (20 Pico mol) Reverse Primer and 15 µl Nuclease free water. Thermal cycles were carried out as follows: Initial denaturation at 94ºC for 6 min followed by 35 cycles at 94º C for 45s, 56ºC for 45s, and 72º C for 1 min with a final extraction at 72º C for 5 min. PCR product was sent to GATC Biotech Company in Konstanz, Germany (https://www.eurofinsgenomics.eu/) for sequencing. The nucleotide sequences result and phylogenetic analysis were compared with bacterial nucleotide sequences in NCBI Genbank databases by using the BLAST tool (http://blast.ncbi.nlm.nih.gov/).

Bacterial survival

Plant debris

The ability of *Xanthomonas* on survives in injured pepper debris saved under field conditions was examined. Thirty grams of the pepper plant debris which was saved for one month and another for nine months each of apart were surface sterilized for 10 seconds in 70% ethanol, then rinsed several times in sterilized water. The sterilized parts were dried on sterilized filter papers for 20 moments, then cracked in a mortar and suspended in 40 ml sterilized water with shaking on a rotary shaker for one hour. Ten microliters of the suspension per plate were streaked on YDC medium and incubated at 28 Cº. The plates were checked after five days to note *Xanthomonas* suspect colonies. The bacterial isolates were evaluated for pathogenicity on pepper plants and confirmed by PCR using specific primers for *Xanthomonas euvesicatoria* which was associated with bacterial spots on pepper plants (Roach *et al*., 2018).

Seeds

Survive of *Xanthomonas* in seeds (produced from symptomatic pepper fruits) was evaluated by isolation. Seeds were stored for one month and another for nine months under room conditions. Ten grams of the stored seeds for one month and another for nine months each apart were used in the experiment. Bacterial isolation from seeds was performed by socking method according to EPPO, 2013. Briefly, seeds were soaked in 10 mM of autoclaved and cooled phosphate-buffered saline (PBS, pH 7.2) contents: /L (2.7g $Na_2HPO_4.12H_2O$, 0.4g $NaH_2PO_4.2H_2O$, 8g NaCl, 1000mL distilled water) with shaking for 2 h at 24ºC. The suspension was filtered through sterilized gauze and centrifuged at 10000–12000g for 20 min at 10°C. The supernatant was discarded and suspended the pellet in 10 mM phosphate-buffered saline (PBS). Tenfold dilutions were prepared, and the isolation was performed from the final concentrate. Ten microliters for each plate of bacterial suspension were streaked on YDC medium in 9 cm plates and incubated at 28º C. The plates were checked after five days to note *Xanthomonas* suspect colonies. Suspect isolates were evaluated for pathogenicity on pepper plants and confirmed by PCR using specific primers for *Xanthomonas euvesicatoria* which was associated with bacterial spots on pepper plants (Roach *et al*., 2018).

Host response

In addition to pepper, the ability of *Xanthomonas euvesicatoria* isolate to induce water soaking and necrotic spots was tested on 11 different healthy plants. The bacterial inoculation was 10^8 CFU/mL concentration was prepared from 48 h old culture grown on YDC medium. Plants were grown in twenty-five centimeter diameter pots and maintained in the greenhouse (Osdaghi *et al*., 2017). Tested plants were: eggplant (*Solanum melongena* L.), black nightshade (*Solanum nigrum* L.), milkweed (*Euphorbia peplus*), chenopodium (*Chenopodium album*), green amaranth (*Amaranthus viridis* L.), barnyard grass or cockspur grass (*Echinochloa crusgalli* L.), fleabane (*Conyza linifolia* L.), tomato (*Solanum lycopersicum* L.), ground cherry (*Physalis pubescens* L.), Sweet orange (*Citrus sinensis* L.) and London rocket (*Sisymbrium irio* L.). Plants were scratched by a piece of cotton with carborundum. Artificially inoculation was performed by spraying the bacterial suspension on the tested plants according to Santos *et al*., 2020. The plants were covered by transparent plastic bags for 5 days for high humidity then removed the covers with regular irrigation and fertilization. Plants were checked after 3 days, then daily to 10 -15 days.

RESULTS AND DISCUSSION

Pathogen isolation

Fruit Samples of sweet pepper (*Capsicum annum* L.) were collected from Egypt during 2019. Samples were showed spots and necrotic lesion spots on fruits as typical bacterial spot (Figure 1).

Figure 1. Typical bacterial spot symptoms on different parts of sweet pepper fruits (Spots and necrotic lesions).

Typical colonies that resembled *Xanthomonas* on nutrient agar medium were appeared after 3-6 days at 27ºC. The colonies were convex, mucoid, glistening, and circular with an entire edge with slightly yellow pigment. In final, twelve *Xanthomonas*-like bacteria were isolated from symptomatic pepper plants.

Characterization of the pathogen

Morphological and microscopical characteristics

In the present study, bacteria isolated from spot lesions of pepper fruits were exhibited typical morphology as

Xanthomonas on YDC medium. Colonies of all isolates were pale yellowish, convex, mucoid, glistening, wet, and circular with an entire edge (Figure 2A). This result agreed with Kyeon *et al*. (2016); Song *et al*. (2019). On mTMB medium, after 3-7 days suspected colonies were yellow, slightly mucoid, and round. Colonies were utilized tween 80 and exhibited clear zone under and around *Xanthomonas* colonies (Figure 2B) agreed with EPPO, 2013.

Bacterial cell under the light microscope was rod shape, had no endospore formation, and negative with Gram staining (Table 1).

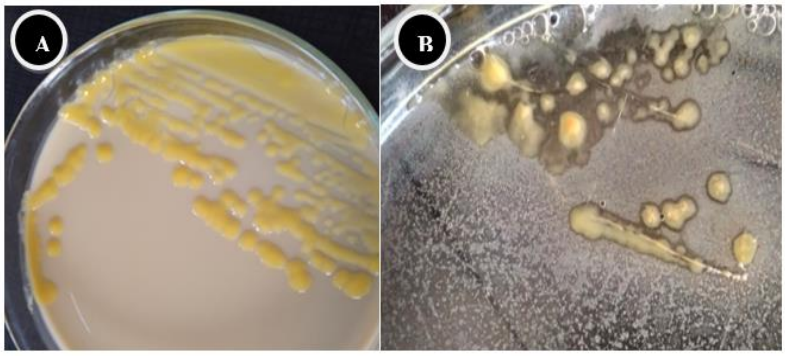

Figure 2. Bacterial isolate colony on: (A) YDC medium and (B) on mTMB medium.

Biochemical tests

Results of biochemical tests were shown in Table 1. Suspect isolates were reacted positively to catalase and potassium hydroxide 3% (KOH) tests, while they showed negatively with oxidase reaction consorted with Roach *et al*., 2017.

Amylolytic and pectolytic activity

In this study, Egyptian bacterial isolates were shown weakly in hydrolyze starch and degrade pectate (Table 1) resemblance with Jones *et al*., 2004; Kyeon *et al*., 2016 who found that the Korean pepper pathogenic strains were negative for pectolytic and amylolytic activity. These features were identical to those of *X. euvesicatoria* and *X. gardneri*. Also; it agrees with Lue *et al*., 2010 who reached 35 strains from 40 isolated from pepper and tomato did not hydrolyze starch and degrade pectate on media. While Osdaghi *et al.* (2016) reported that strains of *X. euvesicatoria* isolated from bacterial spots of pepper plants showed strong amylolytic, but no pectolytic activity. *X euvesicatoria* pv. *euvesicatoria* strains isolated from Grenada were severely in hydrolyzing starch and degrade pectate (Hamza *et al*., 2010).

Hypersensitive reaction

Bacterial isolates were shown water soaking spots as the first reaction after 48-96 h, then yellowish in injection sites. Treated tissues on *Nicotiana* leaves were turned to brown color after 5 days as a positive result with pathogenic isolates (Figure 3A) while on pepper leaves, infiltrated tissues were drained dead and maybe treated tissues felled (Figure 3B).

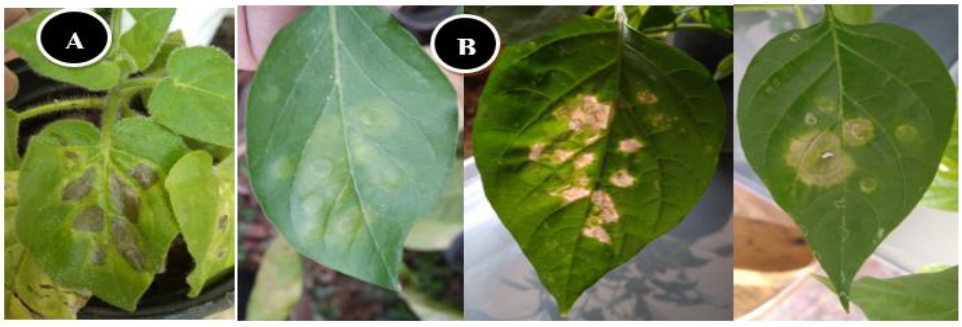

Figure 3. Hypersensitive reaction on (A) *Nicotiana* leaves, yellowish in injection tissues then turned to brown color as a positive result with pathogenic isolates and (B) pepper leaves, yellowish in injection tissues then turned to water-soaked spot and finally turned to brown color may center fall.

Table 1. Characteristics and reactions of bacterial isolates with different tests.

Characteristic	Result
The shape of bacterial cell	rod
Endospore formation	No
Gram staining	Negative
Amylolytic activity	Weak
Pectolytic activity	Negative
Oxidase enzyme	Negative
Catalase enzyme	Positive
Potassium hydroxide 3% (KOH)	Positive

Pathogenicity test

The bacterial isolates which exhibited typical growth of *Xanthomonas* on YDC medium were tested for pathogenicity on pepper plants (*C. annum and C. frutescens*). Water soaking spots on the lower epidermis of leaves as a first positive reaction has appeared after 7-10 days of inoculation. The spots turned to necrotic lesions with dark brown color after 10 days. The affected areas may fall and the leaves were had a perforated appearance (Figure 4A). On the stem, necrotic lesions were appeared then turned to dark color (Figure 4B). On different pepper fruits, raise spots firstly then turned to a dark color on the fruit surface (Figure 4C). The results similar to the symptoms were described by Kyeon *et al*., 2016. While Osdaghi *et al*., 2016 noticed halos surrounding lesions without perforation on infected leaves. Jones *et al*., 2016 and Osdaghi *et al*., 2016 reported a drop of leaves happened with the severity of infection. *Xanthomonas*

enters plant leaves through stomata, then it increases in intercellular spaces and the end, the cells die, and the tissues necrotized, and *Xanthomonas* spread outside infected tissue to new tissues for widespread disease (Potnis *et al*., 2015).

Re-isolated from symptomatic tissues resulted typical colonies of *Xanthomonas* and positive with pathogenicity test.

Figure 4A. Water-soaked spots on lower epidermis of pepper leaves as a first positive reaction then turned to necrotic lesions with dark brown color and in the end, affected areas may fall and the leaves were had perforated appearance.

Figure 4 B. Necrotic lesions were appeared then turned to a dark color on the stem.

Figure 4 C. Raise spots firstly on surface fruits then turned to dark color.

Molecule characterization

Confirmation of the identification for the pathogenic isolate (designated 1X) was performed by BSXeF/BSXeR primers as specific for *Xanthomonas euvesicatoria* was showed amplification of a 173 bp fragment (Figure 5 A). While amplification with Bs-XpF/Bs-XpR as specific primers for *Xanthomonas perforans* was not obtained result (Figure 5 B). This is agreed with Osdaghi *et al*., 2016; Nechwatal and Theil 2020. While Kyeon *et al*., 2016 reported that, Korean pepper was infected by *Xanthomonas euvesicatoria* and *Xanthomonas perforans* is a causing of bacterial spot disease. Roach *et al*., 2018 found that *Xanthomonas euvesicatoria* can infect capsicum and chili plants in eastern Australia causing bacterial spot disease and confirmed that by using BSXeF/BSXeR primers and Australian isolates did not react with Bs-XpF/Bs-XpR specific primers for *Xanthomonas perforans*.

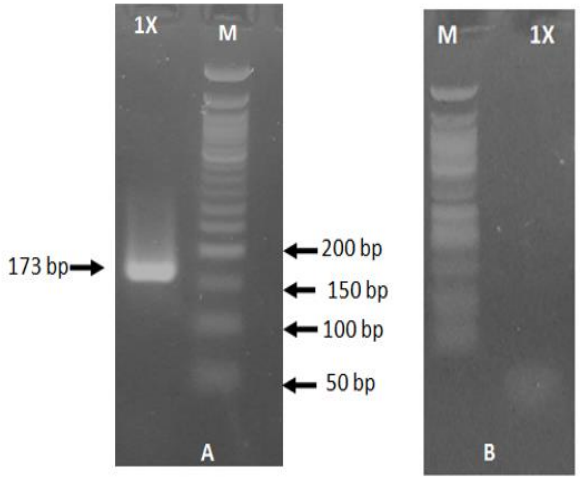

Figure 5. A.PCR amplification using BSXeF/BSXeR primer pars as a specific primer for *Xanthomonas euvesicatoria*. Lan M: ladder marker (GeneRular™ 50 bp DNA ladder) and lan 1X: tested isolate which resulted in bund at 173 bp; (B) PCR amplification using Bs-XpF/Bs-XpR as specific primers for *Xanthomonas perforans*. Lan M: ladder marker (GeneRular™ 50 bp DNA ladder) and lan 1X: tested isolate which no bund resulted.

BLAST search on the NCBI GenBank database with the sequence of 16S rDNA indicated that nucleotide sequence for bacterial isolate obtained from pepper bacterial spot in Egypt belonged to *Xanthomonas*. The isolate was positive with BSXeF/BSXeR primers as specific with *X. euvesicatoria*. Bacterial isolate in this study had homology of *Xanthomonas euvesicatoria* (Figure 6) by percentage identity 96.49% and query cover was 97%. This is agreed with Osdaghi *et al*., 2016; Nechwatal and Theil, 2020; Kyeon *et al*., 2016; Roach *et al*., 2018. Nucleotide sequence for the bacterial isolate was deposited in NCBI GenBank with accession number MZ540733 and bacterial isolate designated *Xanthomonas euvesicatoria* strain 1Xe-MAS.

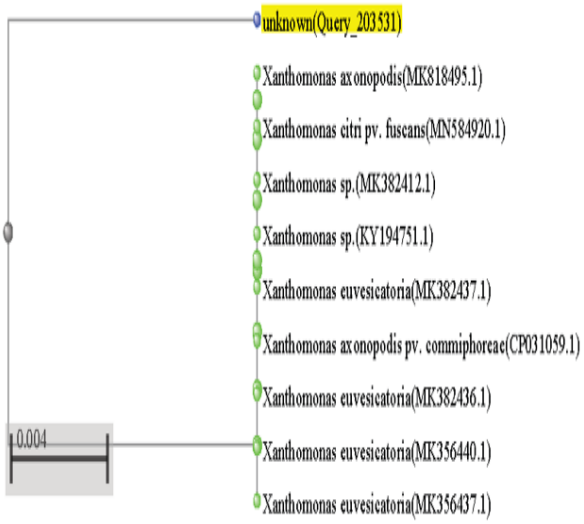

Figure 6. The phylogenetic tree illustrates the similarity of the isolate studied to strains deposited in GenBank database.

Bacterial survival

Plant debris

Experimental isolation of *Xanthomonas* bacterium from pepper Plant debris which was saved for one month showed the largest number of colonies suspected of *Xanthomonas* on YDC medium while the isolation from pepper Plant debris which was saved for nine months was showed fewer numbers of suspected colonies.

Pathogenicity test on pepper plants for resembled isolates isolated from pepper Plant debris whether saved for a month or for nine months showed typical symptoms of bacterial spots on pepper plants (Figure 7).

The isolates were showed Pathogenicity on pepper plants (one pathogenic isolate from a group) were confirmed by PCR using BSXeF/BSXeR as specific primers for *Xanthomonas euvesicatoria* and compared with isolate 1X which was originally isolated from bacterial spots on pepper fruits (Figure 8). Jones *et al*., 1986 found that the pathogenic causal agent can survive in plant debris and can survive in plant residues on the surface of the soil for enough time to infection the seedling in the next season. Gilbertson *et al*., 1990 isolated *Xanthomonas campestris* pv. *Phaseoli* from bean debris during 8 months, highest of bacterial isolates numbers in the first month and the lowest numbers in the last months.

Figure 7. The artificial symptoms on leaves of pepper plants by (A): isolate 1X (identified *Xanthomonas euvesicatoria*); (B): isolate isolated from pepper Plant debris which saved for nine months and (C): isolate isolated from pepper Plant debris which saved for one month.

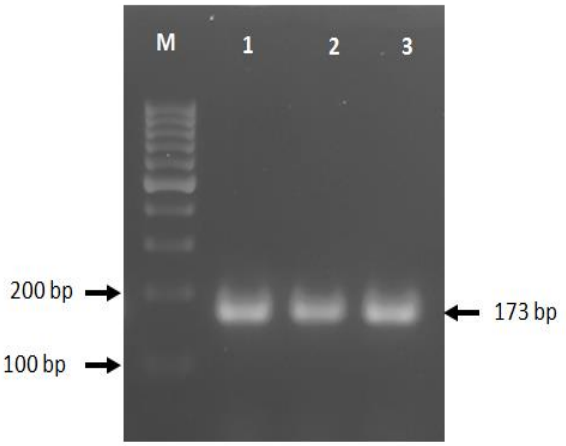

Figure 8. PCR amplification with *Xanthomonas euvescatoria* by specific primers Bs-XeF/ Bs-XeR. Amplification of 173 bp fragment for all isolates studied with specific primers Bs-XeF/ Bs-XeR, M: ladder marker (GeneRular™ 100 pb DNA ladder); (1): isolate 1X as positive isolate; (2): pathogenic isolate isolated from pepper Plant debris which saved for nine months and (3): pathogenic isolate isolated from pepper Plant debris which saved for one month.

Seeds

The attempt of isolation of *Xanthomonas* bacterium from pepper seeds (produced from symptomatic fruits) which were kept for one month and nine months under room conditions were showed no any suspected colonies of being *Xanthomonas* on YDC medium. While Potnis *et al*., 2015 reported that, seeds and seedlings contaminated by *Xanthomonas* spp. are carrying the major sources for bacterial spot disease on tomato and pepper plants. Jones *et al*., 1986 reported that seeds and weeds appear to be questionable inoculum sources. Maybe chemical solutions used in sterilizing the seeds can kill the pathogen which carried on the surface of seeds but difficult that with internally.

Host response

Xanthomonas euvesicatoria isolates were isolated from symptomatic pepper tissues induced symptoms after 10:15 days from inoculation on *Solanum lycopersicum* L., *Physalis pubescens* L., and *Sisymbrium irio* L. (Table 2 & Figure 9). The results agreed with Osdaghi *et al*. (2016) found strains of *X. euvesicatoria* isolated from bacterial spots of pepper plants were infected tomato plants in the greenhouse. Also agreed with Song *et al*., 2019 who reported, *Xanthomonas euvesicatoria* pv. *euvesicatoria* isolates isolated from symptomatic ground cherry was induced symptoms on pepper, tomato, and ground cherry plants by artificial inoculation.

On other hand, *Xanthomonas euvesicatoria* isolates were isolated from symptomatic pepper tissues was not induced symptoms on remaining tested plants (Table 2), on *Solanum melongena* L. agreed with Osdaghi *et al*., 2016, 2017, *Solanum nigrum* L. while Osdaghi *et al*., 2016 documented, Iranian strains of *Xanthomonas euvesicatoria* were able to colonized nightshade plant as a new host. Jones *et al*., 1986 considered black nightshade as a host for *X. vesicatoria*. Also, *Euphorbia peplus*, *Chenopodium album*, and *Amaranthus lividus* L. disagreed with Santos *et al*., 2020 reported, *Xanthomonas euvesicatoria* was isolated from amaranth *Amaranthus lividus* L. plants.

Table 2. Host response to *Xanthomonas euvesicatoria* isolate was isolated from the symptomatic pepper plant.

Tested plant	Family	Reaction
Barnyard grass or cockspur grass (*Echinochloa crusgalli* L.)	Poaceae	-
Black nightshade (*Solanum nigrum* L)	Solanaceae	-
Chenopodium (*Chenopodium album* L.)	Amaranthaceae	-
Eggplant (*Solanum melongena* L.)	Solanaceae	-
Fleabane (*Conyza linifolia* L.)	Asteraceae	-
Green amaranth (*Amaranthus viridis* L.)	Amaranthaceae	-
Ground cherry (*Physalis pubescens* L.)	Solanaceae	+
London rocket (*Sisymbrium irio* L.)	Brassicaceae	+
Milkweed (*Euphorbia peplus* L.)	Euphorbiaceae	-
Sweet orange (*Citrus sinensis* L.)	Rutaceae	-
Tomato (*Solanum lycopersicum* L.	Solanaceae	+

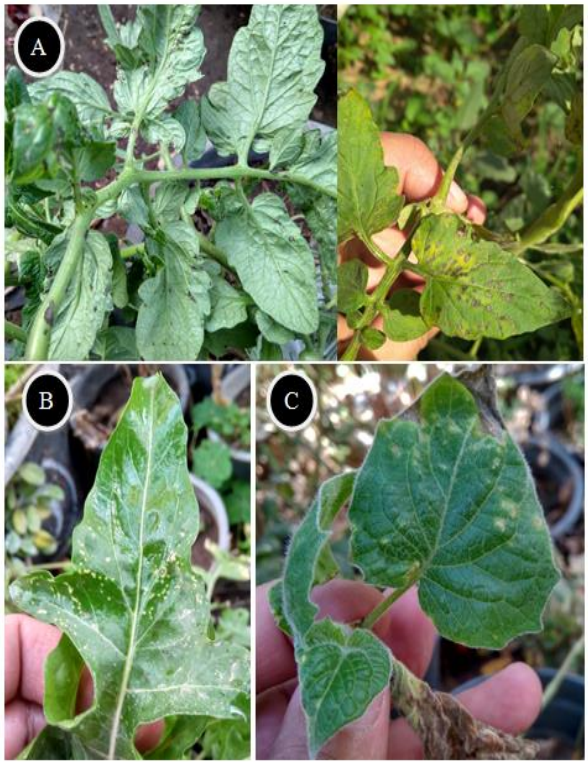

Figure 9. Host response after artificially infected by *Xanthomonas euvesicatoria* and showed a bacterial spot on (A): *Solanum lycopersicum* L.; (B) *Sisymbrium irio* L. and (C) *Physalis pubescens* L.

CONCLUSION

Xanthomonas euvesicatoria was associated with pepper fruits. The fruits were exhibited typical symptoms of bacterial spot disease. *Xanthomonas euvesicatoria* isolated from infected pepper plant debris after one month and after nine months, but not isolated from seeds at the same times. Also, *Xanthomonas euvesicatoria* infected some plants such as ground cherry and London rocket under artificial infection condition.

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مصاحبة *Xanthomonas euvesicatoria* للتبقع البكتيرى على ثمار الفلفل فى مصر

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تعتبر التبقعات المتقرحة على ثمار الفلفل عرضاً نموذجياً للإصابة بمرض التبقع البكتيرى فى الفلفل والذى تسببة بكتيريا *Xanthomonas euvesicatoria* . المستعمرات المعزولة من ثمار الفلفل أظهرت نمواً نموذجيا على بيئات Nutrient agar (NA), yeast dextrose calcium carbonate (YDC), mTMB حيث كانت المستعمرات محدبة ومخاطية ولامعة ورطبة ودائرية مكتملة الحافة ذات صبغة صفراء. العزلات البكتيرية المعزولة كانت متطابقة مع بكتيريا *Xanthomonas* من حيث الإختبارات المجهرية والكيميائية الحيوية. كما ان العزلات اظهرت ضعفاً فى قدرتها على تحليل النشا وعدم مقدرة على تكسير البكتين. مع اختبار القدرة الإمراضية أظهرت العزلات قدرتها على إحداث عرض التبقع المائى على أوراق نباتات الفلفل كأول أعراض الإصابة ، ثم تحولت تلك التبقعات الى مناطق داكنة ومتقرحة والبعض منها ما لبث ان سقط فظهرت الأوراق مثقبة. وعلى السيقان أظهرت العدوى بالعزلات مناطق متقرحة تحولت بعدها الى اللون الداكن وعلى سطح الثمار أيضاً تحولت التبقعات الى اللون الداكن. إعتمد تأكيدالتعريف للعزلات التى أظهرت قدرة إمراضية على إعطاء نتيجة ايجابية مع البادئ المتخصص BSXeF/BSXeR وهومتخصص لبكتيريا *Xanthomonas euvesicatoria* والذى أعطى نتيجة ايجابية عند 173 bp . وبمقارنة التتابع النيوكلتيدى الناتج من استخدام 16S rDNA للعزلة الممرضة ومقارنته مع التتابعات النيكلوتيدية المحفوظة فى قاعدة بيانات بنك الجينات أظهرت ان نسبة التشابة تصل الى 96.49% مع سلالات *Xanthomonas euvesicatoria* المحفوظه. كما تم إيداع التتابع النيوكلتيدى للعزلة المعزولة من ثمار الفلفل ببنك الجينات تحت رقم MZ540733 والسلالة 1Xe-MAS *Xanthomonas euvesicatoria* . كما ان العزلة البكتيرية محل الدراسة أظهرت قدرتها على البقاء فى بقايا نباتات الفلفل من شهر واحد الى تسعة أشهر بينما لم تتمكن من البقاء فى البذور لنفس المدة. كذلك فى أختبار استجابة النباتات وأظهر الأعراض نتيجة العدوى الصناعية بالبكتيريا أظهرت البكتيريا القدرة على إظهار الأعراض على كلاً من نبات الطماطم ونبات الحرنكش ونبات فجل الجمل من بين أحد عشر نباتاً مختبراً تتبع عائلات نباتية مختلفة.