Biological and Chemical Control of bacterial diseases infecting tomato plants

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Thesis

Submitted in Partial Fulfillment of
The Requirements for the Degree of

Doctor of Philosophy

In

PLANT PATHOLOGY

(BACTERIAL DIESEASES)

Agricultural Botany Department
Plant Pathology Branch

Faculty of Agriculture

Benha University

2011

APPROVAL SHEET

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ACKNOWLEDGMENT

All the greatest gratefulness, deepest appreciation and sincerest thanks to ALLAH for all gifts which given me and for enabling to overcome all problems which faced in my and throughout the course of this investigation and helping me to achieve this hard work in the ideal form to bring-forth to light this thesis.

The author wishes to express her deepest gratitude and indebtedness to the supervisor of the present work ***Prof. Dr. Nawal Abd El-Monem Eisa Professor of Plant Pathology Botany Dept., Fac. Agric., Banha Univ****., for her constructive supervision, valuable advice, kind guidance, great assistance in the preparation of this manuscript and for her help in putting thesis in its final from.*

I would like to thank ***Prof. Dr. Nagy Yassien abd El-Ghafar Professor of Plant Pathology, Plant pathology Dept., Fac. Agric., Ain-Shams Univ.****, for his great help, encouragement, invaluable guidance and his kind attitude toward me during all time of this research and in the final preparation of this manuscript.*

The author indebted to ***Prof. Dr. Mohamed Haroun Abdel- Mageed Professor of Plant Pathology, Botany Dept., Fac. Agric****., Banha Univ., for* his *kindness. He has been a great support by his technical observations and effort which were very helpful throughout the work. He was kind enough to share me my problems during this work.*

I'm also indebted to ***Prof. Dr. Fathy Gad Mohamed Professor of Plant Pathology, Botany Dept., Fac. Agric., Banha Univ., for his sincere*** *encouragement, scientific support, keeping interest and his helps in provision of all facilities needed for the present work****.***

Also the progress of love and sincere thanks and gratitude to my dear *Dr.* ***Mohamed Refaat as he stood beside me and encouraging me to work on and access to what I'm into right now.***

Thanks are also due to all staff members of the Fungi and Plant Pathology Branch, Agric. Botany Dept., Fac. Agric., Banha Univ., for their kindness and technical assistance.

And forgotten to thank Department of Plant Pathology in the Faculty of Agriculture, Ain Shams and special plant bacterial diseases, where the peer laboratory to provide all aid to the generous assistance until it is the subject of this picture them all thanks for them.

CONTENTS

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important economical crops, which belonging to the family Solanaceae. Tomato is cultivated for its fruits which having economic importance for domestic consumption, export and food industries. Tomato is grown in open fields and under protected houses. The medium weight of one tomato fruit (about 150g) has: 47% of vitamin C, 23% vitamin A, 8% iron, 5% B6 (Niacin), 5% B7 (Thiamine 1) **(Self. Nutrition Data, 2010).**

Egypt ranks fifth in the world for tomato production (**FAO, 2010**). In 2009/2010, farmers produced about 9,204,097 million tons of tomato from total area of 476.190 feddan plus 2314 protected houses (Year Book of Ministry of Agriculture & Land Reclamation). Tomato also contains important vitamins, minerals and antioxidants.

Bacterial spot disease of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (*Xcv*) is present wherever on tomato and peppers growing's. In general, *Xanthomonas* pathovars have narrow host ranges. *Xcv* consists of different strains that vary in their pathogenicity to tomato, pepper, and nightshade. The bacterium is able to survive on tomato weeds and can overwinter in diseased plant debris. Seed is an important mechanism for survival and dissemination of *Xcv.* Disease development is favored by temperatures between 26.7 and 32°C and by heavy rainfall. The bacterium is spread by wind-driven rain, workers, farm machinery, and aerosols. It penetrates through stomates and wounds created by insects, wind-driven sand, and tools **(Jones *et al.*, 1998; Abd El-Ghafar** and **Mosa, 2001; Abd El-Sayed and Abd El-Ghafar, 2004; El-Hendawy *et al.*, 2005; El-Meneisy *et al.*, 2005 and Dicklow, 2009).**

The disease could be great cause damage to all plant parts including leaves, stems, and fruits with considerable reduction of quality and quantity of tomato yields. Disease control is generally achieved by the use of fungicides, in spite of their side effects. **(Byrnea *et al.*, 2005)**.

Biological agents such as *Pseudomonas fluorescens, Pseudomonas putida, Bacillus subtilis, Rahnella aquatilis* were used for the disease control under greenhouses and field conditions (**Byrnea *et al.*, 2005; El-Hendawy *et al.*, 2005 and Abo-Elyousr and El-Hendawy, 2008).** Recently**,** bacteriophage was used for controlling foliar pathogenic bacteria as described by **Lang *et al.*, (2007**).

Antibiotics such as tetracycline or erythromycin, plant extract of garlic plants (*Allium sativum*), plant oils such as Mentha oil (*Mentha aquatica*), clove oils, as well as, inducing resistant acids like ascorbic acid and salicylic acid could be used to control the bacterial spot of tomato **(Abd El-Ghafar and Abd El-Wahab, 2001; Polizzi *et al.*, 2002; Morais *et al.*, 2002; Pietro *et al.*, 2004** and **Balestra *et al.*, 2009).**

This study aimed to throw the light on the bacterial spot of tomato and peppers which form the great problem on all the foliage parts like leaves, stems, flowers and fruits in Egypt. Thus, we focused our searching on isolation and identification of this bacterium that causing bacterial spot of tomato. Using of different safety methods that able to the inhibited growth of the causal bacterial spot under *in vitro* condition such as: biological agents, antibiotics, plant extracts, plant oils, induction resistance, bactericides and bacteriophages. Also, applying of these treatments for controlling bacterial spot of tomato under *in vivo* conditions.

REVIEW OF LITERATURE

1- Symptoms and isolation of the bacterial spot disease of tomato:

Schaad *et al.* (1980) stated that seed-borne phase of this bacterium *Xanthomonas campestris* pv. *vesicatoria* is an important means of survival and long distance dissemination.

Pohronezny and Volin (1983) revealed that bacterial spot of tomato (*Lycopersicon esculentum*), caused by either *Xanthomonas campestris pv. vesicatoria* or *X. vesicatoria*, is a serious disease, which can affect the foliage, fruit, blossoms, and stems. Causing poor fruit quality and reduced the number of fruits in the US.

Kritzman (1989) studied several detection techniques of bacterial pathogens of tomato seeds. A methods for detecting *X. campestris* pv. *vesicatoria*, including culturing from seed onto a semisolid or differential medium, grow-out tests, inoculating tomato plants with ground seed filtrate, phage plaque counting tests, immunofluorescence (IF), and combination of IF and plant inoculation.

Kritzman (1991) developed a method for detection and quantitative estimation of tomato seed borne pathogenic bacteria. It enables detection in a 79 tomato seed sample of as few as10 cfu/g tomato seed of the previous pathogens. The method employs dry grinding, weighing, bacterial extraction and quantitative calculation on selective or semi selective medium. The efficiency of this method was tested by diluting pathogen-free seed lots with naturally or artificially infested tomato seeds. This procedure enables the determination of the minimal threshold of pathogen which can be detected by this method on media, in comparison with the percentage of diseased seedlings developed from the same seed lots in the growth chamber or in the greenhouse.

Scortichini (1991) detected *Xanthomonas campestris* pv. *vesicatoria* in 15 of 22 seed lots. Recovery of *X. campestris* pv.

vesicatoria from tomato seed ranged from 17.7 to 100% on CKTM (medium) compared with 6.3 to 44.4% on tween B medium. In addition, recovery of *X. campestris* pv. *vesicatoria* on CKTM medium was qualitatively superior to that tween B medium, with a greater reduction of contaminating micro flora explained methods for inspecting tomato seeds for infection by *Corynebacterium michiganensis* subsp. *michiganensis* and *X. campestris* pv. *vesicatoria*, including serological and pathogenicity tests.

Franken *et al.* (1992) mentioned that isolation of *Xanthomonas campestris* pv. *campestris* from seeds has been improved by using semi-selective agar media. However, differentiation of *X. campestris* strains from closely related pathovars of *X. campestris* (Xc) attacking other brassicas is not possible on the basis of morphological and biochemical.

Mortensen (1997) described the bacterial spot diseases on tomatoes as numerous angular spots on the leaves. Initially, the spots are water-soaked. Leaves infected at an early stage became deformed. Often the margins of affected leaves are rimmed with a narrow band of necrotic tissue. Bacterial spots on the fruits are at first small, blister-like and irregular, and later turn brown and develop a warty appearance.

Jones *et al.* (1998) stated that bacterial spot was firstly observed on tomato in South Africa as early as 1914, for almost half a century. A single bacterial species was considered the cause of bacterial spot of pepper and tomato. Recently, it has been shown that bacteria belonging to four distinct groups cause bacterial spot: *Xanthomonas campestris* pv. *vesicatoria* (group A), *Xanthomonas vesicatoria* (group B), *Xanthomonas gardneri* (group D), and group C strains that may represent a subspecies of some group A strains. Strains of groups A and B are the most widely distributed. The vast majority of strains that infect pepper are in group A and possibly some in groups B and D. No pepper strains have been found in group C, however, strains from all four groups have been isolated from

tomato. Some strains infect only pepper (pepper strains), some infect only tomato (tomato strains), and some others infect both pepper and tomato (pepper/tomato strains).

Bouzar *et al.* (1999) isolated four hundred thirty-three Xanthomonads strains from tomato or pepper plants from 32 different fields in four Caribbean and Central American countries.

Abd Alla (2000) assayed the imported tomato seed lots of different cultivars for the presence of seed borne bacterial pathogens, using the liquid assay method for detection of bacteria, where seed extracts were placed on different semi selective media. *X. campestris* pv. *vesicatoria* was detected in 12% of the seed samples tested. All strains from seeds of pepper cv. Yolo Wonder and tomato cv. Calypso were non-pathogenic.

Alvarez (2000) revealed that *Xanthomonas campestris* pv. *campestris* (Xcc), the causal of black rot of crucifers, is a seed-borne bacterium which occurs worldwide.

Abd El-Ghafar and **Abd El-Wahab (2001)** isolated *X. campestris* pv. *vesicatoria* from tomato seeds and plants grown under protected conditions at different locations in Fayoum, Ismailia and Menufia governorates during early summer in 1997 and 1998 growing seasons.

Carrillo-Fasio *et al.* (2001) obtained thirty six isolates of *X. campestris* pv. *vesicatoria* from tomato and pepper leaves and/or fruit showing typical symptoms of bacterial spot, collected in various horticultural zones in the state of Sinaloa.

Jones *et al.* (2004) found that bacterial spot of tomato (*Lycopersicon esculentum*) is one of the most devastating diseases on tomatoes in Florida (incited by two *Xanthomonas* species *i.e.*, *Xanthomonas* perforans tomato races 3 and 4 and *Xanthomonas euvesicatoria* tomato race 1).

Yang *et al.* (2005) found that tomato seeds are borne with a number of bacterial diseases, among which the bacterial spot disease

caused by *Xanthomonas vesicatoria* (Doidge) Dye is a serious problem. The bacterial spot pathogen is seed-borne, and presents as epiphytic populations on asymptomatic seedlings and mature plants. *X. vesicatoria* was more prevalent in regions with high humidity and heavy rainfall.

Fabio *et al*. (2006) revealed that, the bacterial leaf spot caused by *Xanthomonas vesicatoria* is a severe disease favoured by warm, wet weather and is a constant hazard to the commercial production of tomato (*Lycopersicon esculentum* L.).

Roberts *et al*. (2008) stated that, bacterial spot on tomato, caused by *Xanthomonas euvesicatoria* is a major disease on tomato (*Lycopersicon esculentum* L.), in Florida and worldwide. The bacterium infects all of the aerial plant parts causing circular, necrotic lesions on stems, leaves, and fruits. Lesions are generally less than 3 mm in-diameter, infected fruit exhibiting large, scabby, raised lesions are non-marketable. Economic losses have been estimated up to 50% of yield due to either infection of foliage or fruit or both.

Balestra *et al.* (2009) mentioned that, bacterial pathogens are a serious problem on tomato plants. Among them, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*, causal agents of bacterial speck, bacterial spot and bacterial canker, respectively, affect tomato production under greenhouse and field conditions.

Dong *et al.* (2009) reported the Gram-negative bacterium *Xanthomonas campestris pv. vesicatoria* (Xcv) is the causal agent of bacterial spot of pepper and tomato. The primary symptoms are necrotic lesions that occur on leaves, stems, fruits, and flower parts. Isolation of Xanthomonads from plant and seed by conventional techniques is often difficult, usually due to the masking effect of fast-growing, yellow- pigmented bacteria.

2. Identification of pathogenic bacteria:

2.1. Morphological, physiological and biochemical characteristics:

Sijam *et al.* (1991) developed, a new agar medium for isolation and identification of *X. campestris* pv. *vesicatoria*. This medium, designated as CKTM, contained soy peptone, bactotrypton, dextrose, L-glutamine, L-histidine, ammonium phosphate, potassium phosphate, magnesium sulfate, calcium chloride, pourite and agar. Selectivity was afforded with cycloheximide, bacteracin, neomycin, cephalexin, 5-fluorouracil, tobramycin and tween 80. Strains of *X. campestris* pv. *vesicatoria* were easily distinguished from strains of other pathovars of *X. campestris* by the formation of clear ring around their colonies. The ring appear 1-2 day after colony transfer or 3-4 day after serial dilutions were plated. Minute tan to white crystals of variable intensity formed in the clear ring.

Stead *et al.* (1992) compared the fatty acid profiles of 773 strains representing 25 taxa of plant pathogenic and related saprophytic bacteria with two commercially available broad-spectrum libraries and one self-generated library based primarily on cultures from the National Collection of Plant Pathogenic Bacteria. The accuracy of identification at specific level was often 100%, although for some closely related species and infraspecific taxa accuracy was sometimes significantly less than this. The accuracy of identification of *Xanthomonas campestris* pathovars was much better than for *Pseudomonas syringae* pathovars. Almost all identifications were made within 24–48 h, standardization of cultural conditions was essential. Hydroxy fatty acids were of great taxonomic value in classification of Gram-negative bacteria. Improved library development and standardization of cultural and analytical techniques will further increase the accuracy of identification. Fatty acid profiling offers a valuable rapid, accurate method for identification of many bacteria.

Bouzar *et al.* (1999) screened four hundred thirty-three Xanthomonads strains isolated from tomato or pepper plants from 32 different fields in four Caribbean and Central American Countries, were screened for the ability to hydrolyse starch and sodium polypectate. Of these, 95 representative strains were further characterized by various phenetic tests. While, 63 of these strains were then analyzed by genomic fingerprinting. The majority of strains were typical *X. campestris pv. vesicatoria* .

Abd Alla (2000) identified the pathogenic bacteria that isolated from tomato seeds by means of biochemical, physiological and pathogenicity tests as well as the Biolog GN micro plate system for *Xanthomonas vesicatoria*. *X. vesicatoria* was more easily identified on tween B and CKTM media than on other media.

Obradovic *et al.* (2000) detected the causal agent of bacterial spot of pepper and tomato grown in different regions in Yugoslavia. Isolation was made from diseased material and the biochemical and physiological characteristics were studied by standard bacteriological tests. The race of the pathogen was determined on differential cultivars of pepper and tomato. The causal agent of the disease was identified according to the concepts of that time as *X. campestris* pv. *vesicatoria*. Strains isolated from diseased pepper were non-pectolytic and non-amylolytic, and didn't infect tomato plants, these strains belonged to pepper races 1 and 3 of *X. vesicatoria*. Tomato strains showed pectolytic and amylolytic activity and weren't pathogenic to pepper.

Abd El-Ghafar and Abd El-Wahab (2001) isolated and identified *X. campestris* pv. *vesicatoria* from tomato seeds and plants grown under protected conditions at different locations in Fayoum, Ismailia and Menufia governorates according to the morphological and physiological properties, Biolog test, serological tests and pathogenicity tests**.**

Carrillo-Fasio *et al*. (2001) isolated thirty six isolates of *X. c.* pv. *vesicatoria* were obtained from tomato and pepper leaves and/or

fruit the characterized and identified. Previously purified, each isolate was evaluated for Gram reaction, flagella staining and for response to biochemical tests like starch hydrolysis and Catalase production.

Veena and Van Vunrde (2002) developed an indirect immunofluorescence colony staining method for the detection of important seed-borne bacterial pathogens of tomato, such as *Corynbacterium michiganensis* subsp. *michiganensis* and *X. campestersis* pv. *vesicatoria*. The method involves the use of specific antiserum for initial binding of target bacteria and visualization of positive colonies with a commercially available secondary antiserum conjugated with FITC and observed under a fluorescence microscope. The indirect method is especially suitable for laboratories, seed companies and quarantine stations which have no facilities for conjugation of primary antiserum.

Obradovic *et al.* (2004) characterized, twenty-eight strains isolated from pepper and six from tomato. A study of their physiological and pathological characteristics, and fatty acid composition analysis revealed that all of the strains belong to *Xanthomonas campestris* pv. *vesicatoria.* Being non-amylolytic and non-pectolytic, pathogenic on pepper but not on tomato, containing lower amounts of fatty acid 15 : 0 ante–iso fatty acid. The pepper strains were designated as members of the A group of *X. campestris* pv. *vesicatoria*. However, the tomato strains hydrolyzed starch and pectate, caused compatible reactions on tomato but not on pepper, had higher percent of 15: 0 ante–iso fatty acid, and were classified into B phenotypic group and identified as *X. vesicatoria*.

Quezado-Duval *et al.* (2004) observed severe epidemics of bacterial spot in central-west Brazil in fields of processing tomato. Several Xanthomonads, *X. axonopodis* pv. *vesicatoria, X. vesicatoria* or *X. gardneri*, can cause the disease; therefore, attempts were made to identify the pathogen species present in this region. The strains were characterized using pulsed-field gel electrophoresis and

by their amylolytic and pectolytic activities. Carbon source utilization and rRNA sequence comparisons also were performed.

Shenge *et al*. (2007) characterized thirty five strains of *Xanthomonas campestris* pv. *vesicatoria* collected from different tomato-producing areas in three Regions (Morogoro, Arusha and Iringa), representing three different ecological conditions in Tanzania using their carbon source utilization profiles by the GN Microplate system, and their sensitivity to antibiotics. Although most of the strains could not be identified by the Biolog system as *X*. *campestris pv. vesicatoria,* the strains were found to differ in their pattern of carbon source utilization, and were clustered into three major groups. A similar pattern was observed in antibiotic sensitivity of the strains. The results indicated the presence of variations within the Tanzanian populations of *X. campestris* pv. *vesicatoria* on the basis of carbon source utilization patterns and their sensitivity to antibiotics. These findings also indicate the existence of *X. campestris* pv. *vesicatoria* strains in Tanzania that are different from those included in the Biolog database.

Hamza *et al*. (2010) performed bacterial isolates of *Xanthomonas* on KC semi selective agar medium, resulting in isolation of five yellow-pigmented, *Xanthomonas*-like strains. Three strains isolated from tomato or pepper were negative for starch hydrolysis and pectate degradation. Two strains isolated from pepper were strongly amylolytic and degraded pectate.

2.2. Identification of bacterial spot pathogens using bio-techniques:

Jones *et al*. (2000) described the bacteria, which are a major affliction of tomato and pepper crops in warm and humid regions, as a single species, but subsequent research has shown the existence of at least two genetic groups differentiated by physiological, biochemical and pathological characteristics. This work synthesizes the findings from several approaches, including pathogenicity tests, enzymic activity, restriction fragment analysis of the entire genome,

DNA–DNA hybridization and RNA sequence comparisons based on a 2097 base sequence comprising the 16S rRNA gene, the intergenic spacer located between the 16S and 23S rRNA genes and a small region of the 23S rRNA gene. Within the group of Xanthomonads pathogenic on pepper and tomato four distinct phenotypic groups exist, of which three form distinct genomic species. These include *Xanthomonas axonopodis* pv. *vesicatoria* (A and C group), *Xanthomonas vesicatoria* (B group) and *Xanthomonas gardneri* (D group). On the basis of phenotypic and genotypic differences between A- and C-group strains, the C strains should be considered as a subspecies within *Xanthomonas axonopodis* pv. *vesicatoria.*

Said *et al.* (2003) stated that black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), is a major disease constraint to cabbage production by smallholder farmers in Africa. Variability exists within the pathogen, and yet differentiation of *X campestris* pv. *campestris* strains. Great diversity was observed among *X. campestris* pv. *campestris* strains in their Biolog and rep-PCR profiles. Specific rep-PCR genomic fingerprints were linked to some geographical areas in the country. Most of the *X. campestris* pv. *campestris* strains were clustered in two groups based on their fatty acid profiles. Each of the methods allowed a degree of identification from species, pathovars to the strain level. Biolog and MIS identified all *X. campestris* pv. *campestris* strains at least to the genus level. Additionally, the utility of rep-PCR for routine diagnosis of strains was limited, although the procedure was good for delineation of *X. campestris* pv. *campestris* to the strain level. These findings indicate the existence of *X. campestris* pv. *campestris* strains in Tanzania that are distinct from those included in Biolog and MIS databases. The limitations noticed warrant continued improvement of databases and inclusion of pathogenicity testing, using universally susceptible cultivars, as an integral part of strain identification.

Jeffrey *et al.* (2004) identified four phenotypic Xanthomonad groups that are pathogenic to pepper, tomato, or both hosts. These include groups A and C which are found in *Xanthomonas axonopodis* pv. *vesicatoria*, group B found in *X. vesicatoria*, and group D found in '*X. gardneri'*. We present DNA:DNA hybridization data in which *X. axonopodis* pv. *vesicatoria* group A and C strains have less than 70% DNA relatedness with each other, with the type strain of *X. axonopodis*, and with the currently classified species within Xanthomonas and, therefore, should be removed from this species and given species status.

Obradovic *et al.* (2004) developed some PCR primers which amplified conserved DNA regions related to the hrp genes of different strains of *X. campestris* pv. *vesicatoria* associated with pepper and tomato. Restriction analysis of the PCR product resulted in different patterns and enabled grouping of the strains into four groups. The isolated *Xanthomonads* isolated from pepper and tomato in Serbia were analyzed, they clustered into two groups corresponding to the grouping based on their physiological and pathological characteristics. According to the reaction of pepper and tomato differential varieties, the strains from pepper belong to races P7 and P8 and tomato strains belong to the race T2.

Robène-Soustrade *et al.* (2006) developed a nested PCR test from a sequence-characterized amplified region marker identified by randomly amplified polymorphic DNA PCR for the detection of *X. axonopodis* pv. *dieffenbachiae*. Serological and pathogenicity tests were performed concurrently with the nested PCR test with a large collection of *X. axonopodis* pv. *dieffenbachiae* strains which isolated worldwide and are pathogenic to anthurium and/or other aroids. The internal primer pair directed amplification of the expected product (785 bp) for all 70 *X. axonopodis* pv. *dieffenbachiae* strains pathogenic to anthurium tested and for isolates originating from syngonium and not pathogenic to anthurium. Strains originating from

the two host genera can be distinguished by restriction analysis of the amplification product.

Dong *et al.* (2009) described a specific and highly sensitive rapid-PCR assay to detect bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* in pepper and tomato. One set of PCR primer was developed to amplify gene required for an rhs family gene homologous to rhsA, cell envelope biogenesis, outer membrane. Only a PCR product of a 517 bp was produced in PCR reaction with the *Xanthomonas campestris* pv. *vesicatoria* (XCVF/ XCVR) primer set. The protocol can be used as a reliable diagnostic tool for specific detection of *X. campestris* pv. *vesicatoria* in pepper or tomato.

Moretti *et al.* (2009) found a fragment of 1600 bp specific for *X. euvesicatoria* was found by repetitive extragenic palindromic sequence-PCR. Among the primers designed on the basis of the partially sequenced fragment, the primers Xeu2.4 and Xeu2.5 direct amplification of the expected product (208 bp) for all the *X. euvesicatoria* strains and not for other related and unrelated phytopathogenic bacteria or saprophytic bacteria isolated from pepper and tomato phyllosphere. The assay permits the detection of *X. euvesicatoria* in pure culture, with a limit of detection of two bacterial cells and 1 pg of DNA per PCR, and in extracts obtained from asymptomatic inoculated tomato and pepper plants.

3. Host rang

El-Sadek *et al.* (2001) isolated *X. vesicatoria* from pepper plants in El-Minia which was identified by procedures based on morphological, physiological and biochemical characteristics. Host range studies revealed that the leaves of tomato, tobacco, cowpea and bean showed hypersensitive reaction when sprayed with the bacterial inoculum.

Obradovic *et al*. (2008) found that the host range of *Xanthomonas* spp. associated with tomatoes includes tomato

(*Solanum lycopersicum*, formerly *Lycopersicon esculentum*), pepper (*Capsicum annuum*), chili pepper (*Capsicum rutescens)* and *Solanum pimpinellifolium* (formerly *Lycopersicon pimpinellifolium*).

4-Varietal reaction:

Massomo (2004) found that the management of black rot of cabbage, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), has increasingly become very difficult for smallholder growers in Tanzania due to cultivation of susceptible varieties. Generally, varieties with good foliar resistance also showed less black rot in stems and heads. Open pollinated varieties were highly susceptible to black rot.

Kenneth *et al*. (2010) evaluated four tomato (*Lycopersicon esculentum* Mill.) varieties *i.e*., Cal J, Moneymaker, Tanya and Roma VF. which were the commonly grown by tomato farmers in Tanzania for resistance to bacterial speck (*Pseudomonas syringae* pv. *tomato*) and bacterial spot (*Xanthomonas vesicatoria*) diseases, along with five introductions under screen house and field conditions. Seeds of the tomato varieties were purchased from seed vendors in the open market. Results indicated that all the tomato varieties were susceptible to the two diseases, and suffered moderate to severe infection levels. The performance of the introductions against bacterial speck under screen house conditions was variable. All the introductions showed high levels of susceptibility to bacterial spot. Under field conditions, incidence of the diseases was high in all the locally available varieties tested, averaging 87% for bacterial spot and 82.3% for bacterial speck. The results of this study indicate that all the locally available tomato varieties included in the study were highly susceptible to bacterial speck and bacterial spot diseases.

Shenge *et al.* (2010) found that positive correlation between tomato variety and disease, and the locally available varieties were all susceptible to bacterial -speck and bacterial-spot. They added, out of 104 tomato disease samples collected, 65 were found to be affected

by the bacterial speck pathogen (*Pseudomonas syringae pv. tomato*), and 39 fruit samples were found to be affected by the bacterial spot organism (*Xanthomonas campestris pv. vesicatoria*). Screening of tomato seeds procured from the open market showed that they were not infected with *P. syringae pv. tomato* and *X. campestris pv. vesicatoria.*

5. Disease Control:

5.1- Antibiotics:

Abd El-Ghafar and Abd El-Wahab (2001) revealed that**,** application of four chemical compounds (agrimycin, terramycin, kocide 101 and tri-miltox) reduced the growth of *X. c.* pv. *vesicatoria, in vitro* and the severity of bacterial spot by artificial inoculation by field conditions. Interaction between the four chemical compounds proved to be the most effective compounds in reducing bacterial spot incidence and severity disease.

Polizzi *et al.* (2002) tested of Hortocyna 60g/100L, Bion 50WG 5g/100L, Regalis 250g/100L, Biosept 200g/100L, Bioczos BR 1000g/100L, Bio Blatt 25EC 200g/100L Miedzian 50 WG 300g/100L, Copper chloride 25g/100L, Alar 500g/100L, sulfanilic acid 200g/100L, Tytanit 20ml/100L and chitosan 100g/100L for their activity against tomato bacterial canker disease. The best protective and curative activities and the highest yield were obtained from treatments with Bion 50WG, Hortocyna and Regalis. Chitosan and Copper chloride only had protective activity.

Obradovic *et al.* (**2008**) introduced the antibiotics for agricultural use in several countries. The most frequently used antibiotics against plant bacterial diseases were formulations of streptomycin or streptomycin and oxytetracycline. However, soon after widespread application of antibiotic-based compounds in plant protection, resistant strains of the tomato bacterial spot pathogen emerged. In addition, there was a major concern in many countries

that antibiotic application and release in the environment might cause natural resistance in many bacterial species rendering useless, for medical treatments, not only these but also other related antibiotics.

5.2- Plant oils:

Morais *et al.* (2002) tested antimicrobial activity of 45 extracts of medicinal plants against *X. c*. pv. *vesicatoria* and *Corynbacterium michiganensis* subsp. *michiganensis*. Some assays were performed to verify the capability of these plant extracts to show antibiosis. Five extracts (EAFQ, SM1, SM12, SM16 and SA1) showed positive activity against the bacteria. EAFQ expressed bactericidal activity on *C. m*. subsp. *michiganensis*. These active substances can be used in nature or as a model to synthesize industrialized products, intended for field utilization.

Abbasi *et al*. (2003) tested foliar applications of neem (*Azadirachta indica*) oil and fish emulsion, derived from neem seed and menhaden fish for their ability to reduce bacterial spot of tomato and pepper under greenhouse and field conditions. Greenhouse-grown tomato and pepper plants sprayed with aqueous suspensions (0.5%, v/v) of neem oil or fish emulsion and then inoculated with *X. c.* pv. *vesicatoria* showed less disease symptoms than the water-treated controls and reduced disease severity on the foliage of inoculated field-grown tomato and pepper plants.

Pietro *et al*. (2004) assayed *in vitro* the essential oils that extracted from the fruits of *Coriandrum sativum* L. and *Foeniculum vulgare* Miller var. *vulgare* (Miller) for antibacterial activity to *Escherichia coli* and *Bacillus megaterium*, bacteria routinely used for comparison in the antimicrobial assays, and 27 phytopathogenic bacterial species and two mycopathogenic ones responsible for cultivated mushroom diseases. A significant antibacterial activity, as determined with the agar diffusion method, was shown by *C. sativum* essential oil whereas a much reduced effect was observed for *F. vulgare* var. *vulgare* oil. *C. sativum* and *F. vulgare* var.

vulgare. Essential oils may be useful natural bactericides for the control of bacterial diseases of plants and for seed treatment, in particular, in organic agriculture.

Obradovic *et al*. (**2008**) found that weekly sprays of neem oil and fish emulsion reduced disease severity on the foliage of inoculated tomato and pepper plants under both greenhouse and field conditions in two consecutive seasons. The disease incidence on the fruit of these plants was reduced but the effect was not always statistically significant. These results suggested that tested products may enhance efficiency of bacterial spot management programs.

Bajpai *et al*. (2010) revealed that, many of the currently available antimicrobial agents for agriculture are highly toxic and non-biodegradable and cause extended environmental pollution. Therefore, this study was undertaken to assess the *in vitro* and *in vivo* antibacterial efficacy of the essential oil and organic extracts of *Metasequoia glyptostroboides* against plant pathogenic bacteria of *Xanthomonas* spp. The oil (1000 µg/disc) and extracts (1500 µg/disc) displayed potential antibacterial effect *in vitro* as a diameter of zones of inhibition against *Xanthomonas campestris* pv. *campestris*, *X. campestris* pv. *vesicatoria*, *X. oryzae* pv. *oryzae* and *Xanthomonas* sp. which were found in the range of 10–14 and 8–12 mm, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of oil and the extracts were ranged from 125–250 µg/ml and 125 -500 µg/ml and 250–1000 µg/ml and 250–2000 µg/ml, respectively. Also the oil had strong detrimental effect on the viable count of the tested bacteria. Further, the oil displayed remarkable *in vivo* antibacterial effect up to 65 to 100% disease suppression efficacy against the tested strains of *Xanthomonas* spp. on greenhouse-grown oriental melon plants (*Cucumis melo* L. var. *makuwa*).

5.3- Plant extracts:

Satish and Janardhana (1999) screened the aqueous extracts from leaves of 30 higher plants, collected from different localities, *in vitro* for antibacterial activity against different pathovars of the phytopathogenic bacterium, *Xanthomonas campestris.* Eight plant species showed antibacterial activity, based on the zone of inhibition in a diffusion assay. Significant antibacterial activity was observed in the aqueous extracts of *Prosopis juliflora, Oxalis corniculata* and *Lawsonia inermis.* The susceptibility of different pathovars of *X. campestris* to these plant extracts was varied. This study indicates the potential of these plant extracts in the management of diseases caused by *X. campestris* in several important crop plants.

Al-Dahmani *et al*. (2003) investigated the effects of foliar sprays with compost water extracts (compost extracts) on severity of bacterial spot of tomato. Efficacy of the compost extracts ranged from being effective on tomato seedlings in greenhouse bioassays, to marginally effective on fruit and ineffective in controlling foliar symptoms in the field under high disease pressure. Even though some degree of efficacy of compost extracts was observed, it was not comparable to the effect of a mixture of copper hydroxide and chlorothalonil.

Basim *et al*. (**2006**) investigated the "*in vitro*" antibacterial activities of Turkish pollen and propolis extracts against 13 different species of agricultural bacterial pathogens including *Agrobacterium tumefaciens, A. vitis, Clavibacter michiganensis* subsp. *michiganensis, Erwinia amylovora, E. carotovora* pv. *carotovora, Pseudomonas corrugata, P. savastanoi* pv. *savastanoi, P. syringae* pv. *phaseolicola, P. syringae* pv. *syringae, P. syringae* pv. *tomato, Ralstonia solanacearum, Xanthomonas campestris* pv. *campestris* and *X. axonopodis* pv. *vesicatoria*. Among the tested bacteria, *A. tumefaciens* was the most sensitive one to 1/5 concentration of pollen extract, and the sensitivity of the bacteria followed the sequence *A. tumefaciens, P. syringae* pv. *tomato, X. axonopodis* pv. *vesicatoria, E. amylovora, P.*

corrugata, R. solanacearum, X. campestris pv. *campestris , A. vitis, C. michiganensis* subsp. *michiganensis , E. carotovora* pv. *carotovora, P. savastanoi* pv. *savastanoi, P. syringae* pv. *phaseolicola* **,** *P. syringae* pv. *syringae. P. syringae* pv. *phaseolicola* was the most sensitive one to 1/10 concentration of propolis extract, and the sensitivity of the bacteria followed the sequence *P. syringae* pv. *phaseolicola ,P. savastanoi* pv. *savastanoi, P. corrugata, R. solanacearum , E. carotovora* pv. *carotovora, P. syringae* pv. *syringae, E. amylovora, A. tumefaciens, A. vitis, C. michiganensis* subsp. *michiganensis, P. syringae* pv. *tomato, X. campestris* pv. *campestris, X. axonopodis* pv. *vesicatoria.* The least active concentrations towards the tested bacteria were 1/100 of the pollen extract and 1/1000 of the propolis extract. This study is the first report on the antibacterial activities of pollen and propolis against the plant pathogenic bacteria.

5.4- Resistance inducers:

Li *et al.* (1998) concluded the active components of both preparations were malic and citric acids, with minor contributions coming from shikimic and quinic acid based on high performance liquid chromatography analyses and bioassays. Although several compounds including glucose and inositol activated the toxin genes when tested at high concentrations (3 to 5 mM), shikimic and quinic acids were the only ones with activity at concentrations below 0.1 mM. The two acids could not be used as a sole carbon source by strain DC3000. The signal activity of shikimic acid was enhanced 10-fold by the addition of glucose.

Janda *et al.* (2007) investigated the compounds capable of reducing the stress sensitivity of crops. In terms of stress physiology, salicylic acid was first demonstrated to play a role in responses to biotic stress. However, it was gradually found to have more effects that could be of importance for other stress factors, and a great deal of evidence has accumulated in recent years suggesting that salicylic

acid also plays a role in responses to a biotic stress effects (such as low and high temperature, UV-B irradiation, ozone, heavy metals, *etc*.). Most papers, on this subject, have reported on the protective effect of exogenous salicylic acid against a biotic stress. When applied in satisfactory concentrations salicylic acid may cause a temporary low level of oxidative stress in plants, which acts as a hardening process, improving the ant oxidative capacity of the plants and helping to induce the synthesis of protective compounds such as polyamines.

5.5-Biological agent:

McMillan (1987) stated that bacterial spot of tomato caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye was the most serious foliar and fruit disease threatening tomato production in Florida. Pathogen-free seed is essential to seedling production houses and the field tomato grower. Seed treatment in the *in vitro* with Form-A-Turf at 1:250, Formalin at 1:250, NaOCl at 1:4 and hot water at 56°C for 30 min provided 100 percent pathogen-free seed. None of the treatments reduced seed germination. *In vivo* studies percent pathogen-free seed, and seed-germination reduction, varied according to adequacy of grower facilities for adequate hot-water temperature control and flushing of applied NaOCl from the seed.

Buonaurio *et al*. (1994) stated that, pepper bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* is an important disease in many pepper production areas of the world. Like other bacterial diseases, pepper bacterial spot is difficult to control. Chemical sprays, essentially carried out with copper compounds or streptomycin, are not completely effective since copper and streptomycin resistant *X. campestris* pv. *vesicatoria* strains frequently appear.

Chand *et al*. (1994) screened several copper based-compounds and antibiotics grapevine bacterial canker control, but none of these compounds showed a significant reduction in disease severity.

During a four-year trial conducted in a nursery, grape plants with variable levels of infection were monitored with applications of several copper compounds and antibiotics. At the end of the experiment, the pathogen had acquired resistance to both copper and antibiotics. Tolerant strains were viable after exposure to 600-1800µg/mL Cu^{2+}.

Kousik and Ritchie (1996) stated that, the chemical control has been extensively for controlling bacterial spot disease. Growers rely heavily on fixed copper sprays to manage foliar diseases and reduce losses caused by bacterial spot and bacterial speck. Unfortunately, these sprays often are not very effective and their extensive use led to the development of copper resistant strains.

Sahin *et al.* (2000) developed an alternative and effective control method for the bacterial spot diseases of pepper and tomato by using plant activator (Actigard) and 3 different antagonistic biological control agents (BCA): *Burkholderia* sp., *Pseudomonas* sp. and *Bacillus* sp. Their antagonistic effect on the pathogenic *X. c.* pv. *vesicatoria* was investigated under *in vitro* conditions. All treatments had significant reduction in disease severity than the control plants. No phytotoxic effect was observed on the growth of pepper or tomato plants treated with any bioagents.

Abd El-Ghafar and Mosa (2001) compared between bioagents isolates of *Pseudomonas fluorescence, P. putida* and *Bacillus subtilis* and two chemical compounds (Agrimycin and Kocide 101) for their efficiency to control bacterial spot, under laboratory and greenhouse conditions. *In vivo*, two methods were used to apply antagonistic bacteria, foliar or soil treatment. A combination between *Pseudomonas fluorescence* as a soil treatment and Agrimycin as a foliar treatment was studied.

Carrillo-Fasio *et al.* (2001) tested thirty nine *X. c.* pv. *vesicatoria* strains for their sensitivity to several copper formulations and combinations of copper + mancozeb, copper + antibiotics, in

laboratory and field experiments (Mexico). Seventeen out of the 39 strains showed high tolerance to copper formulations under *in vitro* conditions; however, when the strains were tested with other copper formulations plus mancozeb, dithane M-45, Cuprimicin 100 and Cuprimicin 17 marking inhibition zones developed. When these products were tested alone, inhibition zones also developed, although smaller as compared to mixtures of mancozeb and the other products with copper. In vitro assays, 48.7% of the strains tested showed tolerance and/or resistance to copper products.

Santore *et al*. (2001) mentioned that, in North Florida and South Georgia, traditional and current control strategies include weekly applications of copper products and copper plus mancozeb with biweekly acibenzolar-S-methyl (ASM; Actigard 50WG, Syngenta Crop Protection, Inc., Greensboro, NC) application, respectively.

Roberto *et al*. (2002) investigated the ability of acibenzolar-S-methyl to induce resistance in pepper plants against *Xanthomonas campestris* pv. *vesicatoria* in both growth chamber and open field conditions. Growth chamber experiments showed that acibenzolar-S-methyl (300 µM) treatment protects pepper plants systemically and locally against *X. campestris* pv. *vesicatoria*. Evidence for this was a reduction in the number and diameter of bacterial spots and bacterial growth in plant. Systemic protection was also exerted by the acibenzolar-S-methyl acid derivative, CGA 210007, which may be produced by hydrolysis in the plant. The efficacy of acibenzolar-S-methyl was also found in open field conditions, where both leaves and fruit were protected from the disease. The highest efficacy (about 67%) was obtained by spraying the plants 6–7 times every 8–12 days with a mixture of acibenzolar-S-methyl and copper hydroxide (2.5+40 gh^{l-1}active ingredient). Persistence and translocation data obtained from the growth chamber experiments revealed a persistence of acibenzolar-S-methyl lasting five days after treatment with rapid translocation and negligible levels of acid derivative

formation. Since the protection exerted by acibenzolar-S-methyl against bacterial spot disease was observed when the inducer was completely degraded, it would appear to be due to SAR activation.

Werner *et al.* (2002) stated that, application of copper hydroxide alone or mixed with mancozeb or streptomycin limited *Corynbacterium michiganensis* subsp. *michiganensis* populations relative to acibenzolar-S-methyl, acibenzolar-S-methyl mixed with copper hydroxide, and a virulent strains, in greenhouse experiments. Copper hydroxide mixed with streptomycin limited pathogen populations compared with copper hydroxide mixed with mancozeb. Meantime, copper hydroxide mixed with mancozeb limited spread of the pathogen compared with copper hydroxide mixed with streptomycin. Adding copper hydroxide to acibenzolar-S- methyl limited pathogen populations compared with acibenzolar-S- methyl alone. Pathogen spread was also reduced among resistant cultivars compared with the susceptible cultivar treated with streptomycin.

Aguiar *et al.* (2003) detected, copper resistance in *Xanthomonas* and *Pseudomonas*. Resistance is widespread in *X. campestris* pv. *vesicatoria* (Doidge) Dye, causal agent of bacterial spot of pepper and tomato, in several geographical regions, where copper was not effective for disease control. In Brazil, studies have also shown low efficacy of copper compounds to control bacterial spot of sweet pepper.

Obradovic *et al.* (2004) stated that the control strategies are based on combinations of practice such as the use of pathogen free seed and transplants, elimination of volunteer tomato plants, use of resistant cultivars, frequent application of a copper and mancozeb mixture and the use of biological agents. Chemical control has been used extensively for controlling bacterial spot disease. Growers rely heavily on fixed copper sprays to manage foliar diseases and reduce losses caused by bacterial spot and bacterial speck. Unfortunately, these sprays often are not very effective and their extensive use led to

the development of copper resistant strains. Recently, combination of acibenzolar-S-methyl (ASM), a plant activator and bacteriophages were found to be effective against the bacterial spot pathogen in providing better disease control.

Iacobellis *et al*. (2005) mentioned that, the copper compounds represent a problem due to their Phytotoxicity, their accumulation in soil and the necessity of frequent applications. Moreover, according to the recent European restriction (CE Reg .473/2002), the use of cupric salts will be limited. Copper treatments and appropriate agronomic practices such as seed certification, irrigation and fertilization are the main measures presently used to control the above diseases. Since environmental factors and variable colonization strategies play an important role in phytobacteria spread on tomato crops, without effective preventive measures it is difficult to reduce their damage. As an alternative to copper compounds, a few natural substances have recently been proposed, but further studies are needed to optimize their effectiveness. For example, propolis, known as bee-product, has shown interesting antibacterial effects even if its composition (concentration of its main a.p., galangin) is strictly linked to vegetal species and climatic conditions. Also, they added the essential oils have revealed a potential use.

Teixeira *et al*. (2008) stated that, copper compounds have been widely used for management of bacterial diseases of vegetable and fruit crops. These fungicides are capable of inhibiting or delaying bacterial multiplication. However, the efficacy of copper sprays in control of plant bacterial diseases has been variable and is often associated with the occurrence of copper tolerant strains. Resistance to copper was not detected in plant pathogenic bacteria until the 1980s, probably because the presence of copper-resistant strains in the field did not always lead to failure of control with copper sprays. Since then, several studies have focused on characterizing strain sensitivity and the genetic mechanisms of copper resistance in plant-pathogenic bacteria.

Obradovic *et al*. (**2008**) revealed that chemical control of tomato bacterial spot mostly relied on the application of the antibiotic streptomycin and copper-based compounds. The use of copper-based compounds in agriculture started in early 1800's. Copper-containing bactericides proved to be an effective preventive treatment against many bacterial diseases, mostly leaf spots and blights. However, efficacy of copper bactericides for control of tomato bacterial spot was compromised by reduced sensitivity of the bacterium as a result of the excessive application of these chemicals. Copper-tolerant strains became quite prevalent in the 1980s. Although the combination of copper bactericides and ethylene-bis-dithiocarbamates resulted in improved disease control, the combination remained ineffective when weather conditions were optimal for the disease development. Foliar applications of ammonium lignosulfonate (ALS), a product derived from the wood pulping process, and the fertilizer potassium phosphate (KP) were tested for their ability to control the disease under both greenhouse and field conditions. A three-year field study demonstrated that ALS and KP significantly reduced bacterial spot disease severity on tomato and pepper foliage and fruits, compared to untreated control. However, further studies of optimal spray intervals, rates, and adjuvants are needed.

Eder *et al*. (2009) revealed that, bacterial canker, caused by *Xanthomonas campestris* pv. *viticola*, affects grapevines in the irrigated areas of the São Francisco river valley, in the states of Pernambuco and Bahia. Several practices for disease management have been adopted including copper sprays. This is the only available chemical control method and most frequently used in the areas affected by the disease. The objective of this work was to determine the sensitivity to copper in strains of *X. campestris* pv. *viticola* that collected from different locations and over a period of years, from 1998 to 2006. Variation in sensitivity to copper oxychloride and copper sulfate was observed among the 21 strains tested. The

minimum inhibitory concentration (MIC) varied from 10 to 60 µg/ml Cu^{2+}, for both compounds. A general increase in copper tolerance over the years was also observed, with the Brazilian strains being more tolerant than the type-strain, collected in 1972 in India. The differences observed in copper sensitivity may lead to the selection and dominance of the more tolerant strains in the bacterial population as copper compounds continue to be used in the region.

Nisa *et al.* (2010) stated that, copper (Cu)-based biocides are important chemical controls for both fungal and bacterial diseases in crop fields. They showed that, Cu ions at a concentration of 100 µM enhanced *t*-butyl hydroperoxide (*t*BOOH) and hydrogen peroxide (H_2O_2) killing of *Xanthomonas campestris* pv. *campestris* through different mechanisms. The addition of an antilipid peroxidation agent (α-tocopherol) and hydroxyl radical scavengers (glycerol and dimethyl sulphoxide) partially protected the bacteria from the Cu-enhanced *t*BOOH and H_2O_2 killing, respectively.

Flaherty *et al*. (2000) evaluated a mixture of host-range mutant (h-mutant) bacteriophages specific for tomato race 1 (T1) and race 3 (T3) of the bacterial spot pathogen, *Xanthomonas campestris pv. vesicatoria* (Doidge) Dye as biological control of bacterial spot on 'Sunbeam' tomato (*Lycopersicon esculentum* Mill.) transplants and field-grown plants for two seasons. Applications of bacteriophages to field-grown tomatoes decreased disease severity as measured by the area under the disease progress curve (AUDPC) by 17.5% (1997) and 16.8% (1998) compared with untreated control plants. Preharvest plant vigor ratings, taken twice during each field season, were higher in the bacteriophage-treated plants than in either bactericide-treated plants or nontreated controls except for the early vigor ratings in 1998. Use of bacteriophages increased total weight of extra- large fruit 14.9% (1997) and 24.2% (1998) relative to that of nontreated control plants, and 37.8% (1997) and 23.9% (1998) relative to that of plants treated with the chemical bactericides. Chemical names used: manganese, zinc, carboxyethylene bis dithiocarbamate (mancozeb).

Balogh *et al.* (2003) used bacteriophages currently as an alternative method for controlling bacterial spot disease on tomato incited by *Xanthomonas campestris pv. vesicatoria.* However, the efficacy of phage is greatly reduced due to its short residual activity on plant foliage. Three formulations that significantly increased phage longevity on the plant surface were tested in field and greenhouse trials: (i) PCF, 0.5% pregelatinized corn flour (PCF) + 0.5% sucrose; (ii) Casecrete, 0.5% Casecrete NH-400 + 0.5% sucrose + 0.25% PCF; and (iii) skim milk, 0.75% powdered skim milk + 0.5% sucrose. In greenhouse experiments, the nonformulated, PCF-, Casecrete-, and skim milk–formulated phage mixtures reduced disease severity on plants compared with the control by 1, 30, 51, and 62%, respectively. In three consecutive field trials, nonformulated phage caused 15, 20, and 9% reduction in disease on treated plants compared with untreated control plants, whereas plants treated with PCF- and Casecrete-formulated phage had 27, 32, and 12% and 30, 43, and 24% disease reduction, respectively. Plants receiving copper–mancozeb treatments were included in two field trials and had a 20% decrease in disease in the first trial and a 13% increase in the second one. Skim milk–formulated phage was tested only once and caused an 18% disease reduction. PCF-formulated phage was more effective when applied in the evening than in the morning, reducing disease on plants by 27 and 13%, respectively. The Casecrete formulated phage populations were over 1,000-fold higher than the nonformulated phage populations 36 h after phage application.

Jones *et al.* (2007) mentioned that, using of phages for disease control is a fast expanding area of plant protection with great potential to replace the chemical control measures now prevalent. Phages can be used effectively as part of integrated disease management strategies. However, the efficacy of phages, as is true of many biological control agents, depends greatly on prevailing environmental factors as well as on susceptibility of the target

organism. Great care is necessary during development, production and application of phage treatments. In addition, constant monitoring for the emergence of resistant bacterial strains is essential. Phage-based disease control management is a dynamic process with a need for continuous adjustment of the phage preparation in order to effectively fight potentially adapting pathogenic bacteria.

Obradovic *et al*. (**2008**) found that besides the continuous search for an effective chemical treatment, extensive research has focused on identifying biological control agents for use in plant protection. Among the limited number of biological agents commercially available for the control of bacterial diseases, the most encouraging results for control of bacterial spot on tomato were obtained using host specific phages. Application of phage mixture, prepared according to the phage host range and specificity to predominant race, reinforces the importance of timely and accurate diagnosis of the disease and correct identification of the pathogen.

Monk *et al*. (2010) used bacteriophages for almost a century as antimicrobial agents. In the West, their use diminished when chemical antibiotics were introduced, but they remain a common therapeutic approach in parts of eastern Europe. Increasing antibiotic resistance in bacteria has driven the demand for novel therapies to control infections and led to the replacement of antibiotics in animal husbandry. Alongside this, increased pressure to improve food safety has created a need for faster detection of pathogenic bacteria. Hence, there has been a resurgence of interest in bacteriophage applications, and this has encouraged the emergence of a large number of biotech companies hoping to commercialize their use.

Tarasi *et al*. (2010) demonstrated that bacteriophages have been used electively for controlling several Xanthomonas diseases. Bacterium *Xanthomonas campestris* pv. *oryzae* causes bacterial leaf blight, an economically important disease of rice. The use of bacteriophages for controlling bacterial blight in rice was very promising. In comparison to plants in the control plots, plants treated

with bacteriophages had reduced incidence of bacterial blight in the greenhouse.

V- Biochemical changes in tomato plants after control treatments:

Cavalcanti *et al*. (2006) investigated the induced defense responses and protective effects on susceptible tomato (*Lycopersicon esculentum* Mill.) against *Xanthomonas vesicatoria* (Doidge) by a heat-treated aqueous extract (VLA) from dry necrotic tissue of 'Lobeira' (*Solanum lycocarpum* St. Hil.) branches infected with the fungus *Crinipellis perniciosa* (Stahel) compared with acibenzolar-S-methyl (ASM), a commercial inducer of resistance. Plantlets were sprayed with VLA and ASM and challenged 4 days later with a virulent strain of *X. vesicatoria*, under greenhouse conditions. The disease severity, fresh weight of shoots, the activities of phenol peroxidase (POX), polyphenol oxidase (PPO), chitinase (CHI), phenylalanine ammonia-lyase (PAL), lignin deposition, and soluble phenolic contents were evaluated in the leaf tissues. Reduction of the bacterial spot severity was observed in plantlets treated with VLA which conferred 63% of the ASM protection. This protective effect and lesion reduction promoted by VLA were probably associated particularly with POX and PAL activities, lignin deposition on leaf tissues and, to a less extent, CHI activity.

Mahdavian *et al*. (**2008**) stated the effect of salicylic acid (SA) counteracting the UV-A, UV-B, and UV-C-induced action on pepper (*Capsicum annuum* L.) plants was studied. For this purpose, the activities of antioxidant enzymes (peroxidase, polyphenol oxidase, ascorbate peroxidase, catalase, and glutathione reductase) were measured. Plants were sprayed with SA and treated with UV-A (320–390 nm), UV-B (312 nm), and UV-C (254 nm) radiation with a density of 6.1, 5.8, and 5.7 W/m^2. The activities of antioxidant enzymes were enhanced in leaves in response to UV-B and UV-C radiation. SA treatment moderated an increase in the activities of

some antioxidant enzymes (peroxidase, ascorbate peroxidase, catalase, and glutathione reductase) in plants that were treated with UV radiation. The activity of antioxidant enzyme polyphenol oxidase in plants that were treated with UV-B, UV-C, and SA was significantly increased.

MATERIALS AND METHODS

I. Isolation of pathogenic bacteria from:

a. Different parts of tomato plant:

Diseased tomato plants (*i.e.* Leaves) with typical symptoms of bacterial spot diseases were collected from various localities of Qaha, El-Douky, Rashid, Fac. of Agriculture Ain Shams. These samples were transferred to the laboratory in ice-boxes for further studies.

Leaves of tomato with typical symptoms were washed with tap water and air dried. Small pieces of the infected tomato tissues were placed into Petri plates, each one of them containing 10 ml sterile distilled water (SDW), at room temperature (25-30°C). After 30 min. the resultant suspension was streaked onto nutrient agar medium. Also, bacterial spot lesions were excised and surface disinfected by dipping in 95% ethanol for 2 seconds followed by three successive rinses in sterile distilled water (SDW). Then each lesion was crushed in 0.5 ml SDW. The resultant suspension was streaked onto nutrient agar and Tween B medium as a selective medium for *Xanthomonas campestris* pv. *vesicatoria* (**Gore and O'Garro**, **1999**). All inoculated plates were incubated at 28°C for 48-72 hrs. A number of the predominant colonies were selected and purified by re-streaking on the same medium.

b. Tomato seeds:

Seeds of Sixteen tomato cultivars and hybrids *i.e.*, Castle rock, Gs12, Peto 86, Super strain B, Flora-Dade, Money maker, Dora, KTM 141, Niagra, Diamante F1, Super Marmand; Hypride7796,

Mors44, VT916G.SI, HMX4791 and Faqlta 38 were used for isolation the bacteria.

Seedlings of tomato with typical symptoms were washed with tap water and air dried. Then, the two isolation methods which mentioned above were used for isolation from diseased tomato plant parts. As well as, the number of the predominant colonies were selected and purified by re-streaking on the same media.

c. Germinated seeds:

Seeds of different tomato cultivars and hybrids, that mentioned before, were carried out using two methods, as direct planting on selective media (DP) and liquid assay (LA). Hundred seeds of each cultivar were planted on semi-selective medium tween B (TB) (selective medium for *X. vesicatoria*), where the seeds of each cultivar were distributed regulary in ten plates. The plates were incubated at 28°C for 4 – 5 days, (**Fatmi and Schaad, 1988 and Gitaitis *et al.*, 1991**). In liquid assay, 0.1 gram of seeds of each cultivar was soaked in sterile saline solution (10 ml) for 24 h at room temperature and aliquots (0.1 ml) of the suspension was spread onto selective medium as previously mentioned **(Fatmi and Schaad, 1988).**

II- Identification of isolated bacteria:

All bacterial isolates which were resulting by all isolation methods were identified based on their morphological, nutritional and physiological characteristics according to schemes suggested by **Schaad *et al.* (1980); Fahy and Persley (1983); Krieg and Holt (1984) and Leliott and Stead (1987)**.

a. Morphological characteristics

Different morphological characteristics of the subjected bacterial isolates *i.e.*, cell shape, Gram stain, motility, pigment production and spore formation was carried out.

b. Cultural characteristics:

Various cultural properties of the examined isolates, *i.e.*, the growth colony shape on different media, oxygen requirements and growth at different temperatures were also studied.

c. Physiological and biochemical characteristics:

The following physiological characters and biochemical activities were used as bases for bacterial classifications:

- Acid production from sucrose.
- Reducing compounds from (sucrose, glucose, mannose, maltose and sorbitol).
- Acid production from carbohydrates (sucrose, glucose, mannose, maltose and sorbitol).
- Gas from d-glucose.
- Hydrogen sulfide production (H_2S).

Degradation of macromolecules:

- Gelatin hydrolysis test.
- Starch hydrolysis test.
-Arginin hydrolysis.

Other tests:

- Catalase test.
- KOH 3% test.
- Oxidase test.
- Salt tolerance test.

- Phosphates test.
- Levan formation.

The ability to growth on different media

- TB = Tween B medium
- Yeast extract dextrose-$CaCO_2$ medium (YDC).
- Nutrient-broth yeast extract agar medium (NBY).
- King's medium B agar (KB).
- Peptone yeast extract agar medium (PYEA).

II.2- Pathogenicity test:

Two hundred seedlings of either tomato (cv. Balady) and (c.v sweet pepper) (cv. Balady) were grown in a greenhouse in plastic pots (Ø 10 cm) containing a mixture of peat moth and sand (3 : 1) until they reached the six-leaf stage. Inoculum of bacterium was prepared by grown on KB medium (containing: Difco proteose peptone 20g, K_2HPO_4 1.5g, $MgSo_4.7H_2O$ 1.5g, Glycerol 15ml, Agar 15g, Distilled water 1000ml, medium was adjusted to pH 7.2. and incubated at 28°C for 24 h. Bacterial cells were suspended in 0.01M magnesium sulfate (pH 7.2) and the bacterial suspension was adjusted to 10^8 CFU ml^{-1} (Optical Density 660 = 0.06)., and sprayed through stomata leaves of tomato and pepper plants by a high pressure sprayer. On the other hand, other plants were only sprayed with sterile buffer as compared treatment. After that, all plant treatments were covered by plastic sheet for 24hrs. and observed daily for recording the disease symptoms.

- **Variation among bacterial spot pathogens using:**

II.3- Biolog System:

Three pathogenic isolates of those isolated from infected tomato samples and identified as *Xanthomonas* sp. by cultural, morphological, biochemical and physiological characters, as mentioned above, and it confirm by Biolog system. The isolates were identified in Fac. Agricul. Azher University.

II.4- SDS- PAGE analysis:

For emphasizing identification of pathogenic bacteria, bacterial cell suspensions for twelve isolates of identified bacteria as *X. vesicatoria* from different locations, were used for extracting the bacterial proteins. Fractionalization of bacterial protein was achieved using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique as described by **Laemmli (1970)** at the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.

In this study, the tested isolates of pathogenic bacteria were left to grow at 30°C for 48 h in Erlenmeyer flasks (100 ml) where, each one contained 50 ml of Luria broth medium (LB). The LB medium contained 10g bactotryptone, 10g NaCl, 5g yeast extract, 1000 ml distilled water and adjusted at pH 7.0) **Maniatis *et al*.** (**1989**).

II.4.a-Preparation of whole –cell protein extracts:

Protein extracts was prepared according to the method of **Maniatis *et al.*** (**1989**) by the following way; one ml of each one of cultured bacteria was centrifuged at 12000 rpm for 30 seconds in a Microfuge. The supernatant medium was removed and the

precipitated protein pellets were re-suspended by vortexing in 0.5 ml of ice-cold 50 mM Tris.Cl (pH 7.4), then centrifuged again at 12000 rpm for 30 seconds at 0°C in a microfuge. The supernatant was removed and the precipitated protein pellets were left to dry as possible. The extracted protein pellets were re-suspended again by vortexing in 25 µl of H_2O. As soon as, the pellet is dispersed, 25 µl of SDS gel-loading buffer is added and vortexing is continued for 20 seconds. The extracted protein samples containing soluble protein were stored at -20°C until usage.

II.4.b - Electrophoresis of native protein:

The thawed protein-extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (v/v) and 0.1% bromphenol blue (v/v) in 0.15 M Tris–HCl, pH 6.8. Twenty microliters of the resulting suspension (40 µg of protein) was subjected to electrophoresis at room temperature (approximately 20-25°C), for 9 hrs on an 15% polyacrylamide gel with a 6% stacking gel at 10 and 20 mA, respectively, until the dye reached the bottom of the separating gel. Electrophoresis was performed in a vertical slab mold (20 × 18 × 0.2 cm). Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, Mo.) prepared in a 1: 4: 6 mixture of 90% acetic acid, 70% methanol and water then re-stained for 6 hrs with a similar mixture of acetic acid, methanol and water **(Laemmli, 1970)**

Cluster analysis:

Electrophoretic protein patterns of the twelve pathogenic bacteria isolates were clustered (**Hadacova**, ***et al.,* 1980 and Joseph *et al.,* 1992**) by the average linked technique (un-weighed pair-group

method). The results were expressed as phenograms. Cluster analysis was performed with a computerized program.

II.5 - DNA fingerprinting analysis:

II.5.a - The used bacterial isolates:

Three isolates of *Xanthomonas* of those isolated from the tested tomato plants were used in this study. The other isolates were included as positive controls to assure DNA fingerprinting experiments correctly classified isolates to the appropriate pathovars.

- Culturing of bacterial isolates:

Isolates were routinely cultured on nutrient agar or broth during incubated at 26 to 29°C.Also,the isolated *X. vesicatoria* isolates from tomato homogenate were cultured on nutrient agar amended with 60 µg/ml of kasugamycin and 50 µg/ml of cycloheximide to reduce growth of secondary organisms. The addition of these antibiotics to the nutrient agar was not essential to recover *X. vesicatoria* from tissue homogenates, but all isolates used in this study were resistant to both antibiotics and their addition reduced or eliminated the growth of other bacteria or fungi and simplified enumeration of the pathogens. All antibiotics were purchased from Sigma Chemical Co. (St. Louis, MO).

-DNA extraction:

Strains were preserved in 15% nutrient glycerol broth at –80°C for long-term storage. isolates were cultured in 1.5 ml of nutrient broth for 24 h for DNA isolation procedures. The culture was adjusted to an optical density of 0.1 at 600 nm in sterile 0.02 M potassium phosphate buffer pH 7.2 (PB), and DNA was isolated using the CTAB (hexadecyltrimethylammonium bromide) method

(3). DNA was stored in Tris-EDTA buffer (TE [10 mM Tris, 1 mM EDTA, pH 8.0]) at –20°C.

- **RAPD- PCR analysis:**

RAPD-PCR was carried out according to **Williams *et al.*, (1990) and Welsh and McClelland (1990)** at the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.

The used primers were 10–mer oligonucleotide (Eurofins MWG Operon's RAPD 10mer Kits); ten primers were selected as potentially useful as shown in **Table** (1).

PCR reactions were optimized and mixtures (25µl total volume) that composed of dNTPs (200µM), $MgCl_2$ (1.5mM), 1x buffer, primer (0.2µM), DNA (50ng), Taq DNA polymerase (2units).

Amplification was carried out in a thermo Cycler programmed for 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 36°C for 1 min and elongation at 72°C for 1 min. The 40th cycle was followed by an extended primer extension step at 72°C for 4 min and then being held at 4°C until electrophoresis.

Amplification products (15µl) were mixed with 3µl loading buffer and separated on 1.3%agarose gel and stained with 0.5 µg/ml ethidium bromide, and visualized under ultraviolet light and photographed by DNA fragment sizes were determined by comparisons with the 1kbp plus DNA ladder marker.

Table (1): Name and sequences of the used primers with RAPD molecular markers.

Code	Primer name	Nucleotide sequence 5' to 3'
1	OPERON –A03	**5'-AGTCAGCCAC-3'**
2	OPERON –A09	**5'-GGGTAACGCC-3'**
3	OPERON –B10	**5'-CTGCTGGGAC-3'**
4	OPERON –G02	**5'-GGCACTGAGG-3'**
5	OPERON –G08	**5'-TCACGTCCAC-3'**
6	OPERON –G19	**5'-GTCAGGGCAA-3'**
7	OPERON –D-01	**5'-ACCGCGAAGG-3'**
8	OPERON –D-02	**5'-GGACCCAACC-3'**
9	OPERON –E07	**5'-AGATGCAGCC-3'**
10	OPERON –E08	**5'-TCACCACGGT-3'**

– Phylogenetic analysis of the RAPD:

The obtained data of RAPD analysis was entered in a computer file as binary matrices were 0 stands for the absence of a band and in each individual sample. Similarity coefficients were calculated according to dice matrix. Parents were grouped by cluster analysis with the similarity matrix and unweighted pair group method based on arithmetic mean (UPGMA).

III. Host range:

Ten different plants, *i.e*., tubers of potato (*Solanum tuberosum*), cabbage (*Brassica oleracea*), common bean (*Phaseolus vulgaris*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*), lettuce (*Lactuca sativa*), beans (*Vicia faba*), garden strawberry (*Fragaria ananassa*), cantaloup (*Cucumis melo*) and datura (*Datura stramonium*) that belonged to different families were inoculated individually with each one of the three tested bacterial isolates as mentioned before in the pathogenicity test. After inoculation of the

tested plants they covered with plastic sheet for 24 hrs. The reaction with the hypersensitivity or disease symptoms was observed after 24-48 hrs and 5 days post inoculation.

IV. *In vitro studies:*

IV. Effect of different biotic and abiotic treatments on the growth of pathogenic bacteria:

IV.a- Bacteriocides:

Two different copper compounds, Galbin-Cu and Copper oxychloride, at four concentrations (100, 250, 500 and 750 ppm). Nutrient agar medium with each of the tested pathogenic bacteria were used at the rate 0.1 ml of bacterial suspension (1×10^{-8} dilution - 24 hrs-old culture) were spread onto nutrient agar medium. Saturated filter paper disks (5mm) of each one of the selected bactericide at different concentrations were placed on the surface of inoculated plates. Disks without any treatment, (sterilized water only), were used as control. Four disks/plate and 3 replicates for each treatment were done then the plates were incubated at 25-28°C for 48 hrs. Effect of tested bacteriocides was measured in form of inhibition zone surrounding the disks according to **Loo *et al.*** (**1945**) and **Thornberry** (**1960**).

IV.b- Antibiotics:

Two antibiotics namely, tetracycline, erythromycin, were used at four different concentrations (50, 100, 250, 500ppm). Nutrient agar medium was inoculated with each one of the tested pathogenic bacteria at rate (0.1 ml) of bacterial suspension ($1X\ 10^{-8}$ dilution - 24

hrs-old culture) were spread onto nutrient agar medium. Saturated filter paper disks (5mm) of each antibiotic at different concentrations were placed on the surface of inoculated plates. Disks without any treatment, (sterilized water only), were used as control. Four disks/plate and 3 replicates for each treatment were done then the plates were incubated at 28°C for 72 hrs. Effect of tested antibiotics was measured in form of inhibition zone surrounding the disks according to **Fatmi and Schaad (1988).**

IV.c- Resistance inducers:

Two acids namely ascorbic acid and salicylic acid were used at four different concentrations (50, 100, 250, 500ppm) as resistance inducers. Nutrient agar medium was inoculated with each one of the tested pathogenic bacteria at rate 0.1 ml of bacterial suspension (1×10^{-8} dilution - 24 hrs-old culture) were spread onto nutrient agar medium. Saturated filter paper disks (5mm) of each inducer acid at different concentrations were placed onto the surface of the inoculated plates. Disks without any treatment, (sterilized water only), were used as control. Four disks/plate and 3 replicates for each treatment were served as replicates and then the plates were incubated at 28°C for 72 hrs. Effect of tested inducer was measured in form of inhibition zone surrounding the disks according to **Fatmi and Schaad (1988).**

IV.d- Plant oils:

Two plant oils *i.e.*, Mentha oil (*Mentha aquatica*) and Clove oil (*Syzygium aromaticum*)were used at four different concentrations (2.5, 5, 10 and 20%). Nutrient glucose agar medium with each one of

the tested pathogenic bacteria at rate (0.1 ml) of bacterial suspension (1×10^{-8} dilution - 24 hrs-old culture) were spread onto nutrient agar medium. Saturated filter paper disks (5mm) of each plant oil at different concentrations were placed onto the surface of the inoculated plates. Disks without any treatment, (sterilized water only), were used as control. Four disks/plate and 3 replicates for each treatment were served as replicates and then the plates were incubated at 28°C for 72 hrs. Effect of the tested plant oil was measured in form of inhibition zone surrounding the disks according to **Fatmi and Schaad (1988).**

IV.e- Plant extract:

Plant extract of garlic (*Allium sativum*) cloves was prepared as follow: fifty grams of old garlic cloves were washed with sterile distilled water and blotted with paper towels. Samples were then cut into small pieces and blended using a twister blender (Hamilton Beach, 16-speed Turbo- Twister blender) for 10 min at room temperature. The extracts were obtained by centrifuging samples (Sorvall RC5B centrifuge, Newton، CT) at 8000 g for 45 min to remove the bigger particles. The supernatants were collected and sterilized using a Nalgene filter (diameter 0.42 µm, Fisher Scientific). The extracts were then kept at 40°C until use within 24 h. Four different concentrations of garlic extract *i.e*., 2.5, 5, 10, 15%. Nutrient glucose agar medium with each one of the tested pathogenic bacteria at rate (0.1 ml) of bacterial suspension (1×10^{-8} dilution - 24 hrs-old culture) were spread onto nutrient agar medium. Saturated filter paper disks (5mm) of each concentrate of garlic extract was placed on the

surface of inoculated plates. Disks without any treatment, (sterilized water only), were used as control. Four disks/plate and 3 replicates for each treatment were done then the plates were incubated at 28°C for 72 hrs. Effect of each concentrate of garlic extract was measured in form of inhibition zone surrounding the disks according to **Fatmi and Schaad (1988).**

IV.f- Biological agents:

1- *Pseudomonas fluorescens*

Two isolates of *Pseudomonas fluorescens* (*Pf*1 and *Pf2*) which isolated and identified from tomato leaves and seeds were used in this study. Nutrient agar medium with each one of the tested pathogenic bacteria at rate (0.1 ml) of bacterial suspension (1×10^{-8} dilution - 24 hrs-old culture) were spread onto nutrient agar medium. A loop full of bioagents (24 hrs-old cultures) streaked at the center of inoculated plates. Plates without streaks for bacterial bioageant (sterilized water only) were done for control treatment. Four disks/plate and 3 replicates for each bioagents isolates were done then the plates were incubated at 28°C for 72 hrs. Diameter of inhibition zone was measured according **Boudyach *et al*. (2001).**

2- Kombucha:

Kombucha was prepared by fermenting sweetened green tea (100 g sucrose, 10 g Chinese green tea per liter of water) with a symbiotic colony of yeasts and bacteria **(Dipti *et al*., 2003).** Twelve days from incubation at 28°C, mother culture was omitted and filtrate was kept to self-refermented for additional 21 days, filtrate was collected, centrifuged for 10 min at 1000 rpm to separate any debris,

then sterilized using sintered glass (G6) funnel (**Betsy and Sonford, 1996**). Crude (100%) kombucha filtrate was diluted with distilled water to 2.5, 5, 10 and 20% before used.

3- Bacteriophages:

3. a. Bacterial strains and Bacteriophages:

Bacterial strains were grown on nutrient agar (NA) medium (0.8% (w/v) nutrient broth (NB) and 1.5% (w/v) Bacto Agar (Difco) at 28°C. For bacteriophage detection and propagation either semisolid nutrient agar yeast extract medium (NYA), (0.8% Nutrient Broth, 0.6% Bacto Agar and 0.2% Yeast Extract (Difco, Becton Dickinson and Co., Sparks, MD) or liquid nutrient broth medium was used. Sterilized tap water or SM buffer (0.05 M Tris-HCl (pH 7.5), 0.1 M NaCl, 10 mM $MgSO_4$ and 1% (w/v) gelatin) was used for preparing phage suspensions.

Bacterial strains used in this study were stored at -80°C in NB supplemented with 30% glycerol. Bacteriophages were stored at 4°C and protected from light.

3. b. Phage isolation from diseased plant tissue and soil:

Bacteriophages were isolated from spot lesions of tomato leaves. Phage was isolated from diseased tissue at the Laboratory of Department Plant Pathology, Bacteriology branch, Fac. Agric., Ain-Shames Univ. as a part of the tomato spot Eradication Program**.** Diseased tissues were collected directly from infected seedlings located then directly placed in flasks 125 containing 50 ml sterilized tap water were shaken for 20 min. Two milliliters were collected and centrifuged at 10,000 rpm for 10 min to remove debris. The

supernatants then were either treated with chloroform or filter-sterilized and then were checked for the presence of bacteriophages by spotting 20 µl onto freshly prepared lawns of the indicator bacteria. Three isolates were used for detection of diverse origins plus isolates were used. If plaques were observed after 24 h post incubation at 28°C, the phage was purified by three successive single plaque isolations and then propagated and stored, as described above.

3. c. Standard bacteriophage techniques:

Purification and storage

Phages were purified by three subsequent single plaque isolations. Single plaque isolations were carried out by transferring phages from isolated plaques to a fresh lawn of the host bacterium using sterile toothpicks and then quadrant streaking them with sterile plastic transfer loops. Following purification the phages were propagated by mass streaking on fresh lawns of the host. After 24-h incubation at 28°C, the phages were eluted by pouring 5 ml sterilized tap water into the Petri dishes and gently shaking the plates (20.000 rpm for 30 min). The eluate was centrifuged (10,000, rpm for 10 min), treated with chloroform or filter-sterilized, depending on the phage type, then quantified as described below, and stored in 2-ml plastic vials at 4°C in complete darkness. The concentrations of these suspensions were approximately 10^9 plaque forming units (PFU) per ml determination of titer. Phage concentrations were determined by dilution-plating-plaque- count assay on NYA plates without bottom agar as previously described by (**Rizvi and Mora 1963)**. One hundred microlitre aliquots of dilutions of phage suspensions were

mixed with 100 µl of concentrated bacterial suspension in empty Petri dishes and then 12 ml warm (48°C) NYA medium was poured in each dish. The dishes were gently swirled to evenly distribute the bacteria and the phages within the medium. After the medium solidified, the plates were transferred to 28°C incubators and the plaques were counted on the appropriate dilutions after 24 or 48 hrs. The phage concentration was calculated from the plaque number and specific dilution and was expressed as PFU/ml.

Phage propagation

Phages were recovered from storage, purified by single plaque isolations and then mass streaked on the freshly prepared lawn of the propagating host. The next day phages were eluted from the plate, sterilized and enumerated, as described above. The elute used for infecting 500 ml actively growing culture of the propagating grown in NB liquid medium in 1 liter flasks, at 0.1 multiplicity of infection (MOI), (*i.e.*, the phage concentration at the beginning of the incubation was 10^7 PFU/ml). After addition of the phage and 5-min incubation on the bench top, the culture was shaken at 150 rpm/min at 28ºC for 18 h. The resulting culture was sterilized; phages were enumerated and stored at 4ºC in the dark until use. This method yielded phage titers of approximately 10^{10} PFU/ml.

-Phage concentration by high speed centrifugation.

High titer phages lysate (10^{10} PFU/ml) were concentrated and purified according to the method described by **Ackermann (2005**). The supernatant was discarded and replaced with 0.1 M ammonium acetate solution (pH 7.0). Following an additional centrifugation (60 min, 10,000 rpm) the supernatant was discarded and the pellet was

resuspended in 1.5 ml SM buffer. The final phage concentration was approximately 10^{12} PFU/ml.

-Evaluation of bacterial sensitivity to bacteriophage:

Sensitivity of a bacterial strains to phages was determined based on the ability of the phage to produce plaques on the bacterial lawn, and the level of sensitivity was evaluated based on efficiency of plating (EOP) on the test strain in comparison with the propagating host strain of the phage as follows. A phage suspension of known concentration was plated simultaneously on the test and the host strains and EOP was calculated as the number of plaques on the test strain divided by the number of plaques on the host strain.

Phage susceptibility tests:

The qualitative sensitivity tests were done by spotting the phage suspensions with a platinum loop on the surface of an agar layer inoculated with the bacteria in Petri dishes. All positive reactions were confirmed to be the lytic reaction of the phages by forming separate plaques on plates (using the double-layer technique) which were seeded with the mixture of properly diluted phage suspension (about 100 to 300 pfu per plate).

4- Transmission Electron Microscope:

Transmission Electron Microscopy (TEM) was carried out at Laboratory of the Microbiology Department, Faculty of Science, El-Azhar University. The phages were visualized using negative staining protocol with 1% aqueous uranyl acetate, as follows. A drop of the phage suspension was applied to a 300 mesh formvar- coated copper grid. After 2 min, the liquid was blotted away and the grid was rinsed with DI water. A 1% uranyl acetate solution was applied to the grid

and blotted away after 1 min. The phages were observed and photographed on a Zeiss EM-10CA Transmission Electron Microscope operating at 100 K.volt.

V. *In vivo studies:*

V.1- Evaluation of tomato cultivars to bacterial spot infection:

Sixteen tomato cultivars and hydrides (*i.e.*, Castle rock, Gs12, Peto 86 ,Super strain B, Flora-Dade, Money maker, Dora, KTM 141, Niagra, Diamante F1, Super Marmand; Hypride7796, Mors44, VT916G.SI, HMX4791 and Faqlta 38) were evaluated for their susceptibility to bacterial spot, under greenhouse conditions. Pots (Ø 25cm containing 3 kg soil/pot) were planted with one seedling/pot. After three week post transplanting, tomato plants were inoculated individually by bacterial suspension of the three isolates of *Xanthomonas vescatoria* which prepared as pathogenicity test. The inoculated tomato plants were maintained in a humid chamber for 48 h before inoculation. Disease severity, disease incidence, number of spots and disease reduction was recorded, after 3 and 10 days post inoculation.

V.2- Effect of different biotic and abiotic treatments on the disease incidence of pathogenic bacteria:

In all the *in vitro* studies, pots (Ø 25cm containing 3 kg soil/pot) were planted with 6 seedlings of tomato cv. Super strain B (30 days age). After 15 days post transplanting it inoculated with pathogenic bacterial suspensions (1×10^8 cfu) and its sprayed by different compounds that used for disease control at 3 days before spraying by bacterial pathogens. [Each pot contained one plants and six pots were

used as replicates per treatment, **Abd El-Ghafar and Mosa (2001**). Disease severity was recorded after 3 and 10 days from inoculation]. All inoculated plants were maintained in humid chamber and Disease severity was recorded after 10 days from inoculation (**Abbasi *et al.*, 2002).**

Disease assessment:

Disease severity ratings were recorded using two different procedures. The first involved was determining lesion numbers on 30 leaflets. The second ones, disease severity was rating consisted of assessing the percentage of defoliation on two dates using the Horsfall- Barratt scale **(Horsfall and Barratt, 1945).**

Scale ranging content eight degrees from 0-7, where:

- 0	0	represented a sparse plant canopy
- 1	1-10	necrotic spots on the leaves/plant
- 2	11-20	necrotic spots on the leaves/plant
- 3	21-30	necrotic spots on the leaves/plant
- 4	31-40	necrotic spots on the leaves/plant
- 5	41-50	necrotic spots on the leaves/plant
- 6	51-60	necrotic spots on the leaves/plant
- 7	>61	lack of epinasty on new growth

Disease Incidence (DI) were calculated by the following formula:

$$\mathbf{DI} = \frac{\Sigma\, R.t \times 100}{7 \times N}$$

Where, t = number of plants with disease severity scale R (R = 0-7).

N = total number of inoculated plants .

$$\text{Disease Reduction} = \frac{DS_{control} - DS_{treatment} \times 100}{DS_{control}}$$

V.2.a- Bactericides:

Two copper compounds Glaben-Cu (Benalaxyl- Cu) 48% and Copper oxychloride (Copper oxychloride) 64% were used as plant spray at (750 ppm), 3 days before inoculation of the tested pathogenic bacterium. After 45 days from plants, age the disease assessments were recorded according **Loo *et al.*** (**1945**) and **Thornberry** (**1960**) as mentioned before.

V.2.b- Antibiotics:

Two antibiotics namely tetracycline and erythromycin were used as plant spray at 500ppm 3 days before inoculation with the tested pathogenic bacterium. After 45 days from plants age the disease assessments were recorded according to **Fatmi and Schaad (1988)** as mentioned before.

V.2.c- Plant oils:

Two plant oils namely Mentha oil, resulted from plants *Mentha aquatica* and clove oil product from plants *Syzygium aromaticum* were used as plant spray at 20% concentration, 3 days before inoculation of the tested pathogenic bacterium. After 45 days from plants age the disease assessments were recorded according to **Fatmi and Schaad (1988)** as mentioned before.

V.2.d- Plant extract:

Plant extract of garlic cloves (*Allium sativum*) was used as plant sprayed at 20%, 3 days before inoculation with the tested pathogenic bacterium. After 45 days from plants age the disease assessments were recorded according as to **Fatmi and Schaad (1988).**

V.2.e- Resistance inducers:

Two inducted resistance acids *i.e*., ascorbic acid, and salicylic acid were used as plant sprayed at 500 ppm, 3 days before inoculation with the tested pathogenic bacterium. After 45 days from plants age the disease assessments were recorded according as to **Fatmi and Schaad (1988).**

V.2.f- Effect of hot water treatment on the ability of to infect with pathogenic bacteria tomato plants.

Tomato seeds and seedlings (Super strain B c.v) each were dipped for an hour in the pathogenic bacterial suspension (3 concentrations, *i.e.* 1×10^8, 0.5×10^8 and 0.3×10^8), Arabic gum (2g/l) was added. Treated seeds and seedlings were air dried then every 50 seeds or seedlings from each treatment and control were separately treated indirect for 5 min with hot water (45 – 50°C). Five treated seeds or seedlings were sown in the plastic pots (∅25cm). Three pots from each treatment were used as replicate. Disease assessments were done after 45 days from plantation as mentioned before.

V.2.g- Biological agents:

V.2.g.1- ***Pseudomonas fluorescens:***

Two previous bioagents *Pseudomonas fluorescens* (*Pf*1 and *Pf2*) were grown on yeast extract peptone dextrose agar (YPDA) medium for 48 h at 28°C. The bacterial cells were suspended in sterile distilled water and centrifuged at 3000 rpm/min for 30 min.

The pellets were re-suspended in distilled water and adjusted to the density of 10^8 cfu/ml. Tested bioagents were used as foliar application; seedlings were sprayed with bacterial suspension (50 ml/

plant) of bioagent at 4 weeks old before the inoculation with bacterial pathogen. Other tomato seedlings were sprayed with water only as control treatment. Treated plants were placed in humid chamber (70%) for 48 h before inoculation. Each pot contained one plant and six pots were used as replicates per treatment (**Abd El.Ghafar and Mosa, 2001**). Disease severity was recorded after 3 and 10 days post inoculation with bio-agents.

V.2.g.2- Kombucha:

Kombucha filtrate was prepared as mentioned before. Tomato plants were sprayed with kombucha filtrate at 20% before inoculation with individual the three isolates of the tested pathogenic bacteria. Treated plants were placed in humidity chamber for 48 hrs after treating. Each pot contained one plant and six pots were used as replicates per treatment. Disease severity was recorded after 3 and 10 days post inoculation.

V.2.g.3- Bacteriophage.

Phage isolates which purified and identified as mentioned before, 3% corn flour + 5% sucrose was added to the liquid cultures of the tested phage isolates individually the other treatment was mixture of the four phage isolates. Tomato plants (4 weeks old) were sprayed with phage isolates as mixture treat individual and treatment before the inoculation with the three isolates of *Xanthomonas vescatoria* **(Balogh *et al.*, 2003)**. Treated plants were placed in humid chamber (70%) for 48 hrs after treatments. Pot contained one plant and six pots were used as replicates per treatment.

Disease severity was recorded after 3 and 10 days from inoculation as mentioned before.

VI- Some biochemical changes in tomato plants:

Peroxidase and polyphenoloxidase activity were determined in tomato leaves which collected after 3 and 7 and 10 days from inoculation with the three isolates of pathogenic bacteria as follows:

1. Extraction of enzymes:

Samples was ground with 0.2M Tris HCl buffer (PH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20min at 4°C. The supernatant was used to determine enzyme activities **(Tuzun *et al.*, 1989).**

1. Determination of Peroxidase (POD):

Peroxidase activity is routinely assayed by measuring the oxidation in the presence of hydrogen peroxide and the enzyme every 30 sec. intervals using UV- 2401 PC UV- Vis recording Spectrophotometer (Cent. Lab., Fac. Agri., Benha Univ.) in a 4 ml light path cuvettes. The reaction mixture (unless otherwise stated) contained in a volume of 3 ml: 8 µmoles hydrogen peroxidase, 60 µmoles guaiacol, 60 µmoles sodium acetate buffer. pH 5.6 and peroxidase at concentrations which gave a linear response over a period of 3 min. The reaction is initiated by introducing the enzyme and mixing, A unit of peroxidase activity is defined as that amount of enzyme which cause one O.D =430nm/g fresh weigh/30sec) change per minute **(Ghazi, 1976).**

2. Determination of polyphenol oxidase (PPO):

A- Activity:

Polyphenol oxidase activity was determined by measuring the initial rate of quinine formation, as indicated by an increase in

absorbance at 420 nm/g fresh weigh/30sec, **(Coseteng and Lee, 1978)** using Spectrophotometer.

One unit of enzyme activity was defined as the amount of enzyme that caused a change in absorbance of 0.001/min, PPO activity was assayed in triplicate measurements. The sample cuvette contained 2.95 ml of 20 mM catechol solution in 0.1 M phosphate buffer. pH 6.0 and 0.05 ml of the enzyme solution. The blank sample contained only 3 ml of substrate solution.

3. Determination of phenols:

Extraction samples:

Samples of 1g were taken from artificially inoculated leaves 10 days post inoculation. Leaves spot samples without treatment were taken as control for comparison. All taken samples were cut to small portions, transferred to 50 ml of 95% ethanol in brown bottles and kept in darkness at room temperature for one month then homogenized in sterile mortar as recommended by **Bozarth and Diener (1963).** The ethanolic extracts were air dried at room temperature till near dryness and then were quantitatively transferred to 10ml 50% isopropanol and stored in vials at 1°C. The obtained extracts were used for the following determinations:

Free, conjugated and total phenols were colorimetrically determined using the "Folin and Ciocalteu" reagent as described by **Snell** and **Snell** (**1953**).

The reagent was prepared by adding 100g sodium tungestate and 25g sodium molybdate to 700ml water in conical flask then 50ml phosphoric acid (85%) and 100ml HCl were added. Flask containing this mixture was attached to reflective condenser. The mixture was

left to boil gently in a water bath for 10 hrs, then it was left to cool, 150g lithium sulphate and 50ml distilled water were also added.

Few drops of bromine were also added and the mixture was heated again to remove access bromine. Finally, the mixture was completed to 1000ml with distilled water.

Free, conjugated and total phenols were determined in previously prepared extracts of tomato leaves samples. Determination was calculated as catechol in terms of mg phenols per 1-g fresh matter. To determine the free phenols, 1ml of sample extraction was put in a sterilized test tube with 1ml distilled water, 5ml Folin-Ciocalteu reagent and 15ml Na_2CO_3 (20% w/v). The mixture was completed to 50ml with distilled water and the color density was recorded at 520 nm. The total phenols were determined by treating 1ml of extracted sample with 0.25ml HCl and boiled in a water bath for 10 min then cooled. Ten ml of Folin-Ciocalteu and 25ml of Na_2CO_3 were added. The mixture was completed to 100ml with distilled water and the values of color density were measured (Spectronic 20-D) at 520 nm using the Spectronic 20-D). Conjugated phenols were determined by subtracting the free phenols from the total phenols.

VII. Statistical analysis:

Data were statistically analyzed using the (F) test and the value of LSD (P 0.05) was calculated according to **Cochran and Cox (1957).**

EXPERIMENTAL RESULTS

I- Isolation of bacteria caused of tomato leaf spot disease:

A-Isolation from leaves:

Data in **Table (2)** indicate that, sum of 70 bacterial isolates were isolated from different naturally infected tomato leaves, which collected from four localities *i.e.* El-Dokki (cv. Super Marmand,) at Giza Governorate, Qaha (cv. Peto 86 and cv. Super strain B), Fac. of Agriculture Ain Shams at Qalubia Governorate (cvs. Moneymaker, Super Marmand and Super strain B) and Rashid (cv. Mors-44, cv. Niagra) at Domiatt Governorate. All isolated bacteria were found to belong to 4 different groups according to their morphological features.

Group 1 gave the highest frequency among the isolated bacteria (30 isolates), whereas; the Group 2 and 3 were represented by 10 isolates for each of them. Meanwhile, group 4 was represented by 20 isolate of bacteria which varied in their morphology. The highest isolation number of Group 1 was recorded in Qaha location (10 isolates) followed by El-Dokki (8 isolates) and Fac. Agri., Ain Shams (7 isolates). Meanwhile, the least isolation number of group 1 was 5 isolates with frequency in Rashid locality. On the other hand, Group 4 recorded 10 isolates representing the highest isolation number in Rashid locality with frequency followed by Fac. Agri., Ain Shams location (4 isolates). Also, the isolated bacteria which representing group 2 (10 isolates) were recorded only on samples of Qaha locality (5 isolates) and El-Dokki (5 isolates) without any recording of the same group bacteria on samples of Fac. Agric., Ain Shams and Rashid localities. On the other hand, the isolated bacteria which representing group 3 (10 isolates) were recorded only on samples of Qaha (8 isolates) and Rashid (2 isolates) without any recording of the same group bacteria on samples of El-Dokki and Fac. Agric., Ain Shams localities. Generally the highest isolation number of isolated bacteria was recorded in Qaha (28 isolate) followed by Rashid (17 isolate), El-Dokki (16 isolate) and Fac. Agric., Ain Shams (11 isolate) respectively.

Table (2): Number and frequency% of the isolated bacteria from naturally infected tomato leaves with bacterial leaf spot disease at different localities.

Isolated bacteria	Isolation localities								Total
	Qaha		EL -Dokki		Fac. of. Ain Shams		Rashid		
	Fre.	%	Fre.	%.	Fre.	%	Fre.	%	
Group 1	10	**35.7**	**8**	**50**	**7**	**63.6**	**5**	**29.4**	**30**
Group 2	**5**	**17.8**	**5**	**31.3**	**0**	**0.0**	**0**	**0.0**	**10**
Group 3	**8**	**28.5**	**0**	**0.0**	**0**	**0.0**	**2**	**11.8**	**10**
Group 4	**3**	**10.7**	**3**	**18.8**	**4**	**36.4**	**10**	**58.8**	**20**
Total	**26**		**16**		**11**		**17**		**70**

b- Isolation from tomato seeds:

In this experiment, sixteen cultivars and hybrids of tomato seeds were used for isolation the bacteria causing bacterial leaf spot disease on tomato plants. Data in **Table** (**3**) show that none of the bacterial isolates which belong to group 1 were recorded on the tested seeds of tomato cultivars and hybrid *i.e.*, Dora, Gs-12, KTM-141, Niagra, Diamante-F1, Hybrid-7796, Hybrid Super strain B, Mors-44, Hybrid VT916G.SI, Hybrid HMX4791 and Faqlta-38 when directly planted onto the nutrient agar (NA) agar medium. Only 9 bacterial isolates belonging to the same group 1 were recorded on seeds of cv. Super strain B.

On the other hand, none of bacterial isolates which belonging to group 2 were recorded on seeds *i.e.*, Dora, KTM-141, Diamante-F1, Hybrid-7796, Hybrid HMX4791, Faqlta-38, Mors-44, and Hybrid VT916GSI. Meanwhile, the isolated bacteria which belonging to group 2 were recorded only on Gs-12 (2 isolates), Niagra (5 isolates), Super strain B (4 isolates) and Hybrid Super strain B (1 isolate). Also, none of bacterial isolates belong to group 3 were recorded on seeds of any one of tomato cultivars and hybrids. As for group 4 of isolated bacteria from seeds of tomato cultivars and hybrids, 20 bacterial isolates were recorded

only on Dora (2 isolates), Gs-12 (4 isolates), Niagra (1 isolate), Super strain B (8 isolates), Faqlta-38 (3 isolates) and Mors-44 (2 isolates).

While none of the isolated bacteria was recorded on the other tested tomato cultivars and hybrids. The results indicate also that the highest number of bacterial isolates of the 4 isolated groups was recorded on cv. Super strain B (17 isolate) followed by cv. Niagra (11 isolate) and cv. Gs-12 (6 isolates) respectively while the least number of the isolated bacteria was recorded on Hybrid Super strain B (1 isolate).

Table (3): Number and Frequency of isolated bacteria from naturally infected tomato seeds with bacterial spot disease.

Cultivars and hybrids	Different bacterial isolates								Total
	Group 1		Group 2		Group 3		Group 4		
	Fre.	%	Fre.	%.	Fre.	%	Fre.	%	
Dora	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**10**	**2**
Gs-12	**0**	**0.0**	**2**	**16.7**	**0**	**0.0**	**4**	**20**	**6**
KTM -141	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Niagra	**0**	**0.0**	**5**	**41.6**	**0**	**0.0**	**1**	**5**	**6**
Diamante F1	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Super strain B	**5**	**55.6**	**4**	**33.3**	**0**	**0.0**	**8**	**40**	**17**
Hybrid Super strain B	**0**	**0.0**	**1**	**8.3**	**0**	**0.0**	**0**	**0.0**	**1**
Hybrid 7796	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Hybrid HMX4791	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Faqlta 38	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**3**	**15**	**3**
Castle rock	**2**	**22.2**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**
Flora-Dade	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Money maker	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Peto 86	**2**	**22.2**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**
Mors44	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**10**	**2**
Hybrid VT916G.SI	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**0.0**
Total	**9**		**12**		**0**		**20**		**41**

II- Identification of isolated bacteria:

1. Morphological, physiological and biochemical characteristics:

According to the variations in the clear morphological features of the isolated bacteria from naturally infected tomato leaves, they divided to 4 different groups. The first 3 groups (1, 2 and 3) were identified based on the variations in their morphological, physiological and biochemical characteristics. The 4th. Group (group 4) was excluded from identification because of it contained different bacterial isolates which differed in their clear cultural features. In this respect, out of 50 bacterial isolates from tomato leaves were identified and classified while the other isolated bacteria from seeds were excluded from this test, 30 isolates of those belonging to group 1 were identified as *Xanthomonas*, 10 isolates of those belonging to group 2 were identified as *Bacillus* and 10 isolates of those belonging to group 3 were identified as *Pseudomonas*.

In the first scheme **(Tables, 4-a&b)**, the isolated bacteria of group 1 which encoded under serial number (1-30) were identified based on their reactions with the different morphological, physiological and biochemical tests used in identification. The data of **Tables (4-a & b)** indicated that all the bacterial isolates of group 1 could be classified to belong the genus *Xanthomonas*. Therefore, the other biochemical tests confirmed that these bacterial isolates of the group 1 could be classified as *Xanthomonas vesicatoria* where, these isolates were short rod shape, Gram negative, aerobic, positive with KOH 3%, starch hydrolyzed, growth on peptone yeast extract agar (PYEA),catalase, H_2S production, motility, arginine dihydrylase, nitrate reduction, yellow pigment on YDC medium, acid production of glucose, mannose and Levan formation on sucrose medium.

Also, they were negative to gram reaction, gelatin liquefaction, and fluorescent pigment on King's B medium and, utilize arabinose of utilization, glucose, mannose, galactose, maltose and sorbitol galactose, maltose and sorbitol, acid production of arabinose, galactose, maltose and sorbitol.

Data in **Table (4-c)** revealed that these isolates could be classified to the genus *Bacillus*. Therefore, the other biochemical tests as shown in **Table (4-d)** confirmed that these bacterial isolates of the group 2 could be classified as *Bacillus* where these isolates were rod shape, Gram positive, spore former, starch hydrolysis, gelatin liquefaction, growth on yeast extract dextrose $CaCO_3$, catalase, glucose of utilization and lactose, negative with KOH 3%, pigment on King's B medium,H_2S production, arginine dihydroulase, nitrate reduction, acid production of glucose, lactose. Data in **Table (4-e)** reveal that these group 3 isolates could be classified as genus *Pseudomonas.*

The biochemical tests of the group 3 isolates are shown in **Table (4-f)** that this genus could classified as *Pseudomonas fluorescens* where these isolates were short rod shape, Gram negative (G^-), aerobic, non-spore former, starch hydrolyzed, H_2S produced, arginin dihydroulase, nitrate reduction, gave positive reaction with KOH 3%, gelatin liquefaction, fluorescent pigment on King's B medium, pigments diffusible, Levan type colonies on sucrose medium, growth on peptone yeast extract agar (PYEA), yeast extract dextrose $CaCO_3$, Catalase produced.

Table (4-a):Morphological, physiological and biochemical characteristics used for identification and classification group-1 isolated bacteria.

Identification Tests	Group 1														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on common media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Size	----------All Shorts ----------														
KOH 3%,	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pigment on K.B.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spore production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacterial genera	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*

Table (4-b): Biochemical tests used for identification and classification of the isolated bacteria of group 1 at the level of specie.

Identification Tests	Group 1														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Arginin dihydrolase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yellow pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Mannose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levan formation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Relation to O_2	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.
Bacterial genera	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*

X= *Xanthomonas*

Table (4-a): Morphological, physiological and biochemical characteristics used for identification and classification group-1 isolated bacteria.

Identification Tests	Group 1														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on common media	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Size	----------All Shorts ----------														
KOH 3%,	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pigment on K.B.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spore production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacterial genera	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*

Table (4-b): Biochemical tests used for identification and classification of the isolated bacteria of group 1.

Identification Tests	Group 1														
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Arginine dihydrolase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Yellow pigment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Mannose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Utilization of Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid from Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Maltose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Sorbitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Levan formation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Relation to O_2	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.
**Bacterial genera	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*

X.* = *Xanthomonas

Table (4-c): Morphological, physiological and biochemical characteristics used for identification and classification of group2 isolated bacteria.

Identification Tests	Isolate No.									
	31	32	33	34	35	36	37	38	39	40
Gram reaction	+	+	+	+	+	+	+	+	+	+
KOH 3%	-	-	-	-	-	-	-	-	-	-
Growth on common media	+	+	+	+	+	+	+	+	+	+
Size	----------All Longs ----------									
Spore production	+	+	+	+	+	+	+	+	+	+
Pigment K.B.	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+	+	+	+
Levan formation	-	-	-	-	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+	+	+	+	+
****Bacterial genera**	***B.***	***B.***	***B.***	***B.***	***B.***	***B.***	***B.***	***B.***	***B.***	***B.***

Table (4-d): Biochemical tests used for identification and classification of the isolated bacteria of group 2.

Identification Tests	Isolate No.									
	31	32	33	34	35	36	37	38	39	40
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+	+	+	+
utilization of glucose.	+	+	+	+	+	+	+	+	+	+
utilization of lactose	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-
Arginin dihydrolase	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-	-	-	-
Yellow pigment	-	-	-	-	-	-	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+	+	+	+
Acid from lactose	+	+	+	+	+	+	+	+	+	+
Relation to O_2	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.
Motility	+	+	+	+	+	+	+	+	+	+
Bacterial genera	*B.*	*B.*	*B.*	*B.*	*B.*	*B.*	*B.*	*B.*	*B.*	*B.*

B = ***Bacillus***

Table (4-e): Morphological, physiological and biochemical characteristics used for identification and classification of group1 isolated bacteria.

Identification Tests	Isolate No.									
	41	42	43	44	45	46	47	48	49	50
Gram reaction	-	-	-	-	-	-	-	-	-	-
KOH 3%	+	+	+	+	+	+	+	+	+	+
Growth on common media	+	+	+	+	+	+	+	+	+	+
Size	----------All Shorts ----------									
Spore production	-	-	-	-	-	-	-	-	-	-
Pigment K.B.	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+	+	+	+
Levan formation	+	+	+	+	+	+	+	+	+	+
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-
Catalase activity	+	+	+	+	+	+	+	+	+	+
Bacterial genera	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*

Table (4-f): Biochemical tests used for identification and classification of the isolated bacteria of group 1.

Identification Tests	Isolate No.									
	41	42	43	44	45	46	47	48	49	50
Utilization of glucose	+	+	+	+	+	+	+	+	+	+
Utilization of lactose	+	+	+	+	+	+	+	+	+	+
Arginin dihydrolase	-	-	-	-	-	-	-	-	-	-
Pigments diffusible	+	+	+	+	+	+	+	+	+	+
Non diffusible pigments	+	+	+	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-	-	-
Yellow pigment	-	-	-	-	-	-	-	-	-	-
Acid from glucose	+	+	+	+	+	+	+	+	+	+
Acid from lactose	+	+	+	+	+	+	+	+	+	+
Relation to O_2	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.
Motility	+	+	+	+	+	+	+	+	+	+
Bacterial genera	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*	*P.*

P. = *Pseudomonas*

III- Pathogenicity test

Data in **Table (9) and Fig (1)** show that the 30 bacterial isolates of those isolated from infected spots of tomato and were identified as *Xanthomonas sp.* were tested for their pathogenic abilities on tomato and pepper plants. Results indicated that twelve isolates which identified as *Xanthomonas vesicatoria*, *i.e.*, Xv_2, Xv_3, Xv_7, Xv_8, Xv_{10}, Xv_{13}, Xv_{14}, Xv_{20}, Xv_{22}, Xv_{23}, Xv_{26} andXv_{28} were the highly pathogenic where they induce clear bacterial spot on leaves of tomato and pepper. Also, eight isolates of *X. vesicatoria* (*i.e.*, Xv_1, Xv_4, Xv_5, Xv_9, Xv_{11}, Xv_{12}, Xv_{15} and Xv_{24}) caused moderately aggressive effect where they revealed as few spots as symptoms on leaves of tomato and pepper plants. The last ten isolates of *Xanthomonas* (*i.e.*, X_6, X_{16}, X_{17}, X_{18}, X_{19}, X_{21}, X_{25}, X_{27}, X_{29} and X_{30}) were avirulant where no visual symptoms were recorded on tomato and pepper leaves after testing.

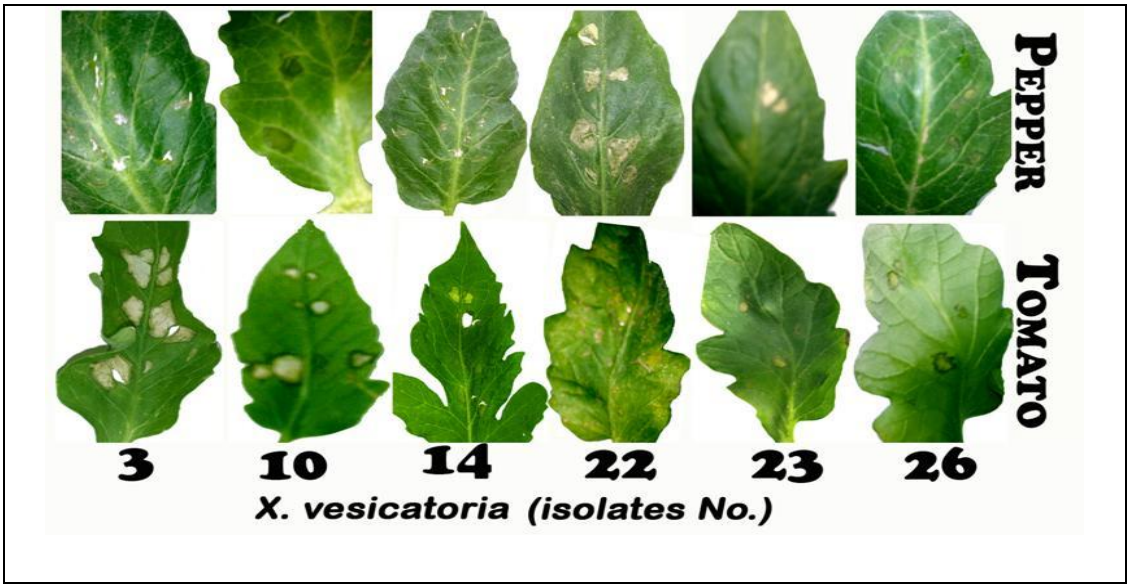


Fig (1): The pathogenicity test for 6 isolates (highly pathogenic).

Table (9): Pathogenicity test of *Xanthomonas vesicatoria* isolates on tomato and pepper plants.

No. of Isolates	Tomato	Pepper
Xv_1	+	+
Xv_2	+++	+++
Xv_3	+++	+++
Xv_4	+	+
Xv_5	+	+
X_6	--	--
Xv_7	+++	+++
Xv_8	+++	+++
Xv_9	+	+
Xv_{10}	+++	+++
Xv_{11}	+	+
Xv_{12}	+	+
Xv_{13}	+++	+++
Xv_{14}	+++	+++
Xv_{15}	+	+
X_{16}	--	--
X_{17}	--	--
X_{18}	--	--
X_{19}	--	--
Xv_{20}	+++	+++
X_{21}	--	--
Xv_{22}	+++	+++
Xv_{23}	+++	+++
Xv_{24}	+	+
X_{25}	--	--
Xv_{26}	+++	+++
X_{27}	--	--
Xv_{28}	+++	+++
X_{29}	--	--
X_{30}	--	--

+++: Highly pathogenic
+: Moderately pathogenic
--: Non pathogenic

IV- Variation among the isolated bacteria using new modern techniques:

1. Electrophoretic analysis using SDS-PAGE:

In this trial, electrophoretic analysis of the extracted proteins of twelve bacterial isolates of X*anthomonas vesicatoria* i.e., *Xv2, Xv8* and Xv_{10} (El-Dokki), Xv_3, Xv_7, Xv_{20}, Xv_{28} and Xv_{23} (Qaha) and Xv_{13}, Xv_{14}, Xv_{26} and Xv_{22} (Rashid) was done using polyacrylamide gel 12%. Data in **Table (10)** and **Fig. (2&3)** reveal that, the fractionated protein bands for the first 3 Xv isolates representing El-Dokki location were 14, 11 and 12 bands respectively with molecular weights ranged between 13.80 - 149.83 kDa and clear diversity between the three isolates, Meanwhile, the fractionated protein bands for the five Xv isolates representing Qaha location were 25, 30, 29, 23 and 24 bands respectively with molecular weights ranged also between 13.80 - 149.83 kDa and high similarity between the five isolates. On the other hand, the fractionated protein bands the four isolates representing Rashid location were 19, 21, 20 and 31 bands respectively with molecular weights ranged between 13.80 -129.09 kDa and moderate similarity between them. It is clear from the obtained results that the molecular weight 17.71 kDa was present in all the fractionated protein bands of all 12 tested *Xanthomonas* isolates to confirm that these isolates of the three locations are belonging to one cluster. On the other hand, the molecular weight 31.11 kDa was present in all fractionated protein bands of 11 *Xanthomonas* isolates except Xv_{10} and this could be increase the similarity level between these 11 isolates. It is pronounced from the obtained results also that the similarity between the 4 *Xanthomonas* isolates representing Rashid location and the 5 isolates of Qaha location was high where there were a lot of similar molecular weights in the fractionated protein bands of these isolates while, the 3 isolates representing El-Dokki were not similar to the both mentioned groups.

Table (10): Molecular weights of fractionated protein profiles of the different bacterial isolates of *Xanthomonas vesicatoria* representing three locations in Egypt.

MW. (KDa)	*Xanthomonas vesicatoria* isolates obtained from;											
	EL.Dokki				Qaha				Rashid			
	Xv 2	Xv 8	Xv10	Xv 3	Xv 7	Xv 20	Xv 28	Xv 23	Xv 13	Xv 14	Xv 26	Xv 22
149.82	+	+	-	+	+	-	-	-	-	-	-	-
129.09	-	-	-	+	-	+	-	-	-	-	-	+
111.68	-	-	-	-	+	-	-	-	-	-	-	+
103.32	-	-	-	-	-	+	-		-	-	-	+
98.28	-	-		+	+	+	+	+	+	-	+	-
91.24	-	-	-	-	-	-	-	+	-	-	+	+
87.19	-	-	+	-	+	+	-	+	-	+	-	-
81.13	+	-	-	+	+	-	+	+	+	+	+	+
78.46	-	-	-	-	+	+	-	-	+	-	+	-
72.19	-	+	+	-	+	+	-	+	-	+	-	+
66.43	-	-	-	+	+	+	+	+	+	+	+	+
63.49	-	+	+	+	+	+	+	+	+	-	+	-
61.14	-	-	-	+	+	+	-	-	+	+	+	+
58.93	-	-	-	+	-	+	+	+	-	-	-	+
57.15	-	-	-	-	-	-	-	-	+	+	+	+
55.09	-	-	-	+	+	+	+	+	+	+	-	-
52.64	-	-	-	-	-	-	-	-	-	+	-	+
51.97	+	+	+	+	-	+	-	-	+	+	+	-
48.84	+	-	-	+	+	+	+	+	-	-	-	+
47.81	+	-	-	-	-	-	-	-	+	+	-	+
46.60	-	-	-	-	-	-	-	+	-	+	+	-
44.41	-	-	-	+	+	+	+	+	-	+	-	+
42.72	-	-	-	+	+	-	-	-	+	+	+	-
40.58	-	+	+	-	-	+	+	-	-	+	-	+
37.36	+	-	-	+	+	+	+	+	+	-	+	+
34.12	+	-	+	+	+	+	-	-	-	-	+	+
32.76	-	-	-	-	-	+	+	+	+	+	+	+
31.11	+	+	-	+	+	+	+	+	+	+	+	+
29.56	-	-	-	+	+	+	+	+	-	+	-	+
28.47	-	-	-	+	+	+	+	+	-	-	-	+
27.83	+	-	-	-	-	-	-	-	+	+	-	+
26.57	-	-	-	-	+	+	+	+	-	-	+	+
25.31	-	-	+	+	+	+	+	+	-	-	-	-
24.41	+	+	-	-	-	-	-	-	-	+	-	+
23.03	-	+	-	+	+	+	+	+	+		-	+
22.23	-	-	+	+	+	-	+	+	-	-	-	-
21.10	+	-	+	-	+	-	-	-	-	-	-	-
19.70	+	+	+	-	-	+	+	+	-	-	+	+
17.71	+	+	+	+	+	+	+	+	+	+	+	+
16.68	-	-	-	+	+	-	+	-	+	-	+	+
16.01	-	-	-	+	-	+	-	+	+	-	-	+
15.51	+	-	-	-	+	+	-	+	+	+	+	+
14.59	-	-	-	+	+	-	+	-	-	-	-	-
14.13	-	+	-	-	+	+	-	-	-	-	-	-
13.80	-	-	+	-	+	-	+	+	-	+	+	+
Total	**14**	**11**	**12**	**25**	**30**	**29**	**23**	**24**	**19**	**21**	**20**	**31**

Isolate No.

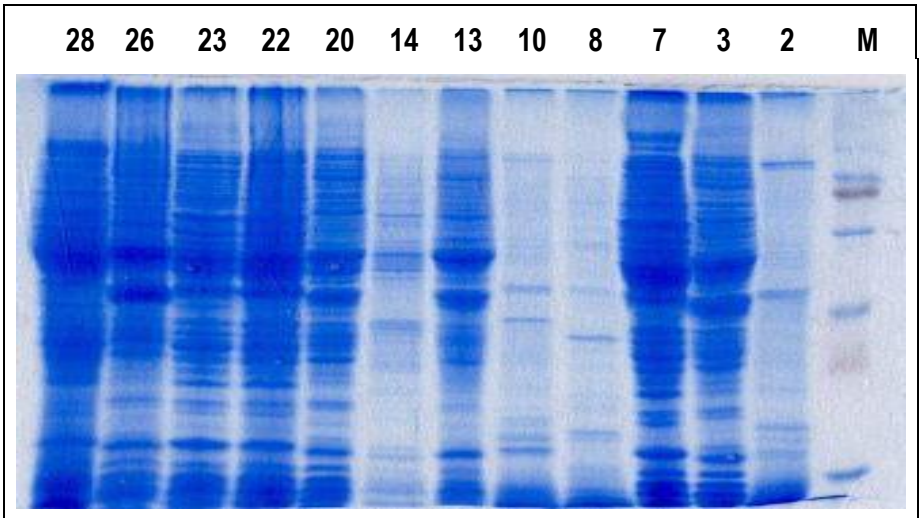


Fig. (2): SDS-PAGE of fractionated protein bands of twelve tested *Xanthomonas vesicatoria* isolates.

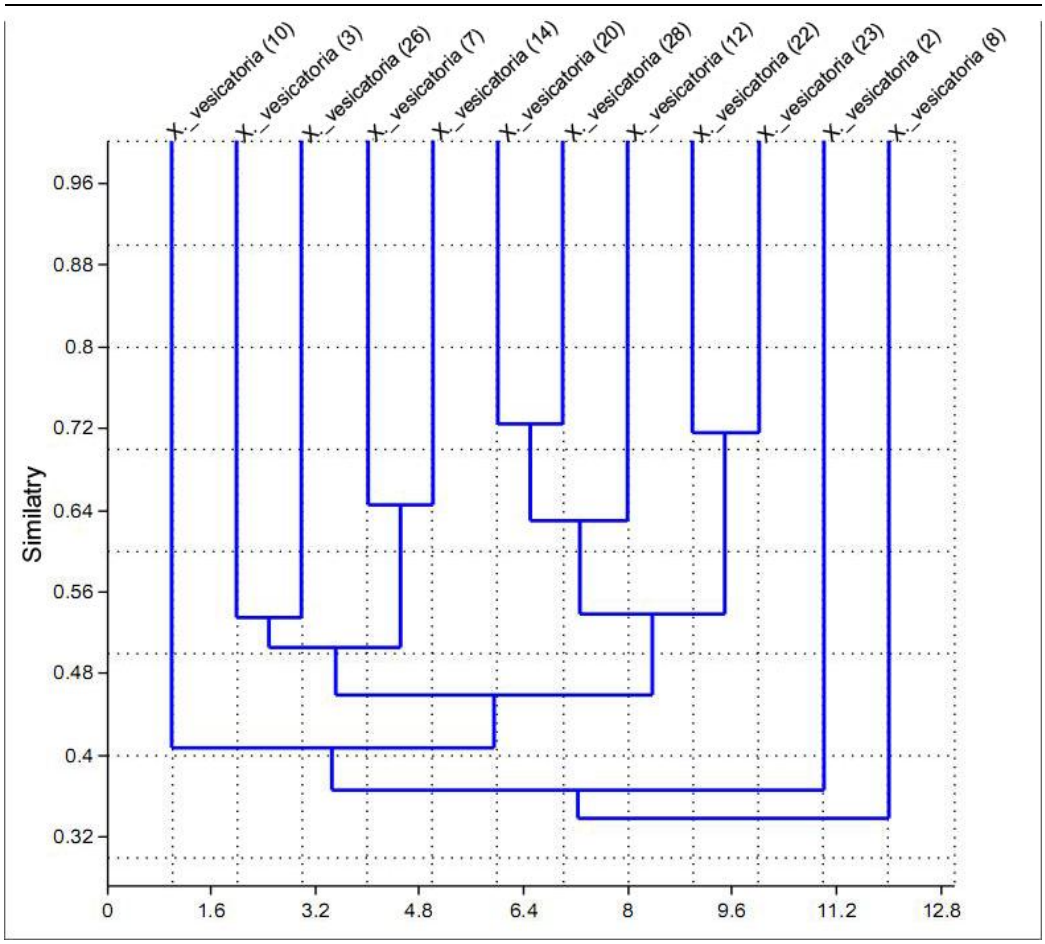


Fig. (3): Dendogram obtained from UPGMA cluster based on SDS-PAGE data from the twelve bacterial strains (*Xanthomonas vesicatoria*) genotypes.

2. Biolog system:

Three bacterial isolates *i.e.*, Xv_{28}, Xv_{22} and Xv_{10} which selected according to their highly pathogenic abilities as well as to represent the three different locations of isolation in Egypt were confirmed for their identification as *Xanthomonas vesicatoria* using the Biolog system based on their sugar contents **Fig. (4)** reveals that there are great similarities between the three tested isolates.

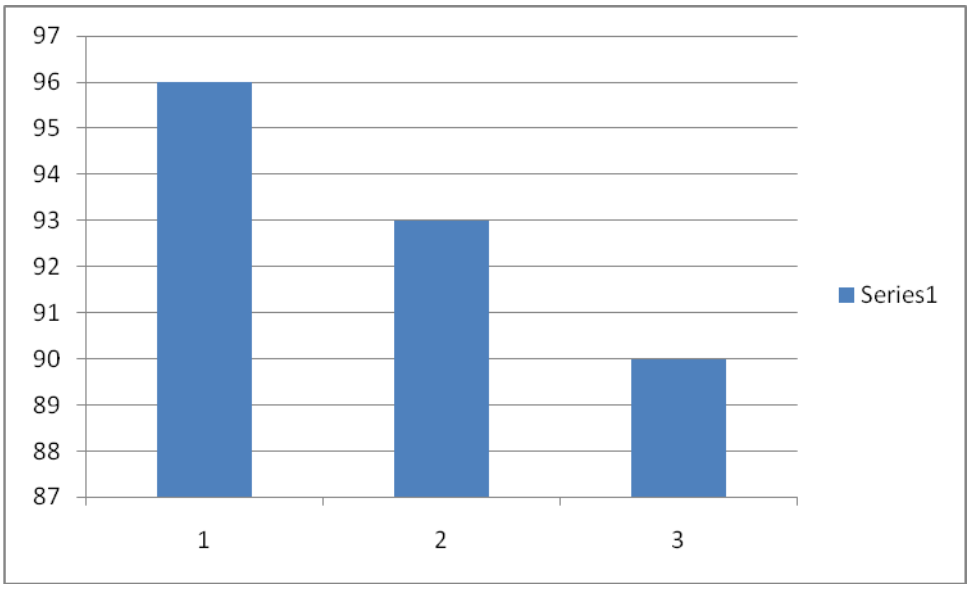


Fig.(4): Variation among the bacterial isolates by Biolog system.
1= Xv_{28} & 2= Xv_{22} and 3= Xv_{10}

3. RAPD-PCR technique:

In this trial, six RAPD primers *i.e.*, A9, B10, A3, G2, G8 and G19 were used to confirm the identification of the three bacterial isolates *i.e.*, Xv_{28}, Xv_{22} and Xv_{10} which, were selected according to their highly pathogenic abilities as well as to represent the three different locations of isolation in Egypt.

Data in **Tables** (11&12) and **Figs. (5 & 6)** illustrated that the first 4 RAPD primers *i.e.*, A9, B10, A3 and G2 were the best in revealing the initiated PCR banding patterns of the fractionated DNA fragments (bp) of

the three tested *X. vesicatoria* isolates. Amplification patterns obtained with primers A9 and A3 revealed three major products of 1580, 1130 and 840 bp. This pattern was common to all three *Xanthomonas vesicatoria* isolates isolated from three various geographical locations.

The similarity values showed clearly substantial differences among the three *Xanthomonas* isolates (Xv_{28}, Xv_{22} and Xv_{10}). In this respect, the genetic similarity ranged from 25.5 to 32.2% among the three bacterial isolates with an average of 28.85%. On the contrary, some bacterial strains displayed low genetic similarity where it was 25.52% for Xv_{28} & Xv_{22} isolates and 27.0% for Xv_{28} & Xv_{10} isolates while it was 32.2% for Xv_{22} x Xv_{10} isolates.

The dendogram analysis which resulting from the UPGMA cluster analysis showed that the three tested bacterial isolates could be divided into two clusters.

Cluster one included all the determined genotype; (Xv_{22}) and (Xv_{10}) isolates from Rashid and El-Dokki. Cluster 2 included only one bacterial spot strain (Xv_2) isolated from Qaha.

This result also confirmed the previously mentioned traditional identification which revealed that the three tested isolates are identified as *Xanthomonas vesicatoria*.

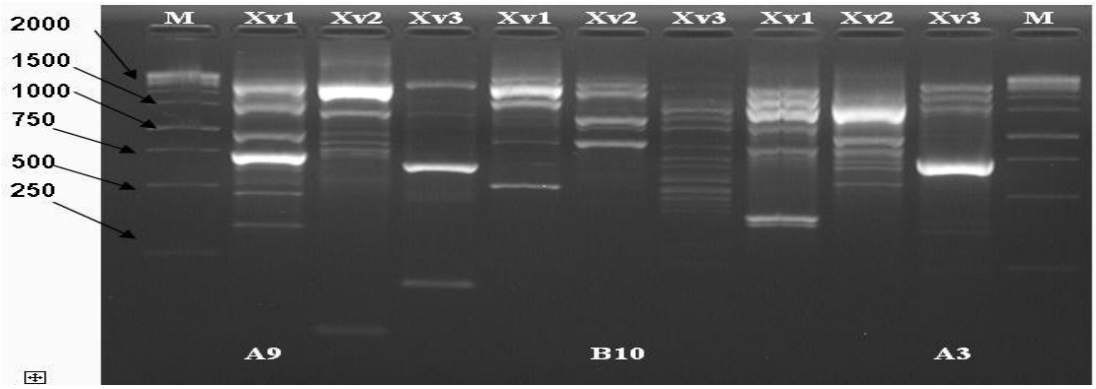


Fig. (5): DNA polymorphism of the three bacterial spot strains ($Xv_{1=Xv28}$, $Xv_{2==Xv22}$ and $Xv_{3==Xv10}$) genotypes using RAPD_PCR with three primers (A9, B10 and A3).

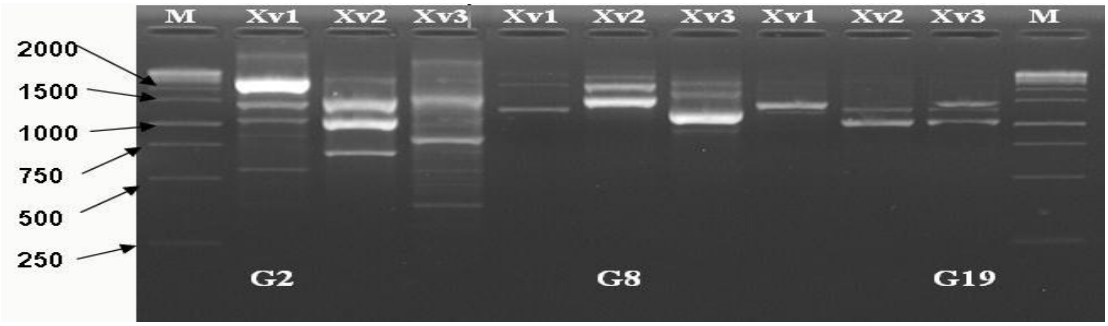


Fig. (6): DNA polymorphism of the three bacterial spot strains ($Xv_{1=Xv28}$, $Xv_{2==Xv22}$ and $Xv_{3==Xv10}$) genotypes using RAPD_PCR with three primers (G2, G8 and G19).

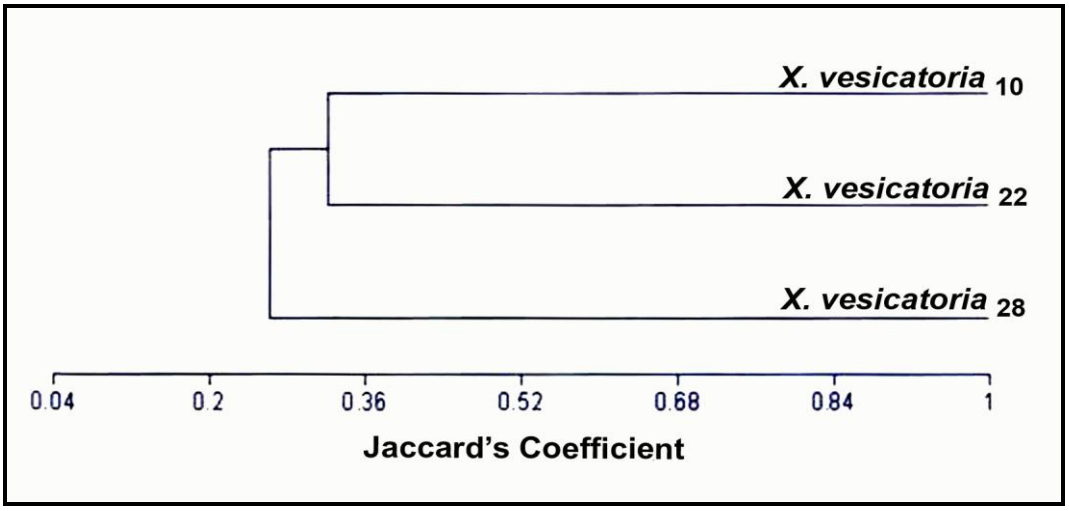


Fig. (7): Dendogram obtained from UPGMA cluster based on RAPD data from the three bacterial spot strains (A=Xv_{28}, B=Xv_{22} and C=Xv_{10}).

Table (11): Analysis of RAPD-PCR products of primer A9, B10 and A3 for three bacterial spot strains (Xv_1, Xv_2 and Xv_3), where (1) means presence and (0) means absence of band.

MW_{bp}	RAPD Primers								
Band size	A9			B10			A3		
	Xanthomonas vesicatoria								
	Xv_{28}	Xv_{22}	Xv_{10}	Xv_{28}	Xv_{22}	Xv_{10}	Xv_{28}	Xv_{22}	Xv_{10}
1716	0	0	0	1	1	0	0	0	0
1578	1	1	1	0	1	1	0	0	0
1578	0	0	0	0	1	1	0	0	0
1513	0	0	0	1	1	0	0	0	0
1451	0	0	0	0	0	0	1	0	1
1392	0	1	0	0	0	0	0	0	0
1334	0	0	0	0	0	0	1	0	1
1280	0	0	0	1	0	1	0	0	0
1227	1	0	0	0	0	0	0	0	0
1177	0	0	0	0	1	1	0	0	0
1128	0	0	0	0	0	0	1	1	1
1082	0	1	1	0	0	0	0	0	0
1038	0	0	0	0	1	1	0	0	0
995	0	1	1	0	0	0	0	0	0
954	0	0	0	0	1	1	0	0	0
915	0	0	0	0	0	0	1	0	0
841	1	1	1	0	0	0	0	0	0
807	0	0	0	0	0	0	1	1	0
774	0	1	1	1	1	0	0	0	0
742	0	0	0	0	0	1	0	0	0
711	0	1	1	0	0	0	0	0	0
682	0	0	0	0	0	0	1	1	0
654	1	0	0	0	0	0	0	0	0
627	0	0	0	0	0	1	1	0	0
602	0	0	0	0	0	0	0	1	0
577	0	0	0	0	0	1	0	0	0
553	1	0	1	0	0	0	0	1	1
488	0	1	0	0	0	1	0	0	0
468	1	0	0	0	0	0	0	0	0
449	0	0	0	1	0	0	0	1	0
430	0	0	0	0	0	1	0	0	0
412	1	0	1	0	0	0	0	0	0
379	0	0	0	0	0	1	0	0	0
349	0	0	0	0	0	1	0	0	0
321	0	0	0	0	0	1	0	0	0
295	0	0	0	0	0	0	1	0	0
271	1	0	0	0	0	0	1	0	1
249	0	0	0	0	0	0	0	0	1
211	0	0	0	0	0	1	0	0	0
171	0	0	0	0	0	1	0	0	0
157	0	0	0	0	0	0	0	0	1
133	0	0	1	0	0	0	0	0	0
74	0	1	0	0	0	0	0	0	0

Table (12): Analysis of RAPD-PCR products of primer G2, G8 and G19 for three bacterial spot strains (Xv_1, Xv_2 and Xv_3), where (1) means presence and (0) means absence of band.

MW_{bp}	RAPD Primers								
Band size	G2			G8			G19		
	Xanthomonas vesicatoria								
	Xv_{28}	Xv_{22}	Xv_{10}	Xv_{28}	Xv_{22}	Xv_{10}	Xv_{28}	Xv_{22}	Xv_{10}
1754	0	0	0	1	1	0	0	0	0
1635	1	1	1	0	0	0	1	1	1
1525	0	0	0	1	1	1	0	0	0
1374	0	0	0	0	0	1	0	0	0
1237	0	0	0	0	1	0	0	0	0
1195	1	1	1	0	0	0	1	0	1
1114	0	0	0	1	0	0	0	1	1
1076	0	0	0	0	0	0	1	0	0
1004	0	0	0	0	0	1	0	0	0
969	1	1	1	0	0	0	0	0	0
936	0	0	0	0	0	0	0	1	1
841	0	0	0	0	0	1	0	0	0
786	1	0	1	0	0	0	0	0	0
638	0	1	1	0	0	0	0	0	0
500	1	0	1	0	0	0	0	0	0
449	0	0	1	0	0	0	0	0	0
420	0	0	1	0	0	0	0	0	0
321	1	0	1	0	0	0	0	0	0

V- Host range:

In this experiment, three isolates of *Xanthomonas vesicatoria* of those caused bacterial leaf spot disease on tomato, as Xv_{28} the Xv_{22} and Xv_{10} which represent Qaha, Rashid and El-Dokki , localities , were used to study their abilities to infect the different tested hosts. The three isolates were the highly pathogenic causing bacterial spot disease on tomato plants. Results in **Table (13)** indicated that, all the three tested isolates were highly pathogenic on tomato, pepper and never affected potato, cabbage, common bean, eggplant, lettuce, broad beans, strawberry and cantaloup. On the other hand, the three tested isolates showed moderately effect on datura plants. It seems that all isolates had a narrow host range and specific to tomato and pepper plants.

Table (13): Reaction of different plant hosts to infection with pathogenic bacteria isolates of *Xanthomonas vesicatoria.*

Hosts of plants	*Xanthomonas vesicatoria* isolates		
	Xv_{28}	Xv_{22}	Xv_{10}
Potato "leaves"	-	-	-
Cabbage	-	-	-
Common bean	-	-	-
Eggplant	-	-	-
Tomato	+++	+++	+++
Pepper	+++	+++	+++
Lettuce	-	-	-
Beans	-	-	-
Datura	+	+	+
Strawberry	-	-	-
Cantaloupe	-	-	-

+++: Highly pathogenic
+: Moderately pathogenic
--: Non pathogenic

VI-Isolation of bacteriophages:

In this trial, four phages of *Xanthomonas vesicatoria* were isolated from infected leaves of tomato, using enrichment technique. These samples were collected from Qalubia governorate. The phages were mostly isolated from infected leaves of tomatoes.

The isolated bacteriophages formed single plaques of different morphological characteristics. The formed plaques were circular with irregular margins or without determined margins.

Particle size and morphology of each phage isolate were examined by electron microscopy. Data in **Fig. (8)** indicate that, the isolated phages were of the head and tail types. Four phage types were detected and designated A, B, C, and D, their head diameters were found to be 57.83, 43.84, 63.43 and 79.25 nm, respectively. Phages A, B, C and D were isolated from tomato leaves.

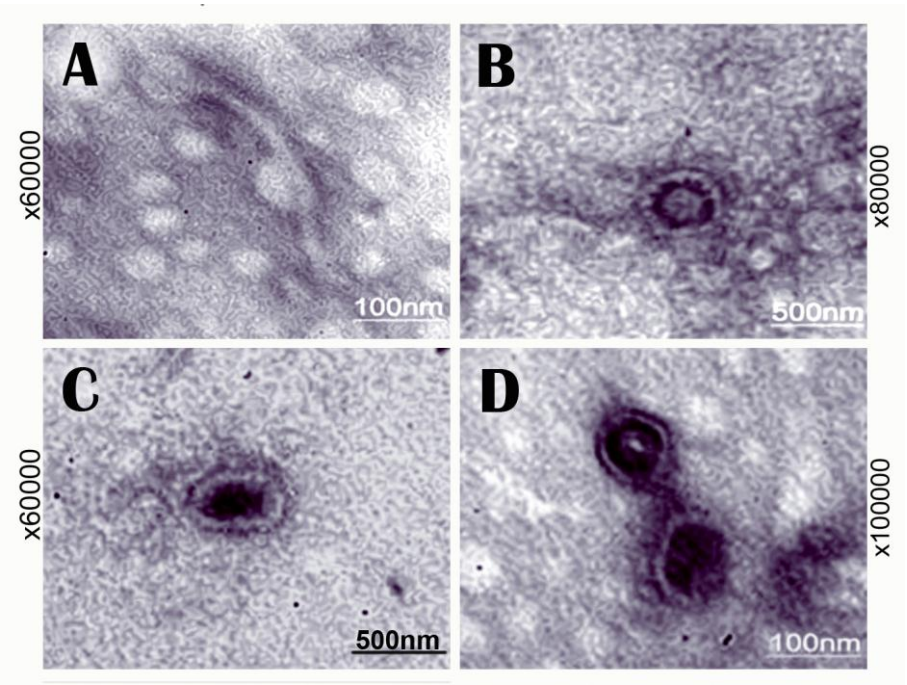


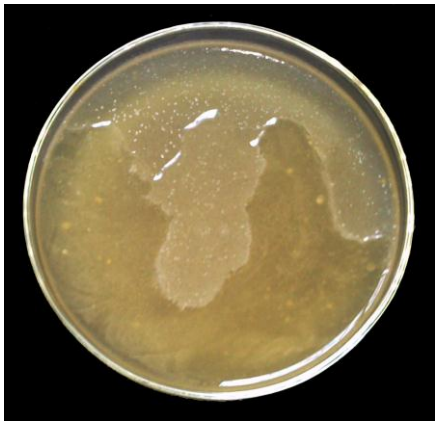
Figure (8): Electron micrographs of *X.vesicatoria* bacteriophages. Phages A and B were isolated from tomato leaves, phages C and D were isolated from tomato rhizosphere soils.

-Evaluation of bacterial sensitivity to bacteriophage:

Sensitivity of a bacterial strains to phages was determined based on the ability of the phage to produce plaques on the bacterial lawn, and the level of sensitivity was evaluated based on efficiency of plating (EOP) on the test strain in comparison with the propagating host strain of the phage as follows. A phage suspension of known concentration was plated simultaneously on the test and the host strains and EOP was calculated as the number of plaques on the test strain divided by the number of plaques on the host strain.

Isolates of bacteriophage were evaluated with three different genera of bacteria *Xanthomonas*, *Bacillus* and *Xanthomonas*. Bacteriophages isolates very characteristically showed a positive lysis on only almost all isolates of *X. vesicatoria* **(Fig., 9).**

Fig. (9): The qualitative sensitivity tests were done as positive lytic reaction by spotting the phage suspensions with a platinum loop on the surface of an agar layer inoculated with the *X. v.* in Petri dishes.



VII- Laboratory "*In vitro*" studies

Different bactericides, antibiotics, oils and plant extracts, resistance inducers, bio-agents were used at different concentrations to study their effects on the growth of tested pathogenic bacteria (*Xanthomonas vesicatoria*) as zone of inhibition (cm) under *in vitro* conditions.

1-Effect of bactericides

Two different concentrations of the tested bactericides (Galbin-Cu48% and Copper oxychloride 54% were tested for their effects on *Xanthomonas vesicatoria isolates*. Data in **Table** (14-a) show that all tested bactericides had an inhibitory effect on growth of the pathogenic bacteria compared with the control. Also, this inhibition was increased by increasing of the bactericide concentrations. On the other hand, different isolates of *X. vesicatoria (*Xv_{28}, Xv_{22} and Xv_{10}*)* varied in their sensitivity to these bactericides. In this respect, Copper oxychloride was more effective than Galbin-cu in reducing the growth of all tested isolates, where, they causing 2.97, 2.27 and 2.42 cm as means of inhibition zones for isolates Xv_{28}, Xv_{22} and Xv_{10}, respectively. Meanwhile, Galbin-Cu was less effective on reducing the growth of the three *X. vesicatoria* where it caused 1.65, 1.97 and 2.17 cm as zone inhibition of Xv_{28}, Xv_{22} and Xv_{10}, respectively.

2- Effect of antibiotics:

Two antibiotics *i.e.,* erythromycin and tetracycline were tested at with different concentrations for their effects on the growth *Xanthomonas vesicatoria* isolates. Data in **Table (14-a)** show that all tested antibiotics had great inhibitory effect on growth of the pathogenic bacteria compared with the control. This inhibition zone was increased by increasing the concentration of antibiotics. Also, different isolates of *Xanthomonas vesicatoria* varied in their tolerance to these antibiotics.

In this respect, tetracycline was more effective than erythromycin in inhibiting the three tested isolates of *Xanthomonas* where they recorded 2.12, 1.67 and 2.67 cm as means of inhibition zone of Xv_{28}, Xv_{22} and Xv_{10}, respectively. Meanwhile, erythromycin was less effective where it reduced the growth of the three isolates to 1.47, 1.72 and 2.22 cm as means of inhibition zone of Xv_{28}, Xv_{22} and Xv_{10}, respectively.

3- Effect of plant extract

Plant extract of garlic (*Allium sativum*) was tested for its effect on growth of *X. vesicatoria* isolates. Data in **Table** (**14-a**) revealed that, all tested concentrations of garlic extract had a good inhibitory effect on growth of the pathogenic bacteria compared with the control. Increasing the concentration increased gradually the inhibition zone. On the other hand, isolates of *Xanthomonas* were varied in their reaction to the tested garlic plant extract where it inhibited the isolate Xv_{22} to 2.12 cm of inhibition zone while it inhibited the isolate Xv_{10} to1.95 cm of inhibition zone whereas, isolate Xv_{28} was more sensitive where it recorded 1.62 cm of inhibition zone.

4- Effect of plant oils:

Different concentrations of two different plant oils *i.e.,* mentha oil (*Mentha aquatica*) and clove oil (*Syzygium aromaticum*) were tested for their inhibitory effect on growth *Xanthomonas vesicatoria*. Data in **Table**

(**14-b**) show that, the two different plant oils had clear inhibitory effect on growth of the tested bacteria compared with the control. In this respect, mentha oil gave 1.64 cm as mean of inhibition zone to be more effective than clove oil, which gave 1.13 cm as mean of inhibition zone for the three tested isolates of *Xanthomonas*. This inhibition effect was increased by increasing the concentration of the tested plant oil.

On the other hand, the three different isolates of *Xanthomonas vesicatoria* varied in their reactions to the different tested plant oils.

Table (14-a): Effect of some antibiotics, bactericides, garlic extract on the growth of the three tested ***Xanthomonas vesicatoria*** the causal agent of tomato bacterial spot ***in vitro***.

Chemical compounds	**Conc. (ppm)**	Inhibition zone (cm) of pathogenic bacterial isolates		
		***Xanthomonas vesicatoria*-$_{28}$**	***Xanthomonas vesicatoria*-$_{22}$**	***Xanthomonas vesicatoria*-$_{10}$**
Tetracycline	**50**	1.3	1.0	1.8
	100	1.9	1.5	2.2
	250	2.4	1.9	3.0
	500	2.9	2.3	3.7
	M	**2.12**	**1.67**	**2.67**
Erythromycin	**50**	0.8	1.0	1.3
	100	1.3	1.5	1.9
	250	1.7	2.0	2.5
	500	2.1	2.4	3.2
	M	**1.47**	**1.72**	**2.22**
Copper oxychloride	**100**	1.8	1.3	1.6
	250	2.5	1.9	2.0
	500	3.4	2.4	2.5
	750	4.2	3.5	3.6
	M	**2.97**	**2.27**	**2.42**
Glaben copper	**100**	0.5	1.1	0.9
	250	1.4	1.6	1.9
	500	2.0	2.2	2.7
	750	2.7	3.0	3.2
	M	**1.65**	**1.97**	**2.17**
Garlic extract *(Allium Sativum)*	**50**	0.6	1.2	1.1
	100	1.5	1.9	1.5
	250	2.0	2.4	2.1
	500	2.4	3.0	3.1
	M	**1.62**	**2.12**	**1.95**

In this respect, *Xanthomonas vesicatoria* -$_{10}$ was more sensitive to mentha oil as it gave 3.5cm as mean of recorded inhibition zone than *Xanthomonas vesicatoria*-$_{28}$ which recorded inhibition zone of 2.5 cm while *Xanthomonas vesicatoria*-$_{22}$ 2.3 cm as mean of recorded inhibition zone. On the other hand, *Xanthomonas* isolate-$_{10}$ was the more sensitive one to clove oil as it gave 3.1 cm as mean of recorded inhibition zone than *Xanthomonas* isolate-$_{22}$, which recorded inhibition zone of 2.2 cm, and *Xanthomonas* isolate-$_{28}$ that scored 1.1 cm as mean of recorded inhibition zone.

5- Resistance inducers:

Two organic acids *i.e.*, ascorbic and salicylic acid with different concentrations were tested on the growth of *Xanthomonas vesicatoria* isolates. **Table** (**14-b**) reveal that the two tested acids had clear inhibitory effect on growth of the pathogenic bacteria comparing to the control treatment. It is clear from the obtained results that increasing the concentration of tested organic acids increased gradually their inhibitory effect on tested *Xanthomonas vesicatoria* isolates. In this respect, ascorbic acid was the more effective, where it recorded 2.77 cm as means of inhibition zone, than salicylic acid, which recorded 1.38 cm of the inhibition zones of the three tested isolates of *Xanthomonas.* Ascorbic acid, was the highest effective in reducing the growth of all tested bacterial isolates, where, it scored 3.5 cm, 3.1 cm and 2.6 cm as means of the recorded inhibition zone for *Xanthomonas vesicatoria* -$_{10}$, v-$_{28}$ andv-$_{22}$, respectively. Meanwhile, 3.4 cm, 2.5 and 2.0 cm as means of recorded of inhibition zones were obtained with using salicylic acid on *Xanthomonas vesicatoria*-$_{10}$, *X. v*-$_{28}$ and *X. v*-$_{22}$, respectively.

6-Kombucha:

Kombucha was known as metabolic secretion of different synergistic microorganisms such as yeasts, fungi and bacteria. Also, it has a clear known inhibitory effect on different microorganisms like bacteria. Thus, it was used with different concentrations for testing their abilities to inhibit the growth of *X. vesicatoria* isolates, *in vitro*. Data in **Table** (**14-b**) indicate that, inhibition of the different isolates of *Xanthomonas* was increased with the increasing of kombucha concentrations. On the other hand, *Xanthomonas* iso-$_{10}$ and iso-$_{28}$ were the more sensitive to kombucha where they scored 2.1cm and 2.0 cm as means of the recorded inhibition zone comparing to *Xanthomonas* iso-$_{22}$ which scored only 1.8 cm of inhibition zone.

7-Bio-agents:

Two isolates of antagonistic bacteria were tested for their abilities to inhibit the growth of *X. vesicatoria* tested isolates, using King's B (KB) medium, *in vitro*. In this respect, *Pseudomonas fluorescens* (Pf1), isolate was the more effective against growth of *X.vesicatoria* bacteria on King's B medium, where the recorded inhibition zones were 2.9 cm Xv_{28}, 3.5cm of Xv_{22}, 4.1 cm of Xv_{10}, respectively. Meanwhile, isolates of *P. fluorescens* (Pf2) affected moderately the tested *X. vesicatoria isolates* on KB medium, where the scored inhibition zones were 3.1cm of Xv_{28}, 3.7cm of Xv_{22} and 4.5cm of Xv_{10}, respectively **Table (14-b)**.

Table (14-b): Effect of some plant oils, resistance inducers and Kombucha and bio-agents on the growth of the three tested *Xanthomonas vesicatoria* isolated *in vitro.*

Chemical compounds	Conc.	Inhibition zone (cm) of pathogenic bacterial isolates		
		X.vesicatoria$_{28}$	*X.vesicatoria*$_{22}$	*X.vesicatoria*$_{10}$
Mentha oil	2.5%	0.0	0.8	1.3
	5%	1.1	1.3	1.7
	10%	1.8	1.5	2.7
	20%	2.5	2.3	3.5
	M	**1.35**	**1.27**	**2.3**
Clove oil	2.5%	0.0	0.0	1.1
	5%	0.0	0.9	1.7
	10%	0.0	1.4	2.2
	20%	1.1	2.2	3.1
	M	**0.27**	**1.12**	**2.02**
Ascorbic acid	100 ppm	2.0	1.6	2.0
	250 ppm	2.6	2.2	2.8
	500 ppm	3.1	2.6	3.5
	750 ppm	3.7	3.1	4.1
	M	**2.85**	**2.37**	**3.1**
Salicylic acid	100 ppm	0.0	0.0	0.8
	250 ppm	1.1	0.0	1.7
	500 ppm	1.9	1.3	2.7
	750 ppm	2.5	2.0	3.4
	M	**1.37**	**0.82**	**1.95**
Kombucha	2.5%	0.0	0.0	0.8
	5%	0.8	0.0	1.2
	10%	1.3	1.1	1.6
	20%	2.0	1.8	2.1
	M	**1.02**	**0.75**	**1.22**
Pseudomonas fluorescens-1	$1x10^8$	**2.9**	**3.5**	**4.1**
Pseudomonas fluorescens-2	$1x10^8$	**3.1**	**3.7**	**4.5**

VIII- Greenhouse Experiments:

1-Evaluation of some tomato cultivars and genotypes infected with *Xanthomonas vesicatoria* tested isolates during 2009season.

Sixteen tomato cultivars and genotypes were evaluated to infection with the *Xanthomonas vesicatoria* isolate-$_{28}$ under greenhouse conditions. Data in **Table** (**15-a**) **and Fig(9)** reveal that, Super strain B and Castle rock cv. were highly susceptible to infection on Super strain B with

Xanthomonas vesicatoria isolate-$_{28}$. In this respect, the recorded disease incidence were 35.7, 64.3% at 3 and 10 days, hence the recorded disease severity were 23.3, 33.3% at the same period. Also, the recorded leaf spot on the same tested cv. were 6.1, 9.5 spots/ leaf at 3 and 10 days. As for reaction of cv. Castle rock, the recorded (DI) were 23.3 and 45.8% at 3 and 10 days. Meanwhile, the scored (DS) were 15.3 and 28.3% at the same period. On the other hand, the recorded leaf spots were 3.3 and 5.7 spots/leaf after 3 and 10 days.

Meanwhile, Peto 86 and Gs12 were low susceptible to infection with *X. vesicatoria*$_{28}$. Concerning Peto 86, the recorded (DI) were 16.3 and 33.3% at 3 and 10 days. Meanwhile, the recorded (DS) were 5.3, and 15.0 at the same period. Also, the recorded leaf spots were 1.8 and 2.3 spots/20 leaf after 3 and 10 days. As for cv. GS12, the recorded (DI) were 7.5 and 30.0 at 3 and 10 days. While, the recorded (DS) were 5.6 and 16.0 at the same period. Also, the recorded leaf spots were 3.1 and 5.1 no. of spots/ leaf at 3 and 10 days. As for cv. Money maker, the recorded DI were 5.0 and 29.0 at 3 and 10 days. While, the recorded (DS) were 5.3 and 16.3 at the same period. Whereas, the recorded leaf spots were 2.5 and 4.0 No. of spots/ leaf after 3 and 10 days. Regarding cv. Dora, the recorded (DI) were 2.5 and 18.3% at 3 and 10 days.

While, the recorded (DS) were 2.3 and 5.3% at the same period. Also, the recorded leaf spots were 1.5 and 3.2 No. spots/ leaf after 3 and 10 days. On the other hand, the next ten of tomato cv. and genotypes, *i.e.*, Diamante F1, Hybrid7796, Mors44, VT916G.SI, HMX4791, Super strain B H, KTM 141, Niagra, Flora-Dade and Faqlta 38, were completely resistant to infection with *Xanthomonas vesicatoria* -$_{28}$.

Table (15-a): Evaluation of some tomato cultivars and genotypes against infection with *Xanthomonas vesicatoria* isolate Xv_{28} after 3 and 10 days, during 2009 season.

Tomato cv. and genotypes	Disease incidence %		Disease severity		No. of spots/ leaf	
	3 days	10 days	3 days	10 days	3 days	10 days
Diamante F1	0.0	0.0	0.0	0.0	0.0	0.0
Hypride7796	0.0	0.0	0.0	0.0	0.0	0.0
Mors44	0.0	0.0	0.0	0.0	0.0	0.0
VT916G.SI	0.0	0.0	0.0	0.0	0.0	0.0
HMX4791	0.0	0.0	0.0	0.0	0.0	0.0
Dora	2.5	18.3	2.3	5.3	1.5	3.2
Super strain B H	0.0	0.0	0.0	0.0	0.0	0.0
Gs12	7.5	30.0	5.6	16.0	3.1	5.1
KTM 141	0.0	0.0	0.0	0.0	0.0	0.0
Niagra	0.0	0.0	0.0	0.0	0.0	0.0
Super strain B	35.7	64.3	23.3	33.3	6.1	9.5
Castle rock	23.3	45.8	15.3	28.3	3.3	5.1
Flora-Dade	0.0	11.3	0.0	4.0	0.0	2.5
Money maker	5.0	29.0	5.3	16.3	2.5	4.0
Peto 86	16.3	33.3	5.3	15.0	1.8	2.3
Faqlta 38	0.0	0.0	0.0	0.0	0.0	0.0

Gs12 | **Flora-Dade** | **Castle rock** | **Super Strain**

Fig (9): The differentiation between four tomato cultivars infected with *X. vesicatoria*-$_{28}$.

Data in **Table** (**15-b**) show that, Super strain B was highly susceptible to infection with *Xanthomonas vesicatoria* -$_{22}$. In this respect, the recorded disease incidence was 37.5 and 71.4% at 3 and 10 days. Meanwhile, the recorded disease severity was 26.6 and 50.0% at the same period. On the other hand, the recorded leaf spot on the same tested cv. were 4.3 and 7.5 no. of spots/ leaf at 3 and 10 days. As for reaction of cv. Castle rock, the recorded (DI)were 8.3 and 38.3% at 3 and 10 days. Meanwhile, the scored (DS) were 4.3 and 18.3% at the same period. On the other hand, the recorded leaf spots were 2.0 and 5.5 no. of spots/ leaf after 3 and 10 days. Meanwhile, cvs. Peto 86, Flora-Dade and Moneymaker were less susceptible to infection with *X.vesicatoria*-$_{22}$. Concerning, cv. Money maker the recorded (DI) were 5.0 and 24.2% at 3 and 10 days. Meanwhile, the recorded (DS) were 3.3, 6.2% at the same period. Also, the recorded leaf spots were 1.5and 2.8 No. of spots/ leaf after 3 and 10 days. As for cv. Dora, the recorded (DI) were 5.0 and 18.8% at 3 and 10 days. While, the recorded (DS) were 3.0 and 5.0 % at the same period. Also, the recorded leaf spots were 1.8 and 2.3no.of spots/ leaf at 3 and 10 days. As for cv. Faqulta38, the recorded (DI) were 2.5 and 13.3% at 3 and 10 days. While, the recorded (DS) 1.7 and 5.3% at the same period.

Whereas, the recorded leaf spots 1.2 and 2.3 no. of spots/ leaf after 3 and 10 days. Regarding cv. Gs12, the recorded (DI) were 2.5 and 12.5% at 3 and 10 days. While, the recorded (DS) were 3.3 and 5.3%at the same period. Also, the recorded leaf spots were 1.3 and 1.8no.of spots/ leaf after 3 and 10 days.

On the other hand, the next seven of tomato cv. and genotypes, *i.e.*, Diamante F1, Hybride7796, Mors44, VT916G.SI, HMX4791, Super

strain B H, KTM 141, were completely resistant to infection with *Xanthomonas vesicatoria*-$_{22}$.

Table (15-b): Evaluation of some tomato cultivars and genotypes against the infection with *Xanthomonas vesicatoria* isolate Xv_{22} at 3 and 10 days, during 2009 season.

Treatment	Disease incidence%		Disease severity%		No of spots/ leaf	
	3 days	10 days	3 days	10 days	3 days	10 days
Diamante F1	0.0	0.0	0.0	0.0	0.0	0.0
Hypride7796	0.0	0.0	0.0	0.0	0.0	0.0
Mors44	0.0	0.0	0.0	0.0	0.0	0.0
VT916G.SI	0.0	0.0	0.0	0.0	0.0	0.0
HMX4791	0.0	0.0	0.0	0.0	0.0	0.0
Gs12	2.5	12.5	3.3	5.3	1.3	1.8
Super strain B H	0.0	0.0	0.0	0.0	0.0	0.0
Dora	5.0	18.8	3.0	5.0	1.8	2.3
KTM 141	0.0	0.0	0.0	0.0	0.0	0.0
Niagra	2.5	15.0	3.3	4.3	13	2.1
Super strain B	37.5	71.4	26.6	50.0	4.3	7.5
Castle rock	8.3	38.3	4.3	18.3	2.0	5.5
Flora-Dade	6.3	28.8	3.7	8.3	1.6	3.5
Money maker	5.0	24.2	3.3	6.2	1.5	2.8
Peto 86	7.5	32.5	4.3	10.3	1.8	4.5
Faqlta 38	2.5	13.3	1.7	5.3	1.2	2.3

Data in **Table** (**15-c**) indicate that, Super strain B was highly susceptible to infection with *Xanthomonas vesicatoria* isolate-$_{10}$.

In this respect, the recorded Disease incidence were 34.3 and 56.4% at 3 and 10 days. Meanwhile, the recorded Disease severity were 15.3 and 30.0% at the same period. On the other hand, the recorded leaf spot on the same tested cv. were 2.1 and 5.3 no. of spots/ leaf at 3 and 10 days. As for reaction of cv. Castle rock, the recorded (DI) were 23.0 and 36.7% at 3 and 10 days. Meanwhile, the scored (DS) were 13.3 and 21.0% at the same period. On the other hand, the recorded leaf spots were 1.8 and 3.2 no. of spots/ leaf after 3 and 10 days. As for, Peto 86 cv. the

recorded (DI) were15.0 and 35.8% at the same period. Also, the scored (DS) were 5.3 and 20.0% after 3 and 10 days.

While, the recorded leaf spots were 1.7 and 2.9 no. of spots/ leaf at 3 and 10 days. On the other hand, cv. Flora-Dade, Moneymaker, Dora, Mors44 and GS12 were less susceptible to infection with *X. vesicatoria*-$_{10.}$ Concerning, cv. Money maker the recorded (DI) were 6.3 and 25.0% at 3 and 10 days. Meanwhile, the recorded (DS) were 2.3 and 6.3% at the same period. Also, the recorded leaf spots were 1.2 and 2.3 spots/20 leaf after 3 and 10 days. As for cv. Dora, the recorded (DI) were 2.5 and 16.6% at 3 and 10 days. While, the recorded (DS) were 1.3 and 15.0% at the same period. Also, the recorded leaf spots were 1.2 and 2.4 no. of spots/leaf at 3 and 10 days.

As for cv. Mors44, the recorded (DI) were 2.5 and 13.3% at 3 and 10 days. While, the recorded (DS) 1.5 and 5.3% at the same period. Whereas, the recorded leaf spots 0.8 and 2.3 no. of spots/leaf after 3 and 10 days. Regarding, cv. GS12, the recorded (DI) were 2.5 and 10.0 % at 3 and 10 days. While, the recorded (DS) 1.5 and 4.3% at the same period. Whereas, the recorded leaf spots 1.0 and 1.8 no. of spots/ leaf after 3 and 10 days.

On the other hand, the next eight of tomato cv. and genotypes, *i.e.*, Diamante F1, Hybrid7796, VT916G.SI, HMX4791, Super strain B H, KTM 141, Niagra and Faqlta 38 were completely resistant to infection with *Xanthomonas vesicatoria*-$_{10}$.

Table (15-c): Evaluation of some tomato cultivars and genotypes against the infection with *Xanthomonas vesicatoria* isolate Xv_{10} after 3 and 10 days, during 2009 season.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf	
	3 days	10 days	3 days	10 days	3 days	10 days
Diamante F1	0.0	0.0	0.0	0.0	0.0	0.0
Hypride7796	0.0	0.0	0.0	0.0	0.0	0.0
Mors44	2.5	13.3	1.5	5.3	0.8	2.3
VT916G.SI	0.0	0.0	0.0	0.0	0.0	0.0
HMX4791	0.0	0.0	0.0	0.0	0.0	0.0
Gs12	2.5	10.0	1.5	4.3	1.0	1.8
Super strain B H	0.0	0.0	0.0	0.0	0.0	0.0
Dora	2.5	16.6	1.3	15.0	1.2	2.4
KTM 141	0.0	0.0	0.0	0.0	0.0	0.0
Niagra	0.0	0.0	0.0	0.0	0.0	0.0
Super strain B	34.3	56.4	15.3	30.0	2.1	5.3
Castle rock	23.0	36.7	13.3	21.0	1.8	3.2
Flora-Dade	10.0	21.3	5.0	8.3	1.5	2.0
Money maker	6.3	25.0	2.3	6.3	1.2	2.3
Peto 86	15.0	35.8	5.3	20.0	1.7	2.9
Faqlta 38	0.0	0.0	0.0	0.0	0.0	0.0

IX- Control studies under *in vivo* conditions:

In all the *in vitro* studies, three isolates of *Xanthomonas vesicatoria* isolates *i.e*; Xv_{28} isolated from Qha, Xv_{22} isolated from Rashid and Xv_{10} isolated from El-Dokki (Giza) were used in this experiments. Different copper compounds, oils and plant extracts, resistance indcuers and bio-agents were used for controlling the bacterial spot disease of tomato cv. Super Strain B after 3 and 10 days of the infestation, during 2009 and 2010 seasons.

Also, disease incidence%, disease severity%, No. of spots/leaf and disease reduction% were recorded as follows:

1. Effect of some treatments on controlling bacterial spot disease of tomato plants at 3 and 10 days post infestation with *X. vesicatoria* (Xv_{28}) during 2009 season.

Data in **Table** (**16**) show that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling bacterial spot disease on tomato leaves. On the other hand, bacteriocides were the more effective in reducing the disease incidence , severity, No. of spots/ leaf and disease reduction% than other treatments, 10 days post infestation with $Xv\text{-}_{28}$.

a- Bactericides:

Concerning the used bactericides, data in **Table** (**16**) revealed that, copper oxychloride, Galbin-cu and tetracycline were the most effective in decreasing the diseases incidence, disease severity , No. of spots/ leaf and increasing the disease reduction% than erythromycin when sprayed on the tomato plants at 3 and 10 days post the infestation with Xv_{28}. Meanwhile, erythromycin was highly effective at 3 days post infestation with Xv_{28} where it gave 91.3% disease reduction.

Copper oxychloride gave 0.0 and 6.3% of disease incidence while it gave 0.0 and 5.7% of disease severity and 0.0 and 1.1 no. of spots/ leaf as well as it gave 100.0 and 85.8% disease reduction at 3 and 10 days post the infestation with Xv_{28}.The least effect was obtained with using erythromycin as bactericide to control bacterial spot on tomato plants where it resulted 5.0 and 21.0% of disease incidence, while it gave 2.0 and 17.0% of disease severity and it caused 1.0 and 4.3 no. of spots/ leaf as well as it reduced the disease reduction to 91.3 and 45.8% at 3 and 10 days post the infestation with Xv_{28}.

b- Plant oils and garlic extract:

Regarding the effect of sprayed oils and plant extracts in controlling leaf spot disease on tomato infested with Xv_{28} at 3 and 10 days, the obtained data in **Table (16)** indicate that, garlic (*Allium sativum*) extract was the more effective on the disease incidence% to be 1.0 and 11.6%, disease severity 1.0 and 9.0% and no. of spots/ leaf being 0.0 and 3.1 and increasing the disease reduction 97.0 and 71.0% than mentha and clove oils at 3 and 10 days post the infestation with Xv_{28} during 2009. Also, mentha and clove oils were effective in reducing disease incidence,7.5, 25.0% and 8.3,28.3%, disease severity and spots/ leaf 1.3,5.1 and 4.4,12.7 as well as increasing the disease reduction % to be 84.8 and 82.6, 37.5% and 82.6, 29.3% at 3 and 10 days post the infestation with Xv_{28} comparing to control treatment.

C- Resistance inducers:

Regarding the effect of the resistance inducers on tomato plants post infestation with *Xanthomonas vesicatoria*-$_{28}$, data in **Table** (**16**) revealed that, ascorbic and salicylic acids were the more effective than the bio-inducer (Kombucha). Data show also, that ascorbic acid was more effective than salicylic acid in all the determined disease parameters, expect only the disease reduction% at 3 and 10 days. In details, ascorbic acid resulted 2.0 and 20.0% disease incidence, 1.0 and 12.3% disease severity, as well as 2.1 and 4.1 no. of spots/ leaf and increased the disease reduction% to 97.0 and 50.0% at 3 and 10 days post infestation of tomato plants with Xv_{28}. While, the bio-inducer (Kombucha) was the least effective in this respect where it gave 18.3 and 30.0% disease incidence, it gave also 4.0 and 23.0% disease severity and the no. of spots/ leaf were 1.9 and 7.3 as well as it gave 66.1 and 25.0% of disease reduction% at 3 and 10 days post infestation of tomato plants with Xv_{28}.

d- Bio-agents:

As for the effect of the tested bio-agents on the bacteria leaf spot disease on tomato plants, data in **Table** (**16**) reveal that, *Ps. fluorescens*-1 was more effective than *Ps. fluorescens* -2, *Ps. fluorescens*-1 reduced the disease incidence to 9.0 and 32.5%, while, it reduced the disease severity to 3.0 and 26.3% and the spots/ leaf to 2.1 and 7.7 as well as it gave 87.0 and 34.3% disease reduction% at 3 and 10 days post the infestation the tomato plants with Xv_{28}. While, *Ps. fluorescens*-2 reduced the disease incidence to 12.5 and 38.3%, while, it reduced the disease severity to 4.0 and 28.3% and the spots/ leaf to 4.3 and 8.2 as well as it gave 82.6 and 29.3% disease reduction% at 3 and 10 days after the infestation of the tomato plants with Xv_{28}.

Generally, the obtained results indicated, that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling bacterial leaf spot disease on tomato plants comparing with the control treatment. On the other hand, bacteriocides was more effective in reducing the diseases incidence%, disease severity, No. of spots/leaf and increasing the disease reduction% than other treatments *i.e.,* oils and plant extracts, inducing resistances and bio-agents, especially at 3 and 10 days post the infestation with Xv_{28}. Also, copper oxychloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic extract was effective in reducing the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than mentha and clove oils.

Regarding the effect of the resistanc inducer on tomato plants post infestation with *Xanthomonas vesicatoria*-$_{28}$, ascorbic and salicylic acids were effective than bio-inducers (Kombucha) in their effects on diseases parameters.

On the other side, *Ps. fluorescens*-1 was more effective than *Ps. fluorescens -2* in its effect on the determined parameters at 3 and 10 days, post the infestation with Xv_{28}.

Table (16): Effect of some treatments on controlling bacterial leaf spot of tomato plants at 3 and 10 days post infestation with *X. vesicatoria* isolate (Xv_{28}) during 2009 season.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf		Disease reduction%	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Bactericides								
Copper oxychloride	0.0	6.3	0.0	5.7	0.0	1.1	100.0	85.8
Galbin-cu	0.0	7.5	0.0	7.3	0.0	1.2	100.0	81.3
Tetracycline	0.0	15	0.0	9.6	0.0	2.9	100.0	62.5
Erythromycin	5.0	21.7	2.0	17.0	1.0	4.3	91.3	45.8
M	**1.25**	**12.6**	**0.25**	**9.9**	**0.25**	**2.4**	**22.8**	**68.9**
LSD 5%	**2.89**		**2.67**		**2.72**		**12.27**	
Oils and plant extracts								
Mentha oil	7.5	25.0	3.5	18.0	1.3	5.1	84.8	37.5
Clove oil	8.3	28.3	4.0	21.0	4.4	12.7	82.6	29.3
Garlic extract	1.0	11.6	1.0	9.0	0.0	3.1	97.0	71.0
M	**0.25**	**21.6**	**2.8**	**16.0**	**1.9**	**7.0**	**88.1**	**50.0**
LSD 5%	**4.08**		**3.68**		**2.11**		**10.66**	
Inducing resistance								
Kombucha	18.3	30.0	4.0	23.0	1.9	7.3	66.1	25.0
Ascorbic acid	2.0	20.0	1.0	12.3	2.1	4.1	97.0	50.0
Salicylic acid	2.5	20.0	3.3	13.3	1.9	3.9	91.3	66.8
M	**7.6**	**23.3**	**2.8**	**16.2**	**2.0**	**5.1**	**84.9**	**47.3**
LSD 5%	**5.41**		**4.64**		**2.01**		**13.70**	
Bio-agents								
Ps. fluorescens -1	9.0	32.5	3.0	26.3	2.1	7.7	87.0	34.3
Ps. flurocences-2	12.5	38.3	4.0	28.3	4.3	8.2	82.6	29.3
M	**10.8**	**35.4**	**3.5**	**27.3**	**3.2**	**7.95**	**84.8**	**31.8**
LSD 5%	**7.92**		**5.66**		**4.63**		**5.29**	
Control Xv_{-28}	**33.0**	**75.7**	**23.0**	**40.0**	**6.6**	**46.3**	**0.0**	**0.0**
Control Healthy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

2- Effect of some treatments on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* (Xv_{28}) during 2010 season.

Data in **Table** (**17**) revealed that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling bacterial spot disease on tomato leaves comparing with the control treatment. On the other hand, bactericides were the more effective in reducing the diseases incidence, disease severity, No. of spots/leaf and increasing disease reduction% than other control treatments, oils and plant extracts, resistance inducers and bio-agents, especially at 3 and 10 days post the infestation with Xv_{28} during 2010 season.

a- Bactericides:

Data in **Table (17)** show that, copper oxychloride, Galbin-cu and tetracycline were most effective in decreasing the disease incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than the sprayed erythromycin after sprayed on the tomato plants at 3 and 10 days post infestation with Xv_{28}. Meanwhile, erythromycin was highly effective at 3 and 10 days post infestation with Xv_{28}. where it gave 94.0 and 61.3% of disease reduction% of. In addition, copper oxychloride gave 0.0 and 7.5% of disease incidence while it gave 0.0 and 4.3% of disease severity and 0.0 and 1.4 no. of spots/ leaf as well as it gave 100.0 and 89.5% disease reduction %,at 3 and 10 days post the infestation with Xv_{28}. Meanwhile, the least effect erythromycin as bactericide to control bacterial leaf spot on tomato plants where it resulted 5.0 and 23.0% of disease incidence, 1.0 and 16.0% of disease severity and 1.0 and 3.6 spot/leaf as well as it gave 94.0 and 61.3% disease reduction% at 3 and 10 days post infestation of tomato plants with Xv_{28}.

b- Plant oils and garlic extract:

Regarding the effect of sprayed oils and plant extract in controlling leaf spot disease on tomato plants infested with Xv-$_{28}$ at 3 and 10 days, the obtained data in **Table** (**17**) reveal that, garlic extract was the more effective in decreasing the disease incidence% to be 0.0 and 10.0%, disease severity to be 0.0 and 7.0% and the no. of spots/ leaf were 0.0 and 1.6 and it increased the disease reduction% to reach 100.0 and 83.1% than mentha and clove oils at 3 and 10 days post the infestation with Xv_{28} during 2010. Also, mentha and clove oils were effective in reducing disease incidence, disease severity and no. of spots/ leaf being and increasing the disease reduction% at 3 and 10 days post infestation with Xv_{28} comparing to control treatment.

c- Resistance inducers:

Regarding the effect of the resistance inducers on tomato plants at 3 and 10 days post the infestation with *X. vesicatoria*-$_{28}$, data in **Table** (**17**) reveal that, ascorbic and salicylic acids were the more effective than bio-inducer (Kombucha). Data show also, that ascorbic acid was more effective than salicylic acid in all the determined disease parameters, expect only the disease reduction% after 3 and 10 days. In details, ascorbic acid resulted 2.5 and 18.3 of disease incidence and 1.3; 11.3% of disease severity as well as it gave 2.0; 4.3 no. of spots/ leaf and increased the reduction% to 85.9 and 72.6% at 3 and 10 days post infestation with Xv_{28} comparing to control treatment. While, biological inducer (Kombucha) was the least effective in this respect where it gave 12.5and 31.7% disease incidence, 4.0 and 22.6% of disease severity, 2.0 and 4.3 spots/leaf of as well as it gave 76.0 and 45.3 of diseases reduction% at 3 and 10 days post the infestation of tomato plants with Xv_{28}.

d- Bio-agents:

As for, the effect of the tested bioagents in controlling the bacteria leaf spot disease on tomato plants, data in **Table** (**17**) revealed that, *Pseudomonas fluorescens*-1 was more effective than *Pseudomonas fluorescens*-2, *Pseudomonas fluorescens*-1 reduced the disease incidence to 11.8 and 33.3%, while, it reduced the disease severity to 6.0 and 25.0% and the no. of spots/ leaf to 3.7 and 4.9 as well as it gave 63.9 and 39.5% disease reduction % at 3 and 10 days post the infestation the tomato plants with Xv_{28}. While, *Ps. fluorescens*-2 reduced the disease incidence to 16.3 and 35.0%, while, it reduced the disease severity to 4.0 and 27.7% and the spots/leaf to 4.3 and 5.1 as well as it gave 76.0 and 32.9% disease reduction% at 3 and 10 days post the infestation the tomato plants with Xv_{28}.

Generally, the obtained results indicated, that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling the bacteria leaf spot disease on tomato plants comparing with the control treatment. On the other hand, bacteriocides was more effective in reducing the diseases incidence, disease severity, No. of spots/leaf and increasing the disease reduction% than other treatments *i.e.,* oils and plant extracts, resistance inducers and bio-agents, especially at 3 and 10 days post the infestation with Xv_{28}. Also, copper oxychloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic extract was effective in reducing the diseases incidence, disease severity, No. of spots/leaf and increasing the disease reduction% than mentha and clove oils. Regarding the effect of the resistanc inducer on tomato plants post the infestation with *Xanthomonas vesicatoria*-$_{28}$, ascorbic and salicylic

acids were effective than bio-inducer (Kombucha), in their effects on determined diseases parameters.

On the other side, *Pseudomonas fluorescens*-1 was more effective than *Pseudomonas fluorescens*-2. In its effect on the determined disease parameters at 3 and 10 days post the infestation with Xv_{28}.

Table (17): Effect of some treatments on controlling bacterial leaf spot of tomato plants at 3 and 10 days post infestation with *X. vesicatoria* isolate (Xv_{28}) during 2010 season.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf		Disease reduction%	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Bactericides								
Copper oxychloride	0.0	7.5	0.0	4.3	0.0	1.4	100.0	89.5
Galbin-cu	0.0	11.6	0.0	5.3	0.0	1.8	100.0	87.2
Tetracycline	1.0	13.3	1.7	9.3	1.2	2.4	97.0	77.5
Erythromycin	5.0	23.0	1.0	16.0	1.0	3.6	94.0	61.3
M	**1.5**	**13.9**	**0.4**	**8.7**	**0.6**	**2.3**	**47.8**	**78.9**
LSD 5%	**8.09**		**1.39**		**0.51**		**6.25**	
Oils and plant extracts								
Mentha oil	8.3	27.5	2.7	18.0	2.2	4.2	84.0	56.4
Clove oil	8.3	23.3	2.0	20.3	5.0	3.8	88.0	50.8
Garlic extract	0.0	10.0	0.0	7.0	0.0	1.6	100.0	83.1
M	**8.3**	**20.3**	**2.4**	**15.1**	**2.4**	**3.2**	**57.3**	**63.4**
LSD 5%	**2.28**		**3.25**		**0.83**		**9.16**	
Inducing resistance								
Kombucha	12.5	31.7	4.0	22.6	2.0	4.3	76.0	45.3
Ascorbic acid	2.5	18.3	1.3	11.3	2.0	4.3	85.9	72.6
Salicylic acid	3.0	23.3	5.3	13.3	2.3	4.1	68.1	67.8
M	**6.0**	**24.4**	**3.5**	**15.7**	**2.1**	**4.2**	**76.7**	**61.9**
LSD 5%	**3.90**		**3.09**		**1.33**		**15.62**	
Bio-agents								
Ps. fluorescens -1	11.8	33.3	6.0	25.0	3.7	4.9	63.9	39.5
Ps. fluorescens -2	16.3	35.0	4.0	27.7	4.3	5.1	76.0	32.9
M	**14.1**	**34.2**	**5.0**	**26.4**	**4.0**	**5.0**	**70.0**	**36.2**
Control Xv_{22}	**35.1**	**71.4**	**16.6**	**41.3**	**15.6**	**48.3**	**0.0**	**0.0**
LSD 5%	**6.91**		**5.24**			**4.41**	**6.39**	
Control Healthy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

3- Effect of some treatments on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* (Xv_{22}) during 2009 season.

Data in **Table (18)** show that all treatments, bactericides, oils and plant extracts, resistances inducer and bio-agents, were effective in controlling bacterial spot disease on leaves of tomato plants comparing with control treatment. On the other side, bacteriocides was more effective in reducing the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than other control treatments. Meanwhile, bio-agents were the least effect on the disease incidence especially at 3 and 10 days post the infestation with Xv_{22} during 2009 season.

a- Bactericides:

Data in **Table (18)** reveal that, copper oxychloride, Galbin-cu and tetracycline were the most effective in decreasing the disease incidence, disease severity, no. of spots/leaf and increasing the disease reduction % than erythromycin when sprayed on the tomato plants at 3 and 10 days post the infestation with Xv_{22}, Meanwhile, tetracycline was highly effective at 3 days from infestation with Xv_{22} where it gave 97.2% of disease reduction%. In this respect, copper oxychloride gave 0.0 and 8.3% of disease incidence, 0.0 and 4.0% disease severity and 0.0 and 1.0 spots/leaf as well as it gave 100.0 and 91.7% of the disease reduction%, respectively. Meanwhile, the least effect was obtained with erythromycin as bactericide to control bacterial spot on tomato plants and it resulted 2.5 and 22.9% the disease incidence, 1.0 and 16.3 % of disease severity%, and 1.5 and 3.9 spots/20 leaf as well as it gave 96.0 and 67.2 % of the diseases reduction % at 3 and 10 days post infestation tomato plants with Xv_{22}.

b- Plant oils and extract:

Regarding the effect of sprayed plant oils and garlic extract in controlling leaf spot disease on tomato plants infested with Xv-$_{22}$ at 3 and 10 days, the obtained data in **Table** (**18**) show that, garlic extract was the more effective on decreasing the disease incidence to be 0.0 and 16.6% , disease severity to be 0.0 and 7.0% and no. of spots/leaf being 0.0 and 2.5 and increasing the disease reduction % to reach 100.0 and 85.9% than mentha and clove oils at 3 and 10 days post the infestation with Xv_{22} during 2009.

In addition, mentha and clove oil were effective in reducing disease incidence and disease severity, No. of spots/leaf and increased the disease reduction% at 3 and 10 days post infestation with Xv-$_{22}$ comparing to control treatment.

c- Resistance inducers:

Regarding the effect of the resistance inducers on tomato plants at 3 and 10 days post the infestation with *X. vesicatoria*-$_{22}$, data in **Table** (**18**) revealed that, ascorbic and salicylic acids were the more effective than the bio-inducer (Kombucha). Data show also, that ascorbic acid was more effective than salicylic acid in all the determined disease parameters, expect only the disease reduction% after 3 and 10 days. In details, ascorbic acid resulted 1.0 and 20.0% disease incidence 1.0 and 11.0% of disease severity as well as it gave 1.2 and 2.8 spots/ leaf and increased the reduction% 96.0 and 77.9% at 3 and 10 days post infestation with Xv_{22} comparing to control treatment. While, bio-inducer (Kombucha) was the least effective in this respect where it gave 15.0 and 36.7% disease incidence, it gave also 4.0 and 24.3% of disease severity, 2.7 and 5.1 spots/ leaf of as well as it gave 84.0 and 51.1% of disease reduction at 3 and 10 days post the infestation of tomato plants with Xv_{22}.

d- Biological agents:

As for the effect of the tested bioagents in controlling the bacterial leaf spot disease on tomato plants, data in **Table** (**18**) show that, *Ps. fluorescens*-1 was more effective than *Ps. fluorescens*-2. In details, *Ps. fluorescens*-1 reduced the disease incidence to 7.5 and 35.8%, while, it reduced the disease severity% to 2.9 and 27.0% and the spots/20leaf to 2.9 and 5.3 as well as it gave 88.4 and 45.7% of disease reduction at 3 and 10 days post the infestation the tomato plants with Xv_{22}.

While, *Ps. fluorescens*-2 reduced the disease incidence to 11.3 and 33.0%, while, it reduced the disease severity to 4.4 and 28.3% and the no. of spots/ leaf to 2.0 and 4.7 as well as it gave 82.4 and 43.1% disease reduction % at 3 and 10 days post the infestation the tomato plants with Xv_{22}.

Abstractly, the obtained results indicate *i.e.,* that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling the bacterial leaf spot disease on tomato plants comparing with the control treatment. On the other hand, bactericides was more effective in reducing the diseases incidence , disease severity, no. of spots/ leaf and increasing the disease reduction % than other treatments *i.e.,* oils and plant extracts, resistance inducers and bio-agents, especially at 3 and 10 days post the infestation with Xv_{22}. In addition, copper chloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic extract was effective in reducing the diseases incidence, disease severity, no. of spots/ leaf and increasing the disease reduction% than mentha and clove oils. Regarding the effect of the resistanc inducer on tomato plants post the infestation with *Xanthomonas vesicatoria*-$_{22}$, ascorbic and salicylic acids were effective than bio-inducer (Kombucha).

In their effects on determined diseases parameters. On the other side, *Ps. fluorescens*-1 was more effective than *Ps. fluorescens-2*. In its effect on the determined disease parameters at 3 and 10 days, post the infestation with Xv_{22}.

Table (18): Effect of some treatments on controlling bacterial leaf spot of tomato plants at 3 and 10 days post infestation with *X. vesicatoria* isolate (Xv_{22}) during 2009 season.

Treatment	Disease index %		Disease Severity (%)		Mean of spots/20 leaf		Disease reduction %	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Bactericides								
Copper oxychloride	0.0	8.3	0.0	4.0	0.0	1.0	100.0	91.7
Galbin-cu	0.0	10.0	0.0	4.6	0.0	1.4	100.0	90.7
Tetracycline	1.0	16.3	0.7	8.7	1.0	2.1	97.2	82.3
Erythromycin	2.5	22.9	1.0	16.3	1.5	3.9	96.0	67.2
M	**0.9**	**14.4**	**0.4**	**8.4**	**0.6**	**2.1**	**48.3**	**83.0**
LSD 5%	**2.08**		**1.59**		**1.08**		**1.58**	
Oils and plant extracts								
Mentha oil	5.0	21.3	2.3	18.7	1.9	3.1	91.0	62.4
Clove oil	10.0	31.7	3.0	20.3	2.0	4.2	88.0	59.2
Allium sativum	0.0	16.6	0.0	7.0	0.0	2.5	100.0	85.9
M	**3.8**	**23.2**	**1.8**	**15.3**	**1.8**	**3.3**	**60.0**	**69.2**
LSD 5%	**2.40**		**2.28**		**1.17**		**11.17**	
Resistance inducers								
Kamboush	15.0	36.7	4.0	24.3	2.7	5.1	84.0	51.1
Ascorbic acid	1.0	20.0	1.0	11.0	1.2	2.8	96.0	77.9
Salicylic acid	1.5	25.0	0.7	13.7	1.4	3.8	97.2	72.4
M	**5.8**	**27.3**	**1.9**	**16.3**	**1.9**	**3.9**	**92.4**	**67.1**
LSD 5%	**2.68**		**3.37**		**1.62**		**11.09**	
Bio-agents								
Ps. fluorescens-1	7.5	35.8	2.9	27.0	2.9	5.3	88.4	45.7
Ps. fluorescens -2	11.3	33.0	4.4	28.3	2.0	4.7	82.4	43.1
M	**9.4**	**34.4**	**3.7**	**27.7**	**2.5**	**5.0**	**85.4**	**44.4**
Control Xv_{-22}.	**30.0**	**82.2**	**25.0**	**49.7**	**9.5**	**43.2**	**0.0**	**0.0**
LSD 5%	**5.45**		**5.01**		**2.93**		**8.84**	
Control Healthy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

4- Effect of some treatments on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* (Xv_{22}) during 2010 season.

Data in **Table (19)** show that all treatments, copper compounds, oils and plant extracts, resistances inducer and bio-agents, were effective in controlling bacterial spot disease on leaves of tomato plants comparing with control treatment. On the other side, bacteriocides was more effective in the diseases incidence, disease severity, no. of spots/leaf and increasing disease reduction% than other control treatments. Meanwhile, bio-agents had the least effect on the disease incidence especially at 3 and 10 days post the infestation with Xv_{22} during 2010 season.

4.a- Bactericides:

Concerning the used bactericides **Table (19)** revealed that, copper oxychloride, Galbin-cu and erythromycin were the most effective in decreasing disease incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than tetracycline when sprayed on the tomato plants at 3 and 10 days post the infestation with Xv_{22}, Meanwhile, erythromycin was highly effective at 3 and 10 days from infestation with Xv_{22} where it gave 100.0% disease reduction%.

In this respect, copper oxychloride gave 0.0 and 10.0% of disease incidence, 0.0 and 4.7% disease severity and 0.0 and 2.1 spots/leaf as well as it gave 100.0 and 90.2 of the disease reduction%, respectively. Meanwhile, the least effect was obtained with tetracycline as bactericide to control bacterial spot on tomato plants and it resulted 2.0 and 18.8% of the disease incidence, 2.0 and 8.0% of disease severity, and 1.0 and 2.9 spots/ leaf as well as it gave 88.0 and 69.2 of the diseases reduction% at 3 and 10 days post infestation of tomato plants with Xv_{22}.

4. b- plant oils and garlic extract:

Regarding the effect of sprayed oils and garlic extract in controlling leaf spot disease on tomato plants infested with Xv-$_{22}$ at 3 and 10 days, the obtained data in **Table** (**19**) show that, garlic (*Allium sativum*) extract was the more effective on decreasing the disease incidence to be 2.5 and 12.5%, disease severity to be 2.0 and 5.7% and no.of spots/ leaf to 1.3 and 2.3 and increased the disease reduction% to reach 92.0 and 88.1% than mentha and clove oils at 3 and 10 days post the infestation with Xv$_{22}$ during 2010. In addition, mentha and clove oils were effective in reducing disease incidence%, disease severity% and no.of spots/ leaf and increasing disease reduction % to reach 80.0 - 61.6%; 76.0- 55.2% disease reduction% at 3 and 10 days post infestation with Xv$_{22}$ comparing to control treatment.

4. c- Resistance inducers:

Regarding the effect of the resistance inducers on tomato plants at 3 and 10 days post the infestation with *X. vesicatoria*-$_{22}$, data in **Table** (**19**) reveal that, ascorbic and salicylic acids were the more effective than bio-inducer (Kombucha). Data show also, that ascorbic acid was more effective than salicylic acid in all the determined disease parameters, expect only the disease reduction% after 3 and 10 days. In details, ascorbic acid resulted in 8.3 and 18.8% of disease incidence and 3.0; 10.0% disease severity as well as it gave 1.9; 3.0 spots/ leaf and increased the reduction% 88.0 and 79.1% at 3 and 10 days post infestation with Xv$_{22}$ comparing to control treatment. While, bio-inducer (Kombucha) was the least effective in this respect where it gave 12.5 and 33.0% of disease incidence, it gave also 4.0 and 24.7% disease severity, 2.0 and 4.6 spots/leaf as well as it gave 84.0 and 48.2 of diseases reduction% at 3 and 10 days post the infestation of tomato plants with Xv$_{22}$.

4. d- Bio-agents:

As for, the effect of the tested bioagents in controlling the bacterial leaf spot disease on tomato plants, data in **Table** (**19**) show that, *Pseudomonas fluorescens*-1 was more effective than *Pseudomonas fluorescens*-2. In details, *Ps. fluorescens*-2 reduced the disease incidence to18.3 and 37.0%, while, it reduced the disease severity to 5.1 and 28.7% and the spots/ leaf to be 3.1 and 5.4 as well as it gave 79.6 and 39.8% disease reduction% at 3 and 10 days post the infestation the tomato plants with Xv_{22}. While, *Ps. fluorescens*-1 reduced the disease incidence to15.0 and 34.3%, while, it reduced the disease severity to 6.7 and 27.7% and the spots/leaf to 2.7 and 5.1 as well as it gave 73.2 and 41.9% disease reduction% at 3 and 10 days post the infestation the tomato plants with Xv_{22}.

Finally, the obtained results indicated, that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling the bacteria leaf spot disease on tomato plants comparing with the control treatment. On the other hand, bacteriocides was more effective in reducing the disease incidence, disease severity, No. of spots/leaf and increasing the disease reduction% than other treatments *i.e.*, oils and garlic extract, inducing resistance inducers and bio-agents, especially at 3 and 10 days post the infestation with Xv_{22}. In addition, copper oxychloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic extract was effective in reducing the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than mentha and clove oils. Regarding the effect of the resistanc inducer on tomato plants post infestation with *Xanthomonas vesicatoria*-$_{22}$, ascorbic and salicylic

acids were effective than bio-inducer (Kombucha) in their effects on determined parameters.

On the other side, *Pseudomonas fluorescens*-1 was more effective than *Pseudomonas fluorescens -2*. In its effect on the determined disease parameters at 3 and 10 days, post the infestation with Xv_{22}.

Table (19): Effect of some treatments on controlling bacterial leaf spot of tomato plants infested with *X. vesicatoria* isolate (Xv_{22}) during 2010 season.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf		Disease reduction%	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Bactericides								
Copper oxychloride	0.0	10.0	0.0	4.7	0.0	2.1	100.0	90.2
Galbin-cu	0.0	12.5	0.0	5.0	0.0	2.6	100.0	89.5
Tetracycline	2.0	18.8	2.0	8.0	1.0	2.9	88.0	83.2
Erythromycin	0.0	24.2	0.0	14.7	0.0	3.2	100.0	69.2
M	**0.5**	**16.4**	**0.5**	**8.1**	**0.25**	**2.7**	**22.0**	**83.0**
LSD 5%	**1.58**		**3.61**		**0.88**		**10.18**	
Oils and plant extracts								
Mentha oil	5.0	22.9	5.3	18.3	2.1	3.1	80.0	61.6
Clove oil	7.5	23.3	6.0	21.3	2.6	3.4	76.0	55.2
Garlic extract	2.5	12.5	2.0	5.7	1.3	2.3	92.0	88.1
M	**5.0**	**19.6**	**4.4**	**15.1**	**2.0**	**2.9**	**82.7**	**68.3**
LSD 5%	**3.84**		**2.47**		**1.30**		**9.35**	
Inducing resistance								
Kombucha	12.5	33.0	4.0	24.7	2.0	4.6	84.0	48.2
Ascorbic acid	8.3	18.8	3.0	10.0	1.9	3.0	88.0	79.1
Salicylic acid	10.0	20.0	3.3	13.3	2.0	3.1	87.0	72.1
M	**10.27**	**23.9**	**3.4**	**16.0**	**1.96**	**10.7**	**86.3**	**66.46**
LSD 5%	**4.95**		**2.94**		**1.14**		**17.49**	
Bio-agents								
Ps. fluorescens -1	15.0	34.3	6.7	27.7	2.7	5.1	73.2	41.9
Ps. fluorescens -2	18.3	37.0	5.1	28.7	3.1	5.4	79.6	39.8
M	**16.7**	**35.7**	**5.9**	**28.2**	**2.9**	**5.3**	**41.4**	**40.9**
LSD 5%	**6.28**		**3.53**		**4.26**		**5.43**	
Control Xv_{22}	**45.3**	**82.2**	**25.0**	**47.7**	**20.0**	**39.8**	**0.0**	**0.0**
Control Healthy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

5- Effect of some treatments on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* (Xv_{10}) during 2009 season.

Data in **Table (20)** show that all treatments, bactericides, plant oils and garlic extracts, resistance inducers and bio-agents, were effective in controlling bacterial spot disease on leaves of tomato plants comparing with control treatment. On the other side, bactericides was more effective in reducing the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than other control treatments. Meanwhile, bio-agents were the least effective in decreasing the disease incidence especially at 3 and 10 days post the infestation with Xv_{10} during 2009 season.

5.a- Bactericides:

Concerning the used bactericides **Table (20)** show that, copper oxychloride, Galbin-cu and tetracycline were the most effective in decreasing the disease incidence, disease severity, no. of spots/leaf leaf and increasing disease reduction% than erythromycin when sprayed on the tomato plants at 3 and 10 days post the infestation with Xv_{10}, Meanwhile, erythromycin was highly effective at 3 and 10 days from infestation with Xv_{10} where it gave 93.0% of disease reduction%.

In this respect, copper oxychloride gave 0.0 and 11.7% of disease incidence, 0.0 and 2.7% disease severity and 0.0 and 1.8 spots/leaf as well as it gave 100.0 and 92.8% of the disease reduction%, respectively. Meanwhile, the least effect was obtained with erythromycin as bactericide to control bacterial spot on tomato plants where it resulted 2.5 and 20.0% of disease incidence, 1.0 and 15.3% disease severity, and 1.3 and 2.7 spots/ leaf as well as it gave 93.0 and 59.4 of the disease reduction% at 3 and 10 days post infestation of tomato plants with Xv_{10}.

5.b- Plant oils and garlic extract:

Regarding the effect of sprayed oils and plant extracts in controlling leaf spot disease on tomato plants infested with Xv_{10} at 3 and 10 days, the obtained data in **Table** (**20**) show that, garlic extract was the more effective in decreasing the disease incidence to 0.0 and 8.8%, disease severity to 0.0 and 5.0% and no. spots/ leaf being 0.0 and 1.4 and increasing the disease reduction% to reach 100.0 and 86.7% than mentha and clove oils at 3 and 10 days post the infestation with Xv_{10} during 2009. In addition, mentha and clove oils were also effective in reducing disease incidence, disease severity, no.of spots/leaf as well as increasing the disease reduction% at 3 and 10 days post infestation with Xv_{10} comparing to control treatment.

5.c- Resistance inducers:

Regarding the effect of the resistance inducers on tomato plants at 3 and 10 days post the infestation with *X. vesicatoria*-**10**, data in **Table** (**20**) revealed that, ascorbic and salicylic acids were the more effective than bio-inducer (Kombucha). Data show also, that ascorbic acid was more effective than salicylic acid in all the determined disease parameters, expect they increased the disease reduction% at 3 and 10 days. In details, ascorbic acid resulted 2.5 and 10.0% of disease incidence and 5.0, 11.0% of disease severity as well as it gave 0.9,1.6 spots/ leaf and increased reduction% to 70.8 and 66.7% at 3 and 10 days post infestation with comparing to control treatment.

While, bio-inducer (Kombucha) was the least effective in this respect where it gave 11.6 and 28.3% of disease incidence, it gave also 3.0 and 20.7% of disease severity, 1.9 and 3.4 no. of spots/leaf of as well as it gave 82.0 and 45.1 of diseases reduction% at 3 and 10 days post the infestation of tomato plants with Xv_{10}.

5.d- Bio- agents:

As for, the effect of the tested bioagents in controlling the bacterial leaf spot disease on tomato plants, data in **Table** (**20**) show that, *Ps. fluorescens*-1 was more effective than *Ps. fluorescens*-2. In details, *Ps. fluorescens*-1 reduced the disease incidence to 7.5 and 22.9%, while, it reduced the disease severity to 3.0 and 22.7% and the spots/leaf to 1.7 and 3.1 as well as it gave 80.0 and 39.8% disease reduction % at 3 and 10 days post the infestation of tomato plants with Xv_{10}. While, *Ps. fluorescens*-2 reduced the disease incidence to10.0 and 29.0%, while, it reduced the disease severity to 5.0 and 24.3% and the spots/leaf to 1.9 and 3.6 as well as it gave 66.7 and 35.5% disease reduction% at 3 and 10 days post the infestation of tomato plants with Xv_{10}.

Finally, the obtained results indicated, that all treatments, bactericides, oils and plant extracts, resistance inducers and bio-agents, were effective in controlling the bacteria leaf spot disease on tomato plants comparing with the control treatment. On the other hand, bacteriocides was more effective in reducing the diseases incidence, disease severity, no. of spots/ leaf and increasing the disease reduction% than other treatments *i.e.*, oils and plant extracts, inducing resistances and bio-agents, especially at 3 and 10 days post the infestation with Xv_{10}. In addition, copper oxychloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic extract was effective in reducing the diseases incidence, disease severity, no. of spots/ leaf and increasing the disease reduction% than mentha and clove oil. Regarding the effect of the resistanc inducer on tomato plants post the infestation with *Xanthomonas vesicatoria*-$_{10}$, ascorbic and salicylic acids were effective than bio-inducer (Kombucha). In their effects on determined diseases parameters.

On the other side, *Ps. fluorescens*-1was more effective than *Ps. fluorescens*-2 in its effect on the determined disease parameters at 3 and 10 days, post the infestation with Xv_{10}.

Table (20): Effect of some treatments on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* isolate (Xv_{10}) during 2009 season.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf		Disease reduction%	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Bactericides								
Copper oxychloride	0.0	11.7	0.0	2.7	0.0	1.8	100.0	92.8
Galbin-cu	0.0	5.0	0.0	3.0	1.0	1.0	100.0	92.0
Tetracycline	0.0	8.3	0.0	8.3	1.2	1.2	100.0	77.9
Erythromycin	2.5	20.0	1.0	15.3	1.3	2.7	93.0	59.4
M	**0.6**	**11.3**	**0.25**	**7.3**	**0.6**	**1.7**	**23.3**	**80.5**
LSD 5%	**1.40**		**1.13**		**0.40**		**13.70**	
Oils and plant extracts								
Mentha oil	3.5	13.8	2.0	17.3	1.2	2.1	86.7	54.1
Clove oil	5.0	16.3	2.1	18.7	2.0	2.4	86.7	50.2
Garlic extract	0.0	8.8	0.0	5.0	0.0	1.4	100.0	86.7
M	**2.8**	**13.0**	**1.4**	**13.7**	**1.1**	**2.0**	**57.8**	**63.7**
LSD 5%	**1.93**		**1.72**		**0.58**		**12.51**	
Inducing resistance								
Kombucha	11.6	28.3	3.0	20.7	1.9	3.4	82.0	45.1
Ascorbic acid	2.5	10	5.0	11.0	0.9	1.6	70.8	66.7
Salicylic acid	4.0	18.8	2.4	13.0	1.1	2.3	84.7	65.5
M	**6.0**	**19.0**	**3.5**	**14.9**	**1.3**	**2.4**	**77.8**	**60.5**
LSD 5%	**3.50**		**1.62**		**0.42**		**11.21**	
Bio-agents								
Ps. fluorescens -1	7.5	22.9	3.0	22.7	1.7	3.1	80.0	39.8
Ps. fluorescens -2	10.0	29.0	5.0	24.3	1.9	3.6	66.7	35.5
M	**8.8**	**26.0**	**4.0**	**23.5**	**1.8**	**3.4**	**73.4**	**37.7**
LSD 5%	**5.75**		**3.38**		**3.46**		**6.67**	
Control Xv_{10}	**34.5**	**65.7**	**15.0**	**37.7**	**7.5**	**29.3**	**0.0**	**0.0**
Control Healthy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

6- Effect of some treatments on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* (Xv_{10}) during 2010 season.

Data in **Table (21)** show that, copper compaunds, plant oil and garlic extract, resistance inducers and bio-agents, were effective in controlling bacterial spot disease on leaves of tomato plants comparing with control treatment. On the other side, bacteriocides was more effective in the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than other control treatments. Meanwhile, bio-agents were the least effect on the disease incidence especially at 3 and 10 days post the infestation with Xv_{10} during 2010 season.

6.a- Bactericides:

Concerning the used bactericides data in **Table (21)** show that, copper oxychloride, Galbin-cu and tetracycline were the most effective decreasing the disease incidence, disease severity, no. of spots/20 leaf and increasing the disease reduction% than erythromycin when sprayed on the tomato plants at 3 and 10 days post the infestation with Xv_{10}, Meanwhile, erythromycin was highly effective at 3 and 10 days post infestation with Xv_{10} where it gave 63.5% of disease reduction% after 3 days.

In this respect, copper oxychloride gave 0.0 and 8.8% of disease incidence, 0.0 and 3.0% disease severity and 0.0 and 1.5 spots/leaf as well as it gave 100and 91.8% disease reduction%, respectively. Meanwhile, the least effect was obtained with using erythromycin as bactericide to control bacterial spot on tomato plants where it resulted 6.3 and 18.8 the disease incidence, 5.0 and 15.0% disease severity, and 0.5 and 2.2 spots/leaf as well as it gave 63.5 and 59.1 disease reduction% at 3 and 10 days post infestation tomato plants with Xv_{10}.

6.b- Plant oils and garlic extract:

Regarding the effect of sprayed oils and plant extracts in controlling leaf spot disease on tomato plants infested with **Xv_{10}** at 3 and 10 days, the obtained data in **Table** (**21**) show that, garlic extract was the more effective in decreasing the disease incidence to be 0.0 and 7.5%, disease severity to be 0.0 and 5.3% and no of spots/leaf being 0.0 and 1.5 and increasing the disease reduction% to reach 100.0 and 85.6% than mentha and clove oils at 3 and 10 days post the infestation with **Xv_{10}** during 2010. In addition, mentha and clove oils were effective in reducing disease incidence, disease severity and mean spots/leaf and increasing the disease reduction% to at 3 and 10 days post infestation with **Xv_{10}** comparing to control treatment.

6.c- Resistance inducers:

Regarding the effect of the resistance inducers on tomato plants at 3 and 10 days post the infestation with *X. vesicatoria*-**$_{10}$**, data in **Table** (**21**) revealed that, ascorbic and salicylic acids were the more effective than bio-inducer (Kombucha). Data show also, that ascorbic acid was more effective than salicylic acid in determined disease parameters, expect the increased the disease reduction% at 3 and 10 days. In details, ascorbic acid resulted 0.0 and 8.8% disease incidence and 0.0, 9.3% of disease severity as well as it gave 0.0, 1.6 spots/leaf and increased disease reduction% to 100.0 and 85.6% at 3 and 10 days post infestation with Xv_{10} comparing to control treatment. While, the bio- inducer (Kombucha) was the least effective in this respect where it gave 11.3 and 25.0% disease incidence, it gave also 7.3 and 20.7% disease severity, 1.9 and 3.2 spots/leaf of as well as it gave 46.7 and 43.6 of disease reduction% at 3 and 10 days post the infestation of tomato plants with Xv_{10}.

6.d- Bio-agents:

As for the effect of the tested bioagents in controlling the bacterial leaf spot disease on tomato plants, data in **Table** (**21**) show that, *Pseudomonas fluorescens*-1 was more effective than *Ps. fluorescens*-2. In details, *Ps. fluorescens*-1 reduced the disease incidence to 7.5 and 21.5%, while, it reduced the disease severity to 3.5 and 23.0% and the spots/leaf to 1.6 and 3.1 as well as it gave 74.5 and 37.3% of disease reduction % at 3 and 10 days post the infestation the tomato plants with Xv_{10}. While, *Ps. fluorescens*-2 reduced the disease incidence to 9.3 and 27.5%, while, it reduced the disease severity to 8.0 and 25.3% and the spots/leaf to 1.9 and 3.8 as well as it gave 41.6 and 31.1% of disease reduction% at 3 and 10 days post infestation tomato plants with Xv_{10}.

Finally, the obtained results indicate that all treatments *i.e.*, bactericides, plant oils and garlic extract, resistance inducers and bio-agents, were effective in controlling the bacteria leaf spot disease on tomato plants comparing with the control treatment. On the other hand, bacteriocides was more effective in reducing the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than other treatments *i.e.*, plant oils and garlic extracts, resistance inducers and bio-agents, especially at 3 and 10 days post the infestation with Xv_{10}. In addition, copper oxychloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic extract was effective in reducing the disease incidence, disease severity, no. of spots/leaf and increasing the disease reduction% than mentha and clove oils. Regarding the effect of the resistanc inducer on tomato plants post the infestation with *Xanthomonas vesicatoria*-$_{10}$, ascorbic and salicylic acids were effective than bio-inducer (Kombucha) in their effects on determined diseases parameters.

On the other side, *Pseudomonas fluorescens*-1 was more effective than *Pseudomonas flurocences*-2. In its effect on the determined disease parameters at 3 and 10 days, post the infestation with Xv_{10}.

Table (21): Effect of some treatments on controlling bacterial leaf spot of tomato plants infested with *X. vesicatoria* isolate (Xv_{10}) during 2010 season.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf		Disease reduction%	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Bactericides								
Copper oxychloride	0.0	8.8	0.0	3.0	0.0	1.5	100.0	91.8
Galbin-cu	0.0	5.0	0.0	3.3	0.0	1.0	100.0	91.0
Tetracycline	2.0	7.5	1.0	7.3	0.7	1.2	92.7	80.1
Erythromycin	6.3	18.8	5.0	15.0	0.5	2.2	63.5	59.1
M	**2.1**	**7.8**	**1.5**	**7.2**	**0.3**	**1.5**	**78.1**	**80.5**
LSD 5%	**3.03**		**1.20**		**0.56**		**9.79**	
Oils and plant extracts								
Mentha oil	5.0	16.6	4.0	16.7	1.5	2.7	71.0	54.5
Clove oil	8.3	18.3	4.5	19.0	1.3	2.4	67.2	48.2
Garlic extract	0.0	7.5	0.0	5.3	0.0	1.5	100.0	85.6
M	**4.4**	**8.5**	**2.8**	**13.7**	**0.9**	**2.2**	**46.0**	**62.8**
LSD 5%	2.98		2.17		0.60		5.44	
Inducing resistance								
Kombucha	11.3	25.0	7.3	20.7	1.9	3.2	46.7	43.6
Ascorbic acid	0.0	8.8	0.0	9.3	0.0	1.4	100.0	74.7
Salicylic acid	7.5	16.3	5.0	12.0	1.0	2.3	63.5	67.3
M	**6.3**	**16.3**	**4.1**	**14.0**	**1.0**	**2.3**	**36.7**	**61.9**
LSD 5%	3.10		3.48		0.45		5.48	
Bio-agents								
Ps. fluorescens-1	7.5	21.5	3.5	23.0	1.6	3.1	74.5	37.3
Ps. fluorescens-2	9.3	27.5	8.0	25.3	1.9	3.8	41.6	31.1
M	**8.4**	**24.5**	**5.8**	**24.2**	**1.8**	**3.5**	**58.1**	**34.2**
LSD 5%	7.06		3.22		1.60		6.64	
Control Xv_{10}	**38.5**	**65.7**	**13.7**	**36.7**	**6.8**	**33.5**	**0.0**	**0.0**
Control Healthy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

IIX. Effect of different bacteriophage isolates on controlling bacterial leaf spot disease of tomato plants infested with *X. vesicatoria* isolates.

Table (22) indicated that all tested bacteriophages *i.e.*, phage 1, phage 2, phage 3, phage 4 and the mixture of different 4 phage isolates were effective in controlling bacterial leaf spot on tomato plants comparing with the control treatment. Also, these phages were effective in decreasing the disease incidence, disease severity%, mean of spots/20 leaf and increasing the disease reduction% comparing with the control treatment at 3 and 10 days post the infestation with *Xanthomonas vesicatoria* isolates (Xv_{28}, Xv_{22}, Xv_{10}).

Concerning of *Xanthomonas vesicatoria*-$_{28}$, data in **Table (22)** revealed that, phage 3 and mixture of the four phages were the more effective in reducing the diseases incidence, disease severity, no. of spots/leaf and increasing the disease reduction % than phage 4, 2 and 1 during spraying of these phages on tomato plants at 3 and 10 days post the infestation with Xv_{28}. Mixed of the four phages gave equal effect of that obtained where these mixed phages disease free plants, 3 and 10 days post infestation with *Xanthomonas vesicatoria*-$_{28}$ with no recording of any bacterial spots on tomato plants. Meanwhile, the least recorded effect was recorded with using phage 4 to control bacterial leaf spot on tomato plants Xv_{28} where it gave 0.0; 10% of diseases incidence and 0.0; 5.0% of disease severity while, the determined no. of spots/leaf 0.0; 1.3 whereas, the recorded disease reduction% were 100.0; 85.7% at 3 and 10 days post infestation of tomato plants with Xv_{28}.

On the other hand, the control treatment (without phages) recorded high value of diseases incidence to be 35.5, 70.0% while, the determined disease severity were 10.0, 21.7 and no. of spots/leaf being 10.4, 22.8 whereas, the determined disease reduction% were 0.0% at 3 and 10 days post infestation of tomato plants with Xv_{28}.

According to the effect of sprayed different phages on tomato plants at 3 and 10 days post the infestation with Xv_{22}. Data in **Table** (**21**) indicate that, all phage treatments whether in single or mixed form were effective in controlling the bacterial leaf spot disease of tomato plants when compared with control treatment. In this respect, all phage treatments reduced greatly the diseases incidence, disease severity, no. of spots/leaf and increased greatly disease reduction% at 3 and 10 days post infestation of tomato plants with Xv_{22}.

As well as, the effect of phage1, phage 2, phage3 and, phage 4 and mixed phage on tomato plants after post infestation with *Xanthomonas vesicatoria*-$_{10}$ was studied. Data in **Table** (**21**) revealed that, mixed the four phages and phage 4 were more effective than other phage treatments on all disease parameters. In this respect, all used phages either single or in mixed form were more effective on decreasing the disease parameters comparing to control treatment at 3 and 10 days post the infestation with Xv_{10}.

Table (21): Effect of different phage isolates in controlling the bacterial leaf spot disease on tomato plants infested with *X.vesicatoria* isolates during season 2010.

Treatment	Disease incidence%		Disease severity%		No. of spots/ leaf		Disease reduction%	
	3 days	10 days	3 days	10 days	3 days	10 days	3 days	10 days
Xv_{28}								
Phage(1)	0.0	5.0	0.0	1.5	0.0	0.5	100.0	92.3
Phage(2)	0.0	7.5	0.0	2.3	0.0	0.7	100.0	89.3
Phage(3)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Phage(4)	0.0	10.0	0.0	5.0	0.0	1.3	100.0	85.7
Phage(1+2+3+4)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Control Xv_{28}	35.5	70.0	10.0	21.7	10.4	22.8	0.0	0.0
M	**3.3**	**8.5**	**0.9**	**2.8**	**1.0**	**2.3**	**27.3**	**24.3**
Xv_{22}								
Phage(1)	0.0	6.3	0.0	3.0	0.0	1.1	100.0	92.2
Phage(2)	0.0	10.0	0.0	5.3	0.0	1.8	100.0	87.5
Phage(3)	0.0	8.3	0.0	4.3	0.0	2.5	100.0	89.9
Phage(4)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Phage(1+2+3+4)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Control Xv_{22}	31.7	80.0	15.0	23.3	13.5	21.5	0.0	0.0
M	**2.9**	**9.5**	**1.4**	**3.3**	**1.3**	**2.5**	**27.3**	**24.5**
Xv_{10}								
Phage(1)	0.0	12.5	0.0	4.3	0.0	2.5	100.0	80.6
Phage(2)	0.0	11.3	0.0	4.0	0.0	2.0	100.0	82.4
Phage(3)	0.0	15.0	0.0	5.3	0.0	3.1	100.0	76.8
Phage(4)	0.0	5.0	0.0	2.0	0.0	0.5	100.0	92.2
Phage(1+2+3+4)	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0
Control Xv_{10}	33.0	64.3	16.3	26.5	9.4	15.5	0.0	0.0
M	3.0	9.8	1.5	3.8	0.9	21.5	36.4	30.2

IIIX- Effect of treating tomato seeds and seedlings with hot water under infestation with the different isolates of *Xanthomonas vesicatoria*.

In this trial, tomato seeds and seedlings of cv. Super strain B were infested with three concentrations of the tested pathogenic bacteria and then treated with hot water (48-50°C/5 min) indirectly to study the effect of hot water treatment on the disease incidence.

Results in **Table** (**22**) revealed that all hot water treatments were effective in increasing the survived plants compared with the control treatment. In addition, increasing the inoculum levels increased gradually the dead of tomato plants whether of seeds and seedlings where, the survived plants in case of the infested seeds with Xv_{28} were 24.0, 80.0, and 94.0% respectively. While, they were 90.0, 92.0, 94.0% in case of the tested seedlings with 1 x 10^{8}, 0.5 x 10^{8} and 0.3 x 10^{8} cfu/ml of the pathogens when treated with hot water. Meanwhile, control treatment (infestation only and untreated with hot water) gave 0.0 and 18.0% of survived tomato plants whether of seeds and seedlings.

Meanwhile, isolate *Xanthomonas* Xv_{22} resulted in 76.0, 80.0, 100.0% and 87.0, 93.0, 100.0% survived tomato plants with use of inoculum levels *i.e.,* 1 x 10^{8}, 0.5 x 10^{8} and 0.3 x 10^{8} cfu/ml of the pathogen during treating with hot water of seeds and seedlings, respectively. Control treatment (infestation only and untreated with hot water) resulted in 13.0 and 32.0% of survived tomato plants of seeds and seedlings, respectively. As for, Xv_{10} it gave 88.0, 94.0, 100.0% and 92.0, 98.0, 100.0% of survived tomato plants after infestation with 1 x 10^{8}, 0.5 x 10^{8} and 0.3 x 10^{8} cfu/ml during treating seeds and seedlings with hot water, respectively, compared with 22.0 and 77.0% of survival plants in

case of control treatment (infested only and untreated with hot water) of tomato seeds and seedlings.

Table (22): Effect of treating seeds and seedlings of tomato plants with hot water on disease incidence% under infestation with three different isolates of *Xanthomonas vesicatoria***.**

Treatment	Seed treatment		Seedling treatment	
	Dead plant %	Survived plant %	Dead plant %	Survived plant %
Xv_{28}				
$1x10^8$	76.0	24.0	10.0	90.0
$0.5x10^8$	20.0	80.0	8.0	92.0
$0.3x10^8$	6.0	94.0	6.0	94.0
Control	**100.0**	**0.0**	**82.0**	**18.0**
Xv_{22}				
$1x10^8$	24.0	76.0	13.0	87.0
$0.5x10^8$	20.0	80.0	7.0	93.0
$0.3x10^8$	0.0	100.0	0.0	100.0
Control	**87.0**	**13.0**	**68.0**	**32.0**
Xv_{10}				
$1x10^8$	12.0	88.0	8.0	92.0
$0.5x10^8$	6.0	94.0	2.0	98.0
$0.3x10^8$	0.0	100.0	0.0	100.0
Control	**78.0**	**22.0**	**33.0**	**77.0**

XIV- Effect of some treatments on enzymes activities in infested tomato plants with *Xanthomonas vesicatoria* isolates.

a- Determination of peroxidase activities 430nm/g fresh weigh/30sec during 2009 and 2010 seasons.

Data in **Table** (**23**) show that, treating the tomato plants with bactericides, plant oils and garlic extract, resistance inducers and bio-agents, in order to control the bacterial leaf spot disease caused by *Xanthomonas* increased the activity of peroxidase enzyme compared to the control treatment. In this respect, the activities of peroxidase enzyme reached their maximum at 7-days post treating with the control treatments. Also, the activities of peroxidase enzyme were high at 3 and

10 days post treating with the control treatments comparing to check treatment (infested without treating).

As for the determined peroxidase activity at 3 days in case of Xv_{28}, copper oxychloride recorded the highest increase in peroxidase activity as mg/g fresh weight followed by Kombucha, garlic extract and ascorbic acid respectively. Meanwhile, the least determined activity of peroxidase enzyme was recorded with the bio-agent (*Ps. fluorescens*-1) at the same period. Moreover, all tested control treatments increased the activities of peroxidase comparing with check treatment (infected without treating). At 7 days post treating with the different control treatments, the highest increase in peroxidase activity was recorded also with copper oxychloride followed by garlic extract, galbin-cu, tetracycline and ascorbic acid respectively. At 10 days post treating with the different control treatments, the highest increase in peroxidase activity was recorded with copper oxychloride followed by garlic extract and galbin-cu respectively. On the other hand, the least activity of peroxidase at 7 and 10 day were recorded with the bio-agent (*Ps. fluorescens*-2).

As for the determined peroxidase activity in case of Xv_{22}, and Xv_{10}, similar trends were obtained where copper oxychloride, galbin-cu, tetracycline, garlic extract, ascorbic acid and clove oil were the best in increasing the peroxidase activities at the three testing periods (3,7 and 10 days) comparing to the other tested control treatments and check treatment (infested without treating).

Regarding the determined peroxidase activities during season 2010, data in **Table (24)** indicate that all used control treatments in controlling the bacterial leaf spot disease on tomato plants increased the activities of peroxidase enzyme at 3, 7 and 10 days post treating. The recorded maximum activities were at 7 days with all cases of infection with Xv_{28},

Xv_{22} and Xv_{10} isolates. It is clear from the obtained results also that all control treatments causing great increases in peroxidase activity during season 2009, caused also similar high increases in peroxidase activities with the three tested isolates of Xanthomonas at the three periods of determination (3, 7 and 10 days).

In this respect, copper oxychloride, galbin-cu, garlic extract, ascorbic acid, tetracycline and clove oil were the best in increasing the peroxidase activities. Moreover, the recorded increases in peroxidase activities in case of infected tomato plants with Xv_{28} and treated with the different control treatments were more than the other cases of Xv_{22} and Xv_{10} during seasons 2009 and 2010.

Table (23): Activity of peroxidase enzyme 430nm/g fresh weigh/30sec in sprayed tomato plants with some control treatments during 2009.

Treatment	Xv_{28}			Xv_{22}			Xv_{10}		
	3 days	7 days	10 days	3 days	7 days	10 days	3 days	7 days	10 days
Bactericides									
Copper oxychloride	20.21	33.19	31.35	15.83	30.47	29.25	11.89	22.57	20.87
Galbin-cu	15.42	29.58	28.33	14.23	28.13	26.98	13.45	26.53	24.98
Tetracycline	14.31	28.98	26.22	13.32	26.11	25.19	12.33	23.14	21.53
Erythromycin	15.63	26.76	24.73	12.56	24.42	22.78	13.12	20.32	18.65
M	**16.39**	**29.63**	**27.65**	**13.99**	**27.28**	**26.05**	**12.70**	**23.14**	**21.51**
Oils and plant extracts									
Mentha oil	13.33	26.49	24.12	13.11	25.12	23.45	11.93	20.11	19.02
Clove oil	14.04	27.22	25.89	14.81	27.91	26.01	13.24	21.02	20.44
Garlic extract	18.15	31.22	28.48	13.21	26.12	24.23	14.11	24.32	23.24
M	**15.17**	**28.31**	**26.16**	**13.71**	**26.38**	**24.56**	**13.11**	**21.82**	**20.90**
Inducing resistance									
Kombucha	19.33	25.10	24.56	12.04	23.58	22.15	11.33	17.65	16.22
Ascorbic acid	17.32	27.34	25.75	13.34	25.21	24.16	13.56	25.46	23.24
Salicylic acid	14.72	25.86	22.89	12.67	26.12	24.56	12.45	19.46	17.45
M	**17.12**	**26.10**	**24.40**	**12.68**	**24.97**	**23.62**	**12.45**	**20.86**	**18.97**
Bio-agents									
Pseudomonas fluorescens-1	13.58	23.49	20.33	14.63	20.78	19.22	12.75	20.23	18.32
Pseudomonas fluorescens-2	15.32	22.65	19.88	13.43	21.65	19.45	12.43	18.33	17.15
M	14.45	23.07	20.11	14.03	21.22	19.34	12.59	19.28	17.74
Control Xv	**11.35**	**15.21**	**14.75**	**9.44**	**13.22**	**12.98**	**7.75**	**10.59**	**9.87**

Table (24): Activity of peroxidase enzyme in sprayed tomato plants with some control treatments 430nm/g fresh weigh/30sec during 2010 season.

Treatment	Xv_{28}			Xv_{22}			Xv_{10}		
	3 days	7 days	10 days	3 days	7 days	10 days	3 days	7 days	10 days
Bactericides									
Copper chloride	19.53	30.47	29.23	16.17	29.89	28.46	12.50	23.65	22.08
Galbin-cu	17.23	28.33	27.49	15.42	27.43	24.76	14.23	28.64	27.44
Tetracycline	14.78	28.73	27.19	12.89	25.61	24.01	13.46	25.34	23.75
Erythromycin	14.95	25.24	23.78	13.03	23.43	22.25	15.23	22.41	21.12
M	**16.26**	**28.19**	**26.92**	**14.38**	**26.59**	**25.11**	**13.86**	**25.01**	**23.60**
Oils and plant extracts									
Mentha oil	15.70	26.16	25.62	15.62	26.15	25.24	13.12	23.73	21.56
Clove oil	14.84	26.86	24. 42	16.73	28.70	27.13	15.20	24.04	22.78
Garlic extract	19.35	29.85	27.12	15.10	25.83	22.76	17.34	26.81	24.30
M	**16.63**	**27.62**	**25.72**	**15.82**	**26.89**	**25.04**	**15.22**	**24.86**	**22.88**
Inducing resistance									
Kombucha	18.56	25.89	23.79	14.33	24.56	22.43	13.24	20.46	19.23
Ascorbic acid	19.77	27.01	26.13	14.89	26.18	25.33	15.67	27.33	25.88
Salicylic acid	15.86	25.86	23.83	13.25	28.43	26.59	14.11	22.13	21.24
M	**18.06**	**26.25**	**24.58**	**14.16**	**26.39**	**24.78**	**14.34**	**23.31**	**22.12**
Bio-agents									
Pseudomonas fluorescens-1	14.66	22.65	21.20	14.61	19.88	17.89	14.05	23.43	21.78
Pseudomonas fluorescens-2	14.98	21.65	20.11	14.02	21.45	19.78	13.66	21.22	19.96
M	**14.82**	**22.15**	**20.66**	**14.32**	**20.67**	**18.84**	**13.86**	**22.33**	**20.87**
Control Xv	**12.53**	**15.89**	**15.07**	**11.05**	**14.22**	**13.88**	**9.23**	**13.29**	**12.89**

b- Determination of polyphenoloxidase activities 420 nm/g fresh weigh/30sec during 2009 and 2010 seasons.

Data in **Table** (**25**) show that, treating the tomato plants with bactericides, plant oils and garlic extract, resistance inducers and bio-agents, in order to control the bacterial leaf spot disease caused by Xanthomonas increased the activity of polyphenoloxidase enzyme compared to the check treatment. In this respect, the activities of polyphenoloxidase enzyme reached their maximum at 7 days post treating with the control treatments. Also, the activities of polyphenoloxidase enzyme were high at 3 and 10 days post treating with the control treatments comparing to check treatment (infested without treating).

As for the determined polyphenoloxidase activity at 3 days in case of Xv_{28}, ascorbic acid followed by copper oxychloride, garlic extract, Kombucha and galbin-cu recorded the highest increase in polyphenoloxidase activity as mg/g fresh weight respectively. Meanwhile, the least determined activity of peroxidase enzyme was recorded with the bio-agent (Ps. fluorescens-1) at the same period. Moreover, all tested control treatments increased the activities of polyphenoloxidase comparing with check treatment (infested without treating). At 7 days post treating with the different control treatments, the highest increase in polyphenoloxidase activity was recorded also with copper oxychloride followed by garlic extract, galbin-cu, tetracycline and ascorbic acid respectively. At 10 days post treating with the different control treatments, the highest increase in polyphenoloxidase activity was recorded with copper oxychloride followed by galbin-cu and garlic extract respectively.

On the other hand, the least activity of polyphenoloxidase at 7 and 10 day were recorded with the bio-agent (*Ps. fluorescens-2*).

As for the determined polyphenoloxidase activity in case of Xv_{22}, and Xv_{10}, similar trends were obtained where copper oxychloride, galbin-cu, tetracycline, garlic extract, ascorbic acid and clove oil were the best in increasing the polyphenoloxidase activities at the three testing periods (3,7 and 10 days) comparing to the other tested control treatments and check treatment (infested without treating).

Regarding the determined polyphenoloxidase activities during season 2010, data in **Table (26)** indicate that all used treatments in controlling the bacterial leaf spot disease on tomato plants increased the activities of polyphenoloxidase enzyme at 3, 7 and 10 days post treating. The recorded maximum activities were at 7 days with all cases of infection with Xv_{28}, Xv_{22} and Xv_{10} isolates.

It is clear from the obtained results also that all control treatments causing great increases in polyphenoloxidase activity during season 2009, caused also similar high increases in polyphenoloxidase activities with the three tested isolates of Xanthomonas at the three periods of determination (3, 7 and 10 days). In this respect, copper oxychloride, galbin-cu, garlic extract, ascorbic acid, tetracycline and clove oil were the best in increasing the polyphenoloxidase activities. Moreover, the recorded increases in polyphenoloxidase activities in case of infected tomato plants with Xv_{28} and treated with the different control treatments were more than the other cases of Xv_{22} and Xv_{10} during seasons 2009 and 2010.

Table (25): Activity of polyphenoloxidase enzyme 420 nm/g fresh weigh/30sec in sprayed tomato plants with some control treatments, during 2009 season.

Treatment	Xv_{28}			Xv_{22}			Xv_{10}		
	3days	7 days	10 days	3days	7 days	10 days	3days	7 days	10 days
Bactericides									
Copper chloride	11.23	20.43	19.15	10.33	13.45	11.23	6.45	8.42	7.73
Galbin-cu	10.80	16.24	15.20	9.24	11.20	10.07	8.23	11.65	10.34
Tetracycline	10.12	14.23	12.76	8.78	10.66	8.87	8.02	10.32	9.23
Erythromycin	8.23	11.20	10.16	7.20	9.84	8.24	7.73	10.01	8.88
M	**10.11**	**15.53**	**14.32**	**8.89**	**9.04**	**9.61**	**7.61**	**10.10**	**9.05**
Oils and plant extracts									
Mentha oil	8.04	11.56	9.87	7.46	10.02	9.11	6.80	8.33	7.62
Clove oil	9.44	14.76	11.32	8.33	12.34	10.46	8.35	11.30	10.11
Garlic extract	12.43	17.67	16.22	9.67	11.56	10.76	10.47	13.74	10.97
M	**9.97**	**14.66**	**12.47**	**8.49**	**11.31**	**10.11**	**8.54**	**11.12**	**9.57**
Inducing resistance									
Kombucha	8.22	12.45	11.04	8. 05	10.76	9.78	7.87	9.64	8.70
Ascorbic acid	11.43	14.72	13.14	9.35	11.70	10.33	9.63	11.54	10.17
Salicylic acid	10.31	13.66	11.65	8.74	11.04	9.73	6.34	10.40	9.32
M	**9.99**	**13.61**	**11.94**	**8.70**	**11.17**	**9.95**	**7.95**	**10.53**	**9.40**
Bio-agents									
Pseudomonas fluorescens-1	9.16	11.32	10.60	6.76	9.76	8.64	5.76	7.57	6.45
Pseudomonas fluorescens-2	9.36	12.46	10.87	7.12	10.23	9.76	6.43	9.36	7.75
M	**9.26**	**11.89**	**10.74**	**6.94**	**9.99**	**9.2**	**6.10**	**8.47**	**7.10**
Control Xv	**6.11**	**8.53**	**9.03**	**4.48**	**5.89**	**6.15**	**3.50**	**4.83**	**5.12**

Table (26): Activity of polyphenoloxidase enzyme420 nm/g fresh weigh/30sec in sprayed tomato plants with some control treatments, during 2010.

Treatment	Xv_{28}			Xv_{22}			Xv_{10}		
	3days	7 days	10 days	3days	7 days	10 days	3days	7 days	10 days
Bactericides									
Copper chloride	12.22	25.86	23.67	11.24	16.23	14.75	8.78	10.98	9.73
Galbin-cu	11.03	23.49	22.15	9.53	12.88	11.13	10.86	13.86	12.13
Tetracycline	10.22	21.75	19.86	10.02	13.08	10.89	10.10	11.76	9.77
Erythromycin	9.53	20.33	19.02	8.45	11.34	9.43	8.46	10.11	9.02
M	**10.75**	**22.86**	**21.18**	**9.81**	**10.55**	**9.19**	**9.55**	**11.68**	**10.16**
Plant Oils and garlic extract									
Mentha oil	10.01	21.15	18.78	8.23	11.43	10.44	7.43	9.76	8.23
Clove oil	10.53	22.65	20.83	10.86	14.56	13.32	8.76	10.73	9.12
Garlic extract	11.24	25.17	23.43	9.13	12.05	10.66	9.84	12.34	10.24
M	**11.0**	**22.99**	**21.01**	**9.41**	**12.68**	**11.47**	**8.68**	**10.94**	**9.20**
Resistance inducers									
Kombucha	7.68	19.25	17.55	8.11	11.19	10.16	7.55	10.23	9.43
Ascorbic acid	9.56	23.01	20.43	9.21	10.35	9.34	8.78	12.02	10.37
Salicylic acid	9.33	20.11	19.66	10.38	13.82	11.32	9.23	11.35	10.12
M	**8.86**	**20.79**	**19.21**	**9.2**	**11.79**	**10.27**	**8.52**	**11.20**	**9.98**
Bio-agents									
Pseudomonas fluorescens-1	8.42	18.76	16.74	7.68	10.13	8.23	6.70	9.22	8.40
Pseudomonas fluorescens-2	9.13	17.36	16.11	7.14	9.12	8.64	7.23	10.25	8.75
M	**8.78**	**18.06**	**32.85**	**7.41**	**9.63**	**8.44**	**6.97**	**9.74**	**9.11**
Control Xv	**5.52**	**6.53**	**8.13**	**4.65**	**5.44**	**6.07**	**3.24**	**4.43**	**5.02**

XV- Effect of some treatments on phenolic contents in infested tomato leaves with *Xanthomonas vesicatoria* isolates during seasons 2009 and 2010

Data in **Table (27)** show that, treating the tomato plants with bactericides, plant oils and garlic extract, resistance inducers and bio-agents, in order to control the bacterial leaf spot disease caused by *Xanthomonas* increased the free, conjugated and total phenols in infested treated tomato leaves at 10 days post treating comparing to check treatment (infested without treating) during seasons 2009 and 2010. The highest increase in total phenols was recorded with treating the tomato plants with copper oxychloride, tetracycline and garlic extract respectively during the two seasons with the three Xanthomonas isolates.

It was clear from the obtained results that the estimated free phenols were more than the determined conjugated ones with all tested control treatments for the three *Xanthomonas* isolates during the two seasons.

Table (27): Effect of biological control with tomato bacterial leaf spot isolates Xv_{28}, Xv_{22}, Xv_{10} on phenols content (mg/g fresh weight) of leaves Season 2009 and 2010.

Years	Treatments	Xv_{28}			Xv_{22}			Xv_{10}		
		Total phenol	Conjugated phenol	Free phenols	Total phenol	Conjugated phenol	Free phenols	Total phenol	Conjugated phenol	Free phenols
2009	Copper oxychloride	36.5	13.2	23.3	40.5	12.3	28.2	30.5	10.1	20.4
	Galbin-cu	31.9	12.7	19.2	34.2	10.1	24.1	31.2	12.6	18.6
	Tetracycline	34.2	13.8	20.4	31.9	10.5	21.4	29.2	10.9	18.3
	Erythromycin	30.5	11.9	18.6	29.7	8.9	20.8	28.4	9.6	18.8
	Mentha oil	29.6	9.1	20.5	27.9	9.3	18.6	27.5	9.8	17.5
	Clove oil	30.2	10.6	19.6	25.3	8.1	17.2	26.3	10.5	15.8
	Garlic extract	33.4	11.2	22.2	30.4	11.5	18.9	32.2	11.8	20.4
	Kombucha	29.7	9.3	20.5	28.4	8.8	19.6	29.2	8.9	20.3
	Ascorbic acid	32.3	11.8	20.5	23.8	7.9	15.9	25.4	8.5	16.9
	Salicylic acid	31.4	12.1	19.4	33.5	10.7	22.8	24.3	10.1	14.9
	Pseudomonas fluorescens -1	30.3	9.6	20.7	25.6	9.3	18.3	23.3	9.1	14.2
	Pseudomonas fluorescens -2	29.5	9.7	19.8	24.8	9.3	15.5	21.2	9.5	11.7
	Control Xv	28.4	10.1	18.3	20.5	6.7	13.8	19.3	7.9	11.4
2010	Copper oxychloride	38.4	10.9	27.5	41.4	14.5	26.9	31.2	11.4	19.3
	Galbin-cu	32.8	10.4	22.4	35.4	7.1	28.3	29.5	9.3	20.2
	Tetracycline	34.3	11.5	22.8	30.4	5.1	25.3	27.2	10.3	16.9
	Erythromycin	29.7	8.3	21.4	22.3	6.2	16.1	29.4	11.1	18.2
	Mentha oil	33.7	8.9	24.8	28.6	8.1	20.5	26.3	9.8	16.4
	Clove oil	31.3	10.5	20.8	26.1	8.5	17.6	25.5	7.6	17.9
	Garlic extract	35.4	7.7	27.7	27.8	9.4	18.4	29.1	8.7	20.4
	Kombucha	32.5	9.7	22.8	25.6	7.4	18.2	27.3	10.7	16.9
	Ascorbic acid	32.7	8.9	23.8	28.9	7.2	21.7	23.4	7.9	14.5
	Salicylic acid	30.8	7.1	28.7	29.5	7.8	21.7	22.9	9.1	13.8
	Pseudomonas fluorescens -1	28.7	9.5	19.2	25.1	4.8	20.3	23.1	7.9	15.1
	Pseudomonas fluorescens -2	27.9	8.8	19.1	24.5	5.4	19.1	20.3	8.8	11.4
	Control Xv	25.3	6.6	18.7	20.5	4.7	15.8	17.2	6.5	10.7

DISCUSSION

Tomatoes (*Lycopersicon esculentum* Mill.) are one of economic and important crops, which cultivated for their fruits of economic importance and nutritional value as well as their importance for domestic consumption, export and food industries.

Sum of 70 bacterial isolates were isolated from different naturally infected tomato leaves which collected from four localities *i.e.* El-Doki (cv. Super Marmand) at Giza Governorate, Qaha (cv. Peto 86 and Super strain B), Fac. of Agriculture Ain Shams at Qalubia Governorate (cv. Money maker, Super Marmand and Super strain B) and Rashid (cv. Mors-44 and Niagra) at Damietta Governorate. All isolated bacteria were found to belong to 4 different groups according to the clear differentiations in their morphological features. Group 1 gave the highest frequent number and frequency% among the isolated bacteria representing the different isolated groups where it represented with 30 isolate, whereas, the group 2 and 3 were represented with 10 isolates for each one. Meanwhile, the group 4 was represented with 20 isolate of bacteria which varied in their morphology. The highest isolation number of Group 1 was recorded in Qaha location followed by EL-Doki and Fac. Agri., Ain Shams. Meanwhile, the least isolation number of group 1 was in Rashid locality. These results could be discussed in light of the findings of **Bouzar *et al.* (1999)** who isolated four hundred thirty-three Xanthomonads strains from tomato and pepper plants from 32 different fields. Also, **Carrillo-Fasio *et al.* (2001)** who obtained thirty six isolates of *X. compestris* pv. *vesicatoria* from tomato and

pepper leaves and/or fruit showing typical symptoms of bacterial spot collected in various horticultural zones in the state of Sinaloa.

As for the isolated bacteria from tomato seeds, non of bacterial isolates which belonging to group 1 were recorded on the tested seeds of tomato cultivars and hybrid used when directly planted onto the nutrient agar (NA) agar media. Only 5 bacterial isolates belonging to the same group 1 were recorded on seeds of cv. Super strain B.

On the other hand, some of bacterial isolates which belonging to group 2 were recorded on seeds of tomato cultivars and hybrids i.e., Dora, KTM-141, Diamante-F1, Hybrid-7796, Hybrid HMX4791, Faqlta-38, Mors-44, and Hybrid VT916G.SI when directly planted onto the n utrient agar (NA) agar media. Meanwhile, the isolated bacteria which belonging to group 2 were recorded only on Gs-12 (2 isolates), Niagra (5 isolates), Super strain B (4 isolates) and Hybrid Super strain B (1 isolate) at the same conditions of isolation. Also, no one of bacterial isolates which belonging to group 3 were recorded on seeds of any one of tomato cultivars and hybrids. As for group 4 of isolated bacteria from seeds of tomato cultivars and hybrids, 20 bacterial isolates were recorded only on Dora (2 isolates), Gs-12 (4 isolates), Niagra (1 isolate), Super strain B (8 isolates), Faqlta-38 (3 isolates) and Mors-44(2 isolates). While no one of them were recorded on the other tested tomato cultivars and hybrids. The results indicated also that the highest number of bacterial isolates of the 4 isolated groups was recorded on cv. Super strain B followed by cv. Niagra and cv. Gs-12 while the least number of the isolated bacteria was recorded on Hybrid Super strain B (1 isolate). These obtained results are in harmony with those obtained by many investigators like **Schaad *et al.*** (**1980), Kritzman (1989)** and **Scortichini (1991)** who

confirmed the presence of *Xanthomonas campestris* pv. *vesicatoria* as important seed-borne phase for survival and long distance dissemination. Also, **Abd El-Ghafar** and **Abd El-Wahab (2001)** isolated *X. c.* pv. *vesicatoria* from tomato seeds and plants grown under protected conditions at different locations in Fayoum, Ismailia and Menufia governorates. Moreover, **Yang *et al.* (2005)** isolated *Xanthomonas vesicatoria* from tomato seeds as seed borne bacterium, which was more prevalent in regions with high humidity and heavy rainfall. Whereas, **Dong *et al.* (2009)** isolated *Xanthomonas campestris* pv. *vesicatoria* (Xcv) from different plant parts and seeds of pepper and tomato and confirmed that it was the causal agent of bacterial spot of pepper and tomato.

Identification of the first 3 groups (1, 2 and 3) based on the variations in their morphological, physiological and biochemical characteristics revealed that out of 50 bacterial isolates which were isolated only from tomato leaves were identified and classified as follows, 30 isolates of those belonging to group 1 were identified as *Xanthomonas vesicatoria*, 10 isolates of those belonging to group 2 were identified as *Bacillus subtilis* and 10 isolates of those belonging to group 3 were identified as *Pseudomonas fluorescence*. Identification and classification of our obtained isolates were achieved based on their morphological and physiological properties according to **Schaad *et al.* (1980)**, **Fahy and Persley (1983)** and **Lelliott and Stead (1987)**. In this respect also, **Abd El-Ghafar** and **Abd El-Wahab (2001)** verified our obtained results where they isolated and identified *X. campestris* pv. *vesicatoria* from tomato seeds and plants grown under protected conditions at different

locations in Fayoum, Ismailia and Menufia Governorates according to the morphological and physiological properties, Biolog test, serological tests and pathogenicity tests**.** While**, Carrillo-Fasio *et al.* (2001)** identified thirty six isolates of *X. c.* pv. *vesicatoria* of which isolated from tomato and pepper leaves and/or fruit according to their Gram reaction, flagella staining and for response to biochemical tests like starch hydrolysis and catalase production. **Obradovic *et al.* (2004)** characterized twenty-eight strains of those isolated from pepper and six from tomato based on their physiological and pathological characteristics and fatty acid composition analysis which revealed that all of the strains belong to *Xanthomonas campestris pv. vesicatoria.* Also, **Opara and Odibo (2009)** isolated bacteria from infected tomato plant parts (leaf, and seed) with bacterial spot lesions and used the cultural, physiological and biochemical analyses for identification of them.

The identification results confirmed that these bacterial isolates were gram negative, yellow, aerobic, rod shaped bacteria with a polar flagella. The bacteria colonies exhibited strong starch hydrolysis, metabolized glucose and produced acid from arabinose, sucrose and cellobiose but not from ducitol or sorbitol.

Also nitrite was not reduced to nitraite based on bacteriological characteristics, the bacteria strains were identified as *Xanthomonas campestris* pv. *vesicatoria* (ex Doidge).

Testing the virulence of 30 bacterial isolates of those isolated from infected spots of tomato and identified as *Xanthomonas vesicatoria* on tomato and pepper plants indicated that twelve isolates which identified as *Xanthomonas vesicatoria, i.e.,* Xv_2, Xv_3, Xv_7, Xv_8, Xv_{10}, Xv_{13}, Xv_{14}, Xv_{20}, Xv_{22}, Xv_{23}, Xv_{26} andXv_{28} were the

highly pathogenic where they caused clear bacterial spot on leaves of tomato and pepper. Also, eight isolates of *X. vesicatoria* (*i.e.*, Xv_1, Xv_4, Xv_5, Xv_9, Xv_{11}, Xv_{12}, Xv_{15} and Xv_{24}) caused moderately pathogenic effect where they revealed a few spots as symptoms on leaves of tomato and pepper plants. The last ten isolates of *X. vesicatoria* (*i.e.*, Xv_6, Xv_{16}, Xv_{17}, Xv_{18}, Xv_{19}, Xv_{21}, Xv_{25}, Xv_{27}, Xv_{29} and Xv_{30}) were non pathogenic where no visual symptoms were recorded on tomato and pepper leaves after testing.

The present results are in harmony with those reported by **Pohronezny and Volin (1983)** who reported that the bacterial spot disease on tomato caused by *Xanthomonas campestris* pv. *vesicatoria* or *X. vesicatoria*, is a serious disease, which can affect the foliage, fruit, blossoms, and stems. Therefore, **Mortensen (1997**) and **Jones *et al*. (1998)** showed that the bacteria caused bacterial spot could be divided to four distinct groups: *Xanthomonas campestris* (*axonopodis* proposed) pv. *vesicatoria* (group A), *Xanthomonas vesicatoria* (group B), *Xanthomonas gardneri* (group D), and group C strains that may represent a subspecies of some group A strains. Strains of groups A and B are most widely distributed. The vast majority of strains that infect pepper are in group A and possibly some in groups B and D. No pepper strains have been found in group C, however, strains from all four groups have been isolated from tomato.

Some strains infect only pepper (pepper strains), some infect only tomato (tomato strains), and some can infect both pepper and tomato (pepper/tomato strains).

Also, all the obtained results of **Bouzar *et al.* (1999); Carrillo *et al*. (2001)** and **Dicklow (2009)** confirmed the virulence of *X.*

vesicatoria isolates on tomato plants causing bacterial leaf spot disease.

Identification of the twelve pathogenic bacterial isolates of *Xanthomonas vesicatoria i.e.*, *Xv2*, *Xv8* and *Xv10* (El-Doki), *Xv3*, *Xv7*, *Xv20*, *Xv28* and *Xv23* (Qaha) and *Xv13*, *Xv14*, *Xv26* and *Xv22* (Rashid) was confirmed using electrophoretic analysis. The fractionated protein bands revealed that the molecular weight 17.71 kDa was present in all the fractionated protein bands of all 12 tested *Xanthmonas* isolates to confirm that these isolates of the three locations are belonging to one cluster. The similarity between the 4 *Xanthmonas* isolates representing Rashid location and the 5 isolates of Qaha location was high where there were a lot of similar molecular weights in the fractionated protein bands of these isolates while, the 3 isolates representing El-Doki were not similar to the both mentioned groups. On the other hand, the molecular weight 31.11 kDa was present in all fractionated protein bands of 11 *Xanthmonas* isolates except Xv10 and this could be increase the similarity level between these 11 isolates. Identification of three bacterial isolates *i.e.*, Xv_{28}, Xv_{22} and Xv_{10} which selected according to their highly pathogenic abilities as well as to represent the three different locations of isolation in Egypt was confirmed using the Biolog system to reveal that there were a great similarities between them.

Identification of the three previously mentioned bacterial isolates *i.e.*, Xv_{28}, Xv_{22} and Xv_{10} was confirmed using six RAPD primers *i.e.*, A9, B10, A3, G2, G8 and G19. The first 4 RAPD primers *i.e.*, A9, B10, A3 and G2 were the best in revealing the

initiated PCR banding patterns of the fractionated DNA fragments (bp) of the three tested *X. vesicatoria* isolates.

Amplification patterns obtained with primers A9 and A3 revealed clearly low genetic similarity among the three *Xanthmonas* isolates.

The dendogram analysis which resulting from the UPGMA cluster analysis showed that the three tested bacterial isolates could be divided into two clusters. Cluster one included all the determined genotype; (Xv_{22}) and (Xv_{10}) isolated from Rashid and El-Doki. Cluster 2 included only one bacterial spot strain (Xv_2) isolate from Qaha.

This result also confirmed the previously mentioned traditional identification which revealed that the three tested isolates are identified as *Xanthomonas vesicatoria*. The obtained data could be interpreting in light of the findings of **Obradovic *et al*. (2004)** who used some PCR primers to identify different strains of *X. campestris* pv. *vesicatoria* associated with pepper and tomato. Restriction analysis of the PCR product resulted in different patterns and enabled grouping of the strains into four groups. The isolated Xanthomonads isolated from pepper and tomato in Serbia were clustered into two groups corresponding to the grouping based on their physiological and pathological characteristics. According to the reaction of pepper and tomato differential varieties, the strains from pepper belong to races P7 and P8 and tomato strains belong to the race T2. Moreover, **Dong *et al.* (2009)** described a specific and highly sensitive rapid -

PCR assay to detect bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* in pepper and tomato.

One set of PCR primer was developed to amplify gene required for family gene homologous to rhsA, cell envelope biogenesis, outer membrane. Only a PCR product of a 517 bp was produced in PCR reaction with the *Xanthomonas campestris* pv. *vesicatoria* (XCVF/ XCVR) primer set. The protocol can be used as a reliable diagnostic tool for specific detection of *X. campestris* pv. *vesicatoria* in pepper or tomato.

Concerning the host range of the tested *Xanthmonas* bacteria, all of three tested isolates were highly pathogenic on tomato, pepper and never affected potato, cabbage, common bean, eggplant, lettuce, beans, strawberry and cantaloup. On the other hand, the three isolates showed moderately effect on datura plants.

These results are in agreement with those obtained by **El-Sadek *et al.* (2001)** who stated that the isolated *X. vesicatoria* from pepper plants in El-Minia and identified based on their morphological, physiological and biochemical characteristics, infected the leaves of tomato, tobacco, cowpea and bean showing hypersensitive reaction when sprayed with the bacterial inoculum.

Evaluation of sixteen tomato cultivars and genotypes to infection with the *Xanthomonas vesicatoria* $-_{28}$ under greenhouse conditions revealed that, cvs Super strain B and Castle rock were highly susceptible to infection with *Xanthomonas vesicatoria*-$_{28}$ than the other tested tomato cvs and genotypes like Peto 86, Gs12, Money maker, Dora. While, the next ten tomato cvs and genotypes, *i.e.*, Diamante F1, Hybride7796, Mors44, VT916G.SI, HMX4791, Super strain BH, KTM 141, Niagra, Flora-Dade and Faqlta 38, were

completely resistant to infection with *Xanthomonas vesicatoria*-$_{28}$. As recording *Xanthomonas vesicatoria*-$_{22}$ Super strain B and Castle rock cvs were the highly susceptible to infection with *Xanthomonas vesicatoria*-$_{22}$. While, cvs. Peto 86, Flora-Dade, Dora, Faqulta38, Gs12 and Money maker were low susceptible to inflectionally the same isolate.

On the other hand, the other tomato cvs and genotypes, *i.e.*, Diamante F1, Hybride7796, Mors44, VT916G.SI, HMX4791, Super strain B H, KTM 141, were completely resistant to infection with *X. vesicatoria* -$_{22}$. Also, Super strain B was highly susceptible to infection with *Xanthomonas vesicatoria* isolate-$_{10}$ followed by cvs Castle rock and Peto 86. While cvs, Flora-Dade, Money maker, Dora, Mors44 and GS12 were low susceptible. On the other hand, the next eight of tomato cvs and genotypes, *i.e.,* Diamante F1, Hybride7796, VT916G.SI, HMX4791, Super strain BH, KTM 141, Niagra and Faqlta 38 were completely resistant to infection with *Xanthomonas vesicato*ria$_{10}$.

These obtained results could be supported in light of the findings of **Massomo (2004), Kenneth *et al*. (2010)** and **Shenge *et al*.** (**2010**) who evaluated four tomato varieties *i.e*., Cal J, Moneymaker, Tanya and Roma VF., which were the commonly grown by tomato farmers in Tanzania for resistance to bacterial speck (*Pseudomonas syringae* pv. *tomato*) and bacterial spot (*Xanthomonas vesicatoria*) diseases, along with, five introductions under screen house and field conditions. Results indicated that all the tomato varieties were susceptible to the two diseases, and suffered moderate to severe infection levels.

The results indicated also that all the locally available tomato varieties included in the study were highly susceptible to bacterial speck and bacterial spot diseases.

Concerning the effect of control treatments on growth of the tested pathogenic bacteria *in vitro*, different bactericides, antibiotics, plant oils and garlic extract, resistance inducers and bio-agents were used at different concentrations to study their effects on the growth of tested pathogenic bacteria (*Xanthomonas vesicatoria)* as zone of inhibition (cm) under *in vitro* conditions. In this respect, Copper oxychloride was more effective than Galbin-cu in reducing the growth of all tested *X. vesicatoria* isolates compared with the control. Also, Tetracycline was the more effective than Erythromycin in increasing the inhibition zone of the three isolates of Xanthomonas. Garlic extract was also highly effective in inhibiting the growth of tested *X. vesicatoria* isolates. On the other hand, mentha and clove oils had clear inhibitory effects on growth of the pathogenic bacteria compared to control with superiority of mentha oil. It was clear also that ascorbic and salicylic acids had clear inhibitory effect on growth of the pathogenic bacteria comparing to the control treatment with superiority of ascorbic acid. Kombucha was also effective as bio-inducer in reducing the growth rates of tested pathogenic bacteria but lesser than the other tested control treatments.

Also, increasing the concentrations of all tested treatments increased the inhibition zones of the resulted growth. Also, *Pseudomonas fluorescence* (Pf1) isolate was the more effective than *P. fluorescens* (Pf2) isolate in inhibiting the growth of *X. vesicatoria* bacteria.

Regarding the effect of treatments used in controlling the bacterial spot disease on tomato during two growing seasons 2009 and 2010 under greehouse conditions, copper oxychloride, Galbin-cu and tetracycline were the most effective in decreasing the diseases incidence%, disease severity%, no. of spots/leaf and increasing the disease reduction% than erythromycin when sprayed on the tomato plants at 3 and 10 days post the infestation with *X. vesicatoria* isolates.

Also, garlic extract was more effective than the tested oils in spite of mentha and clove oils were also effective in reducing infection of leaf spot disease. Also, ascorbic and salicylic acids were more effective than the bio-inducer (Kombusha) in reducing infection of leaf spot disease with superiority of ascorbic acid than others inducers. On the other hand, *Ps. fluorescence*-1 was more effective than *Ps. fluorescence* -2 in reducing the disease parameters. In this respect, similar results were reported by **Kousik and Ritchie (1996)** who stated that, chemical control has been extensively for controlling bacterial spot disease where copper sprays could be manage foliar diseases and reduce losses caused by bacterial spot and bacterial speck. Unfortunately, these sprays often are not very effective and their extensive use led to the development of copper resistant strains.

Also, **Carrillo-Fasio *et al.* (2001)** tested thirty nine *X. compestris* pv. *vesicatoria* strains for their sensitivity to several copper formulations and combinations of copper + mancozeb, copper + antibiotics, in laboratory and field experiments (Mexico). Seventeen out of the 39 strains showed high tolerance to copper formulations under *in vitro* conditions; however, when the strains

were tested with other copper formulations plus mancozeb, dithane M-45, Cuprimicin 100 and Cuprimicin 17 marked inhibition zones developed. Meanwhile, these obtained results could be interpreting in light the findings of are confirmed with **Abd El Ghafar** and **Abd El Wahab (2001**) who revealed that**,** application of four chemical compounds (agrimycin, terramycin, kocide 101 and tri-miltox) reduced the growth of *X. c*. pv. *vesicatoria*, *in vitro* and the severity of bacterial spot under artificial inoculation at field conditions. Interaction between the four chemical compounds proved to be the most effective compounds in reducing bacterial spot incidence and severity disease. The obtained results are also in harmony with those reported by **Morais *et al*. (2002)** who tested the antimicrobial activity of 45 extracts of medicinal plants against *X. c*. pv. *vesicatoria* and *C. m*. subsp. *michiganensis*. Five extracts (EAFQ, SM1, SM12, SM16 and SA1) showed positive activity against the bacteria.

On the other hand, the obtained findings of **Basim *et al***. (**2006**) confirmed the antimicrobial effect of plant extracts against *Xanthomonas campestris* the causal of bacterial leaf spot disease where they screened the effect of aqueous extracts from leaves of 30 higher plants, collected from different localities, *in vitro* for their antibacterial activity against different pathovars of the phytopathogenic bacterium, *Xanthomonas campestris.* Eight plant species showed antibacterial activity, based on the zone of inhibition in a diffusion assay. Significant antibacterial activity was observed in the aqueous extracts of *Prosopis juliflora*, *Oxalis corniculata and Lawsonia inermis.* The susceptibility of different pathovars of *X. campestris* to these plant extracts was varied.

Moreover**, Janda *et al.* (2007)** demonstrated that salicylic acid play a role in responses to biotic stress effects (such as low and high temperature, UV-B irradiation, ozone, heavy metals, etc.). When applied in satisfactory concentrations, salicylic acid may cause a temporary low level of oxidative stress in plants, which acts as a hardening process, improving the ant oxidative capacity of the plants and helping to induce the synthesis of protective compounds such as polyamines.

Also, similar results were obtained by **Bajpai *et al*. (2010)** who confirmed *in vitro* and *in vivo* the antibacterial efficacy of the essential oil and organic extracts of *Metasequoia glyptostroboides* against plant pathogenic bacteria of *Xanthomonas* spp. The oil (1000 µg/disc) and extracts (1500 µg/disc) displayed potential antibacterial effect *in vitro* as a diameter of zones of inhibition against *Xanthomonas campestris* pv. *campestris*, *X. campestris* pv. *vesicatoria, X. oryzae* pv. *oryzae* and *X.* sp. , which were found in the range of 10–14 and 8–12 mm, respectively.

The effect of using the different control treatments could be interpreting in light the findings of **Matias (2009)** who revealed that, the attempted infection of plants by pathogens elicits a complex defensive response. In many non-host and incompatible host interactions it includes the induction of defense-associated genes and a form of localized cell death (LCD), purportedly designed to restrict pathogen advance, collectively known as the hypersensitive response (HR).

It is preceded by an oxidative burst, generating reactive oxygen species (ROS) that are proposed to cue subsequent deployment of the

HR, although neither the origin nor the precise role played by ROS in the execution of this response are completely understood. Also, the obtained results could be interpret in light the findings of **Nisa *et al.* (2010)** who stated that copper (Cu)-based biocides are important chemical controls for both fungal and bacterial diseases in crop fields. They showed that, Cu ions at a concentration of 100 μM enhanced *t*-butyl hydroperoxide (*t*BOOH) and hydrogen peroxide (H_2O_2) killing of *Xanthomonas campestris* pv. *campestris* through different mechanisms.

The addition of an antilipid peroxidation agent (α-tocopherol) and hydroxyl radical scavengers (glycerol and dimethyl sulphoxide) partially protected the bacteria from the Cu-enhanced *t*BOOH and H_2O_2 killing, respectively. Inactivation of the alkyl hydroperoxide reductase gene rendered the mutant vulnerable to lethal doses of copper sulphate, which could be alleviated by the addition of an H_2O_2 scavenger (pyruvate) and α-tocopherol. Taken together, the data suggest that Cu ions influence the killing effect of *t*BOOH through the stimulation of lipid peroxidation, while hydroxyl radical production is the underlying mechanism responsible for the Cu-ion-enhanced H_2O_2 killing effects.

As for the isolated phages of *Xanthomonas vesicatoria* from infected leaves of tomato and from tomato soil rhizosphere samples collected from Qalubia governorate. The isolated phages produced two different types of plaques.

The first type was the most common, where its plaque was circular with an irregular margin while, the second type was circular plaques without determined margin. Also, four types of phages were observed superficially the bacterial cells. The four types of phages

were distinguished as follows, two types having symmetrical particles and the other two types having icosahedral heads particles. The phages infecting tomato plants were found to belong four morphological groups (tailed, polyhedral, filamentous and pleomorphic).

However, based on morphology, the large icosahedral particles were placed in the family podoviridae but further proof of the presence of short tails is needed, while the small icosahedral particles could be classified in the family Micrviridae. The tailed particles could be placed in the family Siphoviridae and the filamentous particles were placed in the family Inoviridae.

As for the effect of tested bacteriophages *i.e.,* phage 1, phage 2, phage 3, phage 4 and the mixture of different phage isolates (1+2+3+4), all tested phages were effective in controlling bacterial leaf spot on tomato plants comparing with the check treatment. Also, these phages were effective in decreasing the disease incidence%, disease severity%, no. of spots/ leaf and increasing the disease reduction % comparing with the control treatment at 3 and 10 days post the infestation with *Xanthomonas vesicatoria* isolates (Xv_{-28}, Xv_{22}, Xv_{10}).

These results similar to those obtained by **Flaherty *et al.* (2000)** who used a mixture of specific bacteriophages to control the bacterial spot pathogen (*Xanthomonas campestris pv. vesicatoria*) as biological control of bacterial spot on 'Sunbeam' tomato (*Lycopersicon esculentum* Mill.) transplants and field-grown plants for two seasons. Applications of bacteriophages to field-grown tomatoes decreased disease severity as measured by the area under

the disease progress curve (AUDPC) by 17.5% (1997) and 16.8% (1998) compared with untreated control plants. Pre-harvest plant vigor ratings, taken twice during each field season, were higher in the bacteriophage-treated plants than in either bactericide-treated plants or non-treated controls except for the early vigor ratings in 1998.

Use of bacteriophages increased total weight of extra-large fruit when compared with non–treated control plants or plants treated with the chemical bactericides. Also, the obtained results of **Jones *et al.* (2007**), **Monk *et al*. (2010) and Tarasi *et al.* (2010)** supported our obtained results.

As for using of hot water (48-50°C/5min) in treating tomato seeds and seedlings of cv. Super strain B to control the disease incidence% of the pathogenic bacteria indirectly. All hot water treatments were effective in increasing the survived plants compared with the control treatment.

Also, increasing the inoculum levels increased gradually the dead % of tomato plants whether of seeds or seedlings when treated with hot water. These results could be discussed in light the findings of **McMillan (1987)** who confirmed the role of hot water in controlling the bacterial spot pathogen on tomato seeds.

Regarding the effect of control treatments on enzymes activities correlated to resistance of tomato plants (cv. Super strain B) against infection with leaf spot disease caused by *X. vesicatoria* during two growing seasons 2009 and 2010 under greenhouse conditions, it was found that bactericides, plant oils and garlic extract, resistance inducers and bio-agents, increased the activity of peroxidase enzyme compared to the control treatment. In this respect,

the activities of peroxidase enzyme reached their maximum at 7 days post treating with the control treatments.

Also, the activities of peroxidase enzyme were high at 3 and 10 days post treating with the control treatments comparing to check treatment (infested without treating). Copper oxychloride recorded the highest increase in peroxidase activity as mg/g fresh weight followed by Kombusha, garlic extract and ascorbic acid respectively. Meanwhile, the least determined activity of peroxidase enzyme was recorded with the bio-agent (*Ps. fluorescence*-1) at the same period.

Moreover, all tested treatments increased the activities of peroxidase comparing with check treatment (infected without treating). On the other hand, all tested control treatments increased the activity of polyphenoloxidase enzyme compared to the check treatment. In this respect, the activities of polyphenoloxidase enzyme reached their maximum also at 7 days post treating with the control treatments. Also, the activities of polyphenoloxidase enzyme were high at 3 and 10 days post treating with the control treatments comparing to check treatment (infested without treating). Ascorbic acid, copper oxychloride, garlic extract, Kombusha and galbin-cu recorded the highest increase in polyphenoloxidase activity as mg/g fresh weight respectively.

Meanwhile, the least determined activity of peroxidase enzyme was recorded with the bio-agent (*Ps. fluorescence*-1) at the same period. The highest increase in polyphenoloxidase activity was recorded at 7 days post treating with copper oxychloride followed by garlic extract, galbin-cu, tetracycline and ascorbic acid respectively. At 10 days post treating with the different control treatments, the

highest increase in polyphenoloxidase activity was recorded with copper oxychloride followed by galbin-cu and garlic extract respectively.

On the other hand, the least activity of polyphenoloxidase at 7 and 10 day were recorded with the bio-agent (*Ps. fluorescence-2*). Treating the tomato plants with bactericides, plant oils and garlic extract, resistance inducers and bio-agents, in order to control the bacterial leaf spot disease caused by *Xanthomonas* increased the free, conjugated and total phenols in infested treated tomato leaves at 10 days post treating comparing to check treatment (infested without treating) during seasons 2009 and 2010. The highest increase in total phenols was recorded with treating the tomato plants with copper oxychloride, tetracycline and garlic extract respectively during the two seasons with the three *Xanthomonas* isolates. It was clear from the obtained results that the estimated free phenols were more than the determined conjugated ones with all tested control treatments for the three *Xanthomonas* isolates during the two seasons.

Treating the tomato plants with bactericides, plant oils and garlic extract, resistance inducers and bio-agents, in order to control the bacterial leaf spot disease caused by *Xanthomonas* increased the free, conjugated and total phenols in infested treated tomato leaves at 10 days post treating comparing to check treatment (infested without treating) during seasons 2009 and 2010. The highest increase in total phenols was recorded with treating the tomato plants with copper oxychloride, tetracycline and garlic extract respectively during the two seasons with the three *Xanthomonas* isolates.

It was clear from the obtained results that the estimated free phenols were more than the determined conjugated ones with all

tested control treatments for the three *Xanthomonas* isolates during the two seasons.

These results could be discussed in light the findings of **Cavalcanti *et al*. (2006)** who investigated the induced defense responses and protective effects on susceptible tomato against *Xanthomonas vesicatoria* by a heat-treated aqueous extract (VLA) from dry necrotic tissue of 'Lobeira' (*Solanum lycocarpum* St. Hil.) branches infected with the fungus *Crinipellis perniciosa* (Stahel) compared with acibenzolar-S-methyl (ASM), a commercial inducer of resistance. Plantlets were sprayed with VLA and ASM and challenged 4 days later with a virulent strain of *X. vesicatoria*, under greenhouse conditions.

The disease severity, fresh weight of shoots, the activities of phenol peroxidase (POX), polyphenoloxidase (PPO), chitinase (CHI), phenylalanine ammonia-lyase (PAL), lignin deposition, and soluble phenolic contents were evaluated in the leaf tissues. Reduction of the bacterial spot severity was observed in plantlets treated with VLA which conferred 63% of the ASM protection. This protective effect and lesion reduction promoted by VLA were probably associated particularly with POX and PAL activities, lignin deposition on leaf tissues and, to a less extent, CHI activity.

SUMMARY

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important economical crops, which belong to family Solanaceae. Tomato cultivated for its fruits of economic importance and nutritional value and high tomato plants important in domestic consumption , export and food industries. Bacterial spot of tomato caused by *Xanthomonas campestris pv. vesicatoria* (Doidge) Dye (*Xvv*) is present wherever tomato and peppers are grown. In general, *Xanthomonas* pathovars have narrow host ranges.

The obtained results of the present study could be summarized as follows:

1. Sum of 60 bacterial isolates were obtained from naturally infected tomato collected from naturally infected tomato leaves and seeds in four locations *i.e.* El-Doki (Super Marmand, cv), Qaha (Peto 86 cv and super strain B cv), Fac. of Agriculture Ain Shams -Kalubia Governorate (Money maker, Super Marmand and Super strain B cv) and Rashid (Mors44, Niagra) Domiata Governorate. The isolated bacteria belong to 4 genera and 4 species.

2. *Xanthomonas vesicatoria* was the more occurred (30 trials of frequency) in all tested samples comparing to the other isolated bacteria. The highest isolation number and frequency% of *X. vesicatoria* was recorded in Fac. of agriculture Ain Shams (63.6%) followed by EL-Doki (50%) and Qaha location (35.7%), meanwhile, the least frequency (29.4%) of *X. vesicatoria* was recorded in Rashid location. Other bacteria were isolated and Rashid location showed the highest number and frequency % (58.8%) followed by Fac. of agriculture Ain Shams location (36.4%).

3. Sixteen of cultivars and hybrids were used for the isolation of pathogenic bacteria that caused spots on tomato plants. All tested cultivars, Dora, Gs12, KTM 141, Niagra, Diamante F1, Hypride7796, Super strain B hybrids, Mors44, VT916G.SI Hypride, HMX4791 Hypride and faqlta 38 gave negative reaction when direct planting on the Nutrient agar (NA). Meanwhile, Super strain B was the only resulted positive reaction for *X. vesicatoria* isolated. On the other hand, all used seeds of cultivars and hybrids of tomato were showed gave negative reaction for *Pseudomonas* isolation. Meanwhile, other bacteria that isolated from tomato seeds of cvs and hybrids were different according the cvs, hence seeds of Super strain B, Gs12 and faqlta 38 were the only results for other bacteria isolated.

4. According the morphological, physiological and biochemical characteristics, out of 60 bacterial isolates which isolated from tomato seeds, leaves, stems and fruits, 30 isolates were identified as *Xanthomonas*, 10 identified to each *Bacillus* and *Pseudomonas*. Other lasted of 10 isolates were identified as non-pathogenic bacteria.

5. Regarding of pathogenicity test, 30 isolates of bacteria that isolated from bacterial spot of tomato was tested for their ability to infect of tomato and pepper plants. Twelve isolates of *Xanthomonas vesicatoria*, numbers: Xv_2, Xv_3, Xv_7, Xv_8, Xv_{10}, Xv_{13}, Xv_{14}, Xv_{20}, Xv_{22}, Xv_{23}, Xv_{26} and Xv_{28} were the highly pathogenic and caused bacterial spot on tomato (leaves) and pepper (leaves), meanwhile, eight isolates of *X. vesicatoria* (No. Xv_1, Xv_4, Xv_5, Xv_9, Xv_{11}, Xv_{12}, Xv_{15} and Xv_{24}) were resulted the moderated pathogenic effect by showing a few symptoms of leaves spot on tomato and pepper plants. The last of the ten isolates of *X. vesicatoria* (No. X_6, X_{16}, X_{17}, X_{18},

X_{19}, X_{21}, X_{25}, X_{27}, $X_{29}$and X_{30}) were non-pathogenic and no symptoms was showed on tomato and pepper leaves after their tested.

6. Concerning the host range, three isolates of *Xanthomonas vesicatoria* that caused bacterial spot of tomato, No. Xv_{28} was highly pathogenic in Qaha, Xv_{22} which was highly pathogenic in Rashid and Xv_{10} that highly pathogenic in El-Doki, were used to study of their ability to infect of different hosts. Data indicated that, the three isolated were highly pathogenic and caused bacterial spot on tomato plants, meanwhile, the remained isolates were moderate pathogenic and showed little symptoms on tomato leaves when tested for their pathogenicity. All these tested isolates were highly pathogenic on tomato and pepper and never affected potato and cabbage, common bean, eggplant, lettuce, beans, strawberry and cantaloupe. On the other hand, the three isolates showed moderately effect on datura plants.

7. Different bactericides, antibiotics, oils and plant extracts, inducing resistance, bio-agents were used at different concentrations to study there effect on the growth of pathogenic bacteria *Xanthomonas vesicatoria* isolates (as inhibition zone cm) under *in vitro* conditions. As for the bactericides**,** Galbin-Cu48% and Copper oxychloride 54% had inhibitory effect on growth of the pathogenic bacteria compared with the control. Also, this inhibition was increased according to the increase of concentrations of bactericides. On the other hand, the different isolates of *X. vesicatoria (*Xv_{28}, Xv_{22} and Xv_{10}*)* were varied for their sensitivity to these bactericides. In this respect, Copper oxychloride was more effective than Galbin-cu for the reduction in growth of all isolates.

8. Two antibiotics Erythromycin and Tetracycline were tested at different concentrations for their effect on the growth *Xanthomonas vesicatoria* isolates. All tested antibiotics had inhibitory effect on growth of the pathogenic bacteria compared with the control. This inhibition zone was increased with the increase of rates of antibiotics. Also, different isolates of *Xanthomonas vesicatoria* were varied in tolerance of these antibiotics. In this respect, Tetracycline was more effective than Erythromycin for the increasing of zone inhibition for the three isolates of Xanthomonas. Meanwhile, Erythromycin caused moderately effective to reduce the growth on the three isolates of Xv_{28}, Xv_{22} and Xv_{10}, respectively.

9. Concentrations of the plant extract of garlic (*Allium sativum*) had inhibitory effect on growth of the pathogenic bacteria compared with the control. This inhibition was increased accorded with increasing of rates of plant extract. On the other hand, isolates of *Xanthomonas* were varied for the effect of plant extract.

10. Different concentrations of two different plant oil, mentha oil (*Mentha aquatica*) and clove oil (*Syzygium aromaticum*) were tested on growth *Xanthomonas vesicatoria*. The two different plant oils had inhibitory effect on growth of the pathogenic bacteria compared with the control. This inhibition was increased with the increasing rates of plant oil. On the other hand, the three different isolates of *Xanthomonas vesicatoria* were varied for their sensitivity different plant oils.

11. Acids ascorbic acid and salicylic acid had inhibitory effect on growth of the pathogenic bacteria when compared to the control treatment. The inhibition zone was increased with increasing the rates

of acids. Also, ascorbic acid more effective, (2.77 cm as means of inhibition zone), than salicylic acid (1.38 cm) for inhibition zone the three isolates of *Xanthomonas*. Also, ascorbic acid, was highest effective on the reduce the growth of all isolates, where, it caused 3.5 cm zone inhibition, 2.6 and 3.1 cm of zone inhibition for *Xanthomonas vesicatoria*-$_{10}$,-$_{28}$and-$_{22}$, respectively. Meanwhile, 3.4cm, 2.5 and 2.0 cm of inhibition zone were obtained from salicylic acid on *Xanthomonas vesicatoria*-$_{10}$&*X.v*-$_{28}$ and *X.v*-$_{22}$ respectively.

12. Kombucha was known as metabolic of different microorganisms such as yeasts, fungi and bacteria on tea. Also, it has inhibition effect on different microorganisms as bacteria. In this respect, it was used at different concentrations for testing of their ability to inhibit growth of *X. vesicatoria* isolates, *in vitro*. Results indicated that, inhibition zone of different isolates of *Xanthomonas* were increasing according to the increase of kombucha concentrations. On the other hand, *Xanthomonas vesicatoria*-$_{10}$and *X.v*-$_{28}$ were more sensitive to kombucha and resulted 2.1 cm and 2.0 cm inhibition zone than *X.v* -$_{22}$ gave only 1.8 cm inhibition zone.

13. Two isolates of antagonistic bacteria were tested for their ability to inhibit growth of *X. vesicatoria* isolates, using King's B (KB) medium, *in vitro*. And indicated that tested *Pseudomonas fluorescense* (i.e. Pf1), isolate were more effective against growth of *X. vesicatoria* bacteria on King's B medium, where inhibition zone were 2.9cm,3.5cm and 4.1cm on isolates Xv$_{28}$, Xv$_{22}$andXv$_{10}$, respectively. Meanwhile, isolates of *P. fluorescence* (Pf2) was moderately effective on KB medium against two pathogens, where

inhibition zone were 3.1cm, 3.7cm ,4.5cm on isolates Xv_{28}, $Xv_{22}$and Xv_{10}, respectively.

14. Phages of *Xanthomonas vesicatoria* were isolated from infected leaves of tomato and from tomato rhizosphere soil samples,4- Phages of *Xanthomonas vesicatoria* were isolated from infected leaves of tomato and from tomato rhizosphere soil samples, using enrichment technique. These samples were collected from Qalubia governorate. The phages were mostly isolated from infected leaves of tomatoes and also from the rhizosphere soil of tomato plants in the field. The phages produced different types of plaques. The first type was the mostly frequent commercial, where the plaque was circular with an irregular margin and the second type produced circular plaques without determined margin.

15. Under greenhouse conditions, sixteen of tomato cultivars and genotypes were evaluated to infection with three *Xanthomonas vesicatoria* isolates under greenhouse conditions. Data revealed that, Super strain B and Castle rock were highly susceptible to the infection with *Xanthomonas vesicatoria* isolates. Meanwhile, Peto 86 and Gs12 were moderates susceptible to the infection. Money maker and Dora were tolerant to the infections with *Xanthomonas vesicatoria* isolate. On the other hand, the next ten of tomato cvs and genotypes, *i.e.*, Diamante F1, Hypride7796, Mors44, VT916G.SI, HMX4791, Super strain B H, KTM 141, Niagra, Flora-Dade and faqlta 38, showed resistant to infection with *Xanthomonas vesicatoria* isolates

16. In conclusion, when comparing the effect of different bactericides, oils and plant extracts, inducing resistances and bio-

agents for controlling the bacterial spot of tomato plants that caused by three *Xanthomonas vesicatoria* isolates Xv_{28}, Xv_{22} and Xv_{10} during 2009 and 2010 seasons. Results indicated that all treatments, bactericides, oils and plant extracts, inducing resistances and bio-agents, were effective on controlling the disease on tomato plants when compared with the control treatment. On the other hand, bacteriocides was more effective in the diseases incedence, disease severity, no. of spots/leaf and disease reduction % than other control treatments, oils and plant extracts, inducing resistances and bio-agents, especially after 10 days from the infestation with Xv_{28}. Also, copper chloride, Galbin-cu and tetracycline were the most effective than erythromycin. On the other hand, garlic (*Allium sativum*) extracts was more effective on the diseases incedence, disease severity, no. of spots/leaf and disease reduction % than mentha and clove oils. Regarding the effect of the inducer resistance on tomato plants after the infestation with *Xanthomonas vesicatoria* isolate Xv_{28}, ascorbic and acidic acids were more effective than biological inducer (Kamboushe) in the diseases incedence, disease severity, no. of spots/leaf and disease reduction %. On the other side, *Pseudomonas flurocences* isolate No. 1 was more effective than *Pseudomonas flurocences* isolate No. 2. for the diseases incedence, disease severity, no. of spots/leaf and disease reduction % after 3 and 10 days from the infestation with Xv_{28}, respectively.

17. Comparison between different bacteriophages for controlling the infection of tomato plants after 3 and 10 days form infestation with *Xanthomonas vesicatoria* isolate Xv_{28}, $Xv_{22,}$ Xv_{10}. Obtained results indicated that, all treatments, *i.e*, phage 1, phage 2, phage 3,

phage 4 and the mixed of different phage isolates (1+2+3+4) were effective on controlling bacterial spot on tomato when compared with the control treatment. Also, these treatments were effective in the diseases indx, disease severity, mean of spots/20 leaf and disease reduction % when compared with control treatment after 3 and 10 days from the infestation with *Xanthomonas vesicatoria* isolates Xv_{28}, $Xv_{22,}$ Xv_{10}.

18. Effect of treated tomato seeds or seedling with hot water under infestation of different isolates of *Xanthomonas vesicatoria*. Tomato seeds or seedlings of cv Super strain B were infestated with the three concentration of pathogenic bacteria and then treated with hot water (48-50 ^{0}C/ 5 min) indirect way to study the effect on the disease incidence. Results revealed that all water hot treatment were effective for increasing survived plant compared with control treatment. Data also showed that, positive relationship between the inoculum increasing of *Xanthomonas* isolates and death of tomato plants in the two cases (seeds and seedlings).

19. Concerning the relationship between using different bactericides, oils and plant extracts, inducing resistances and bio-agents on controlling of bacterial spot diseases of tomato and some biochemical changing treated plants (activity enzymes). All treatments i.e., spraying with bactericides, oils and plant extracts, inducing resistances and bio-agents, which sprayed on tomato plants to control bacterial spot disease caused by *Xanthomonas* isolates Xv_{28}, $Xv_{22,}$ and Xv_{10}, increased peroxidase and polyphenoloxidase activity compared with control treatment.

The highest peroxidase activity was detected after seven and ten days compared with three days from spraying in all the three tested isolates of *Xanthomonas.*

20. Copper oxychloride caused the highest increased in peroxidase and polyphenoloxidase through controlling bacterial spots that caused by *Xanthomonas* isolates after three, seven and ten days, respectively. Meanwhile, in general the least effect on the increasing of peroxidase and polyphenoloxidase activity was recorded when erythromycin was sprayed on tomato plants to control Xv_{28}, Xv_{22} and Xv_{10}, respectively. On the other hand, galbin –cu was the highest effective on increasing in peroxidase and polyphenoloxidase activity compared with other bactericides treatments when it used for controlling of Xv_{10}.

21. According of the effect of using oils and plant extracts, *Allium sativum* was the highest effect on increasing of peroxidase and polyphenoloxidase activity through control of bacterial spot that caused by *Xanthomonas* isolates. Also, clove oil was more effective on the increasing of peroxideas and polyphenoloxidase when compared by mentha oil when sprayed on tomato plants to control the bacterial spot that caused by Xv_{28}, Xv_{22} and Xv_{10}, respectively. Regarding of sprayed inducing resistances on tomato plants to control bacterial spot that caused by Xv_{28}, Xv_{22} and Xv_{10} caused increasing of perioxidase and polyphenoloxidase activity. In this respect, ascorbic and salicylic acid were more effective on increasing of perioxidase and polyphenoloxidase activity than kambousha treatment after the three, seven and ten days of inoculation of bacterial spot disease Xv_{28}, Xv_{22} and Xv_{10}.

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الملخص العربي

يعتبر الطماطم واحداً من أهم محاصيل العائلة الباذنجانية الاقتصادية في مصر و العالم. يزرع الطماطم من اجل ثماره الطازجة والتي تتصف بانها غنيه في محتواها من العناصر الغذائية والفيتامينات لذلك تلعب دورا هاما في الاستهلاك المحلي وكذلك تصديرها للسوق الاوربية كما انها تلعب دورا هاما في تصنيعها باشكال مختلفة كعصائر أو معجون. ويتواجد مرض التبقع البكتيري علي نباتات الطماطم والمتسبب عن بكتريا زانثوموناس كامبستر سلالة فيزيكاتوريا يوجد اينما تزرع محاصيل الطماطم والفلفل وبعض النباتات الاخري التي تصاب بهذه البكتريا ويسبب هذا المرض نقصا حادا في المحصول وكذلك يقلل من جودة الثمار الناتجة.

ويمكن تلخيص أهم النتائج المتحصل عليها من هذه الدراسة كالتالي:

١- تم عزل ٦٠ عزله بكتيرية من نباتات طماطم مصابه بمرض التبقع البكتيري من أربعه مناطق مختلفة منزرعة باصناف مختلفة وهى كالتالي : ١- الدقي حيث تم العزل من الصنف سوبر مرماند ٢- منطقه قها حيث تم العزل من الأصناف التالية (بيتو ٨٦ و سوبر سترين B) ٣- كليه الزراعة جامعه عين شمس حيث تم العزل من الأصناف التالية (منى ميكر وسوبر مرماند وسوبر سترين B) ٤- منطقه رشيد حيث تم العزل من الأصناف التالية (مورس ٤٤ و نيجر)، وقد أظهرت نتائج العزل والتعريف ان العزلات المعزولة تتبع ٤ أجناس وأربع أنواع:

٢- كانت العزلة الأكثر تكرار فى الأربعة مناطق والمسببة للمرض هي عزلة *Xanthomonas vesicatoria* حيث كانت نسبه التكرار من كليه الزراعة جامعة عين شمس هي (٦٣.٦%) ومنطقه الدقي ٥٠.٠% و منطقه قها (٣٥.٧%) ومنطقه رشيد (٢٩.٤%) . وقد تم عمل اختبار القدرة الامراضيه لتحديد أعلى العزلات ضراوة في احداث المرض.

٣- تم العزل أيضا من بذور مجموعه من الأصناف والهجن Flora-Dade , Money maker, Castle rock, Peto86 وتم عزل بكتيريا *Xanthomonas vesicatoria* و التى كانت الأكثر تكرارا على هذه البذور،أما باقي البذور

المستخدمة فلم يتم عزل البكتيريا منها وهى: KTM, Dora, Gs12141, Niagra, Diamante F1, Hypride7796, Mors44, VT916G.SI, Hybrid HMX4791, Hybrid Faqlta 38.

٤- استخدمت الطرق التقليديه في تعريف العزلات البكتيرية المعزولة باستخدام الاختبارات البيوكيميائيه والكيميائية واعتمدت هذه الاختبارات على مورفولجيه وفسيولوجية العزلات البكتيرية وقد أكدت تلك الاختبارات ان ٣٠ عزله تنتمي لبكتريا *Xanthomonas vesicatoria* و١٠ عزلات تنتمي لبكتريا *Bacillus* و١٠ عزلات أخرى تنتمي لبكتريا *Pseudomonas*.

٥- بعد إجراء اختبارات القدرة المرضية على الـ ٣٠ عزله من بكتيريا *Xanthomonas vesicatoria* وجد ان ١٢ عزله منها لها قدره امراضيه شديدة وقد تم التعريف لتلك العزلات .

٦- درس المدى العوائلى للعزلات المختبرة باستخدام بعض النباتات من عائلات نباتيه مختلفة وهي البطاطس والفراولة والطماطم والفلفل والكرنب والفاصوليا والداتورة والباذنجان والخس والفول البلدي حيث ظهر ان العزلات كانت شديدة المرضية على كل الطماطم والفلفل ومتوسطه التأثير على الداتورة.

٧- أظهرت الاختبارات المعمليه أن استخدام مستخلص الثوم بأربعه تركيزات مختلفة هي ٢.٥، ٥، ١٥ و ٢٠% . كانت أفضل النتائج عند التركيز الأعلى مع الثلاث عزلات المختبرة حيث كانت اكبر مساحة تثبيط للبكتيريا حيث أعطت العزلة -٢٢ (٣ سم) والعزلة -١٠ (٣.١سم) والعزلة -٢٨(٢.٤سم) .

٨- اختبر زيت النعناع وزيت القرنفل كزيوت طبيعيه ضد البكتيريا المختبرة معمليا على أطباق بتري باستخدام أربعه تركيزات مختلفة(٢.٥، ٥، ١٥ و ٢٠ %) وقد وجد أن زيت النعناع كان الأعلى تأثيرا من زيت القرنفل على الثلاث عزلات البكتيرية الممرضة حيث دلت نتائج زيت النعناع أنها ثبطت العزلة -٢٨ لتكون مساحة التثبيط كانت(٢.٥سم) والعزلة -٢٢ (٢.٣سم) والعزلة -١٠(٣.٥سم) أما زيت القرنفل فكانت مساحة التثبيط علي العزلة -٢٨ (١.١سم) و-٢٢ (٢.٢ سم) و العزلة - ١٠(٣.١سم).

٩- استخدم نوعين من الأحماض العضوية (كمحثات للمقاومة في النبات) وهي أحماض الاسكوربيك والسالسليك معمليا باربعه تركيزات وهى ٥٠ و١٠٠ و٢٥٠

و٥٠٠ جزء في المليون. وقد أظهرت النتائج أن حمض الاسكوربيك هو الأكثر تأثير من حمض السالسليك حيث كان التركيز الرابع ٥٠٠ جزء في المليون هو أفضل التركيزات وأكثرها تأثير على بكتيريا *Xanthomonas vesicatoria* كما كان مقدار التثبيط مختلف من عزله لأخرى فقد كانت مساحة التثبيط لحمض الاسكوربيك علي العزلة- ١٠ هو (٣.٥ سم) والعزلة -٢٢هو (٢.٦ سم) والعزلة -٢٨ هو(٣.١ سم) أما حمض السالسليك فكان الأقل تأثيرا على هذه العزلات المختبرة حيث كانت مساحة التثبيط علي العزلة -١٠ هو(٣.٤ سم) والعزلة -٢٨هو(٢.٥ سم) والعزلة -٢٢ هو(٢.٠ سم).

١٠-استخدمت الكمبوشيا وهى عبارة عن ناتج ايضى من تفاعل عدد من الكائنات الحية (خميره +فطريات+ بكتيريا) يستخدم في تثبيط عدد من الكائنات الدقيقة مثل(الفطريات والبكتريا) في تثبيط البكتيريا . واستخدمت أربعه تركيزات هي ٢.٥ و٥ و ١٥ و٢٠% حيث أظهر التركيز ٢٠% أعلي مساحة تثبيط علي العزلة -١٠ هو (٢.١سم) والعزلة -٢٨ وهو (٢.٠سم) بينما ثبطت العزلة – ٢٢ بمعدل (١.٨ سم).

١١-تم استخدام نوعين من المضادات الحيوية وهما تتراسيكلين واريثريومايسين حيث كانت التتراسيكلين الأعلى تأثير على العزلات البكتيرية مقارنه بالارثيروميسين. حيث كانت نسبه التثبيط أعلى في التتراسيكلين حيث كان تثبيط العزلة -٢٨ هو ٢.٩والعزلة-٢٢ هو٢.٣ والعزلة-١٠ هو٣.٧سم و أما تأثير الايثرومايسين كان تثبيط العزلة -٢٨ هو٢.١والعزلة-٢٢ هو٢.٤ والعزلة-١٠ هو٣.٢سم

١٢- ايضا استخدم نوعين من المبيدات البكتيرية وهو اوكسى كلورو النحاس وجالبين نحاس حيث كان مبيد اوكسى كلورو النحاس أكثر تأثير على العزلات الممرضة لهذه البكتريا وكانت التركيزات المستخدمه١٠٠، ٢٥٠ ، ٥٠٠ و٧٥٠ جزء في المليون وكانت نسبة التثبيط علي العزلة -٢٨ ٤.٢سم والعزلة الثانية ٣.٥ سم والعزلة الثالثة ٣.٦ سم بينما كانت مساحة التثبيط لمبيد جالبين نحاس الأقل تأثير علي العزلة -٢٨ ٢.٧ سم والعزلة الثانية ٣.٠سم والعزلة الثالثة ٣.٢-سم .

١٣-عند استخدام المبيدات البكتيرية في الصوبة كانت أفضل المعاملات لمبيد اوكسى كلورو النحاس على الثلاث عزلات البكتيرية المختبرة. يليها مبيد جالبين نحاس ثم المضادات الحيويه.

١٤- تم عزل الفاج (لاقمات البكتريا) المتطفل علي بكتريا زانثوموناس فيزيكاتوريا من عينات الطماطم المصابة بالتبقع البكتيري (الأوراق ومنطقة الريزوسفير) من منطقة محافظة القليوبية. لهذا الفاج صفات مورفولوجية محدده تم وصفها من خلال الميكروسكوب الالكتروني. وتم استخدامة كاسلوب حديث في مقاومة مرض التبقع البكتيري علي الطماطم.

١٥- قيمت ١٦ صنف ونوع من الطماطم تحت ظروف الصوبة والمحقونة ب ٣ عزلات من الزانثمونس فزكتوريا وكانت النتائج كالتالي : كل من سوبر استرين بى وكاسل روك كانوا الاكثر حساسية للعزلات بينما كان بيتو ٨٦وجى اس ١٢ كانوا متوسطى الاصابه اما مونى ميكر ودورا كانوا الاكثر تحملا للمرض . ومن ناحية أخرى وجد عشره أنواع وهى Diamante F1, Hypride7796, Mors44, VT916G.SI, HMX4791, Super strain B H, KTM 141, Niagra, Flora-Dade and faqlta 38 كانت الاكثر مقاومه للثلاث عزلات.

١٦- تلخيصا لما سبق ، وجد عند المقارنه بين كل من المبيدات البكتيرية والزيوت الطبيعية والمستخلصات النباتية والمستحثات والكائنات البيولوجية فى مقاومه مرض التبقع البكتيري فى الطماطم (الثلاث عزلات لبكتيريا الزانثمونس فزكتوريا خلال ٢٠٠٩/٢٠١٠) وكانت النتائج كالتالي: وجد المبيدات البكتيرية والزيوت والمستحثات كانت اكثر كفاءه فى مقاومه المرض مقارنه بالكنترول ومن ناحيه اخرى كانت المبيدات البكترية أكثر كفاءه من حيث التأثير على الشدة المرضية ومتوسط عدد البقع فى ٢٠ ورقه مقارنه بالكنترول اما بالنسبة للعزلة- ١ كان كل من كلوريد النحاس وجالبين نحاس وتتراسيكلين كانت اكثر كفاءه من الاريثروميسن بعد ١٠ ايام من الحقن ومن ناحيه اخرى كان مستخلص الثوم اكثر كفاءه فى مقاومه المرض مقارنه بزيت القرنفل والنعناع .اما عن كل من الاسكوربيك والسلسيلك كانوا اكثر كفاءه من الكمبوشا وظهر ذلك فى كل من الشدة

المرضية ومتوسط عدد البقع ل٢٠ ورقه أما في حاله المقاومة البيولوجية فقد كانت العزلة بسيدومونس رقم١ اكثر كفاءه من العزلة - ٢ على التوالى .

١٧ - بالمقارنة بين أربعه أنواع من البكتيرويوفاج المعزولة من نباتات طماطم مصابه بمرض التبقع البكتيري في الطماطم والمستخدمة فى مقاومه المرض فقد تم استخدام كل منهما على حده فى المقاومة ثم خليط من الأربعة وكانت النتائج أن كل المعاملات كانت ذات كفاءه عالية في عمليه المقاومة من حيث الشدة المرضية ومتوسط عدد البقع واختزال المرض بعد ١٠ أيام من المعاملة وذلك على النباتات المحقونة بالثلاث عزلات البكتيرية المستخدمة مع ملاحظة إن استخدام خليط الفاج أعطى ١٠٠% نقص فى المرضية.

١٨ - تأثير معامله البذور والبادرات (سوبر أسترين) بالماء الساخن بعد الحقن بثلاث عزلات من الزنثموناث بثلاث تركيزات مختلفه ثم معاملتها بالماء الساخن لمده ٥ ق بطريق غير مباشر ودراسه تأثير تلك المعاملة فقد وكانت النتائج كالتالي : المعاملة بالماء الساخن كانت مؤثره فى قدرتها على بقاء النباتات مقارنه بالكنترول ودلت النتائج على وجود علاقة بين زيادة تركيز اللقاح وموت النباتات.

١٩- تأثير المعاملات المختلفة للمبيدات البكتيرية والزيوت والمستخلصات والمستحثات والمعاملة بكائنات الدقيقة فى المقاومة وتأثير ذلك على بعض التغيرات الكيماوية (نشاط الأنزيمات) فى النباتات المعاملة وكانت النتائج تدل على زيادة النشاط الانزيمى للبيروكسيديز والبولى فينول اوكسيديز مقارنه بالكنترول. وكانت أعلى نشاط للأنزيم البيروكسيديز بعد ٧ أيام من الرش مقارنه بنشاط الأنزيم بعد ٣ أيام وذلك بالنسبة للثلاث عزلات الزنثمونس .

٢٠- المعاملة باوكسى كلوروالنحاس آدت إلى زيادة فى أنزيم البيروكسيديز والبولى فينول اوكسيديز بعد ٣ و٧ و١٠ ايام من الحقن بثلاث عزلات من البكتيريا . بينما كان اقل تأثير فى زيادة نشاط كل من الأنزيمين فى المعاملة بالايثروميسين مقارنه بالكنترول . ومن ناحيه اخرى ،كانت الزيادة فى نشاط الأنزيمات ملحوظة بعد الحقن بالعزلة رقم ٣ مقارنه بالكنترول .

٢١ –تأثير كل من المعاملات بالزيوت والمستخلصات النباتية (مستخلص الثوم) والتى أدت إلى زيادة نشاط انزيمى البيروكسيديز والبولى فينول اوكسييديز مقارنه بالكنترول المحقون بكتيريا التبقع البكتيرى فى الطماطم ووجد أيضا أن المعاملة بزيت القرنفل كان لها تأثير فى زيادة نشاط الأنزيم الالبيروكسيديز والبولى فينول اوكسيديز مقارنه بالنباتات المحقونة بثلاث عزلات المسببه لمرض التبقع البكتيرى وعن معامله هذه النباتات بزيت النعناع ، وجد أيضا زيادة فى نشاط الانزيمى فى لمعامله بكل من الاسكوربيك والسالسيلك وكانت أكثر تأثيرا مقارنه بمعامله الكمبوشا بعد ٣ و٧و١٠ أيام من الحقن بالثلاث عزلات .

إهداء

إلى من أوجب الله علىّ طاعتهما ، وفرض علىّ برهما .

إلى أبى رحمه الله تعالي رحمة واسعة و غفر له و أسكنه العلى من الجنان .

وإلى أمى متعها الله بالصحة والعافية وبارك فى عمرها ورزقها حسن الخاتمة . وإلى زوجى العزيز الذى طالما ساندنى و أيدنى و كان دافعاً لى طوال مشوارى الأكاديمى نصحاً و إرشاداً و مساعدة .

إليهم جميعاً أهدى هذا العمل المتواضع .راجية الله عز و جل أن يتقبل منا أعمالنا و أن يهدينا إلى سواء السبيل إنه نعم المولى و نعم النصير .

وصلى اللهم وسلم وبارك على سيدنا محمد وعلى آله وصحبه أجمعين

صفحة الموافقة علي الرسالة

المقاومة البيولوجية والكيماوية لأمراض التبقع البكتيري في الطماطم

رسالة مقدمة من

إيمان عثمان حسن علي

بكالوريوس أمراض النبات
كلية الزراعة بمشتهر – جامعة الزقازيق/فرع بنها (١٩٩٨)
ماجستير أمراض النبات/جامعة الزقازيق/فرع بنها (٢٠٠٤)

للحصول على درجة الدكتوراة للعلوم الزراعية
في أمراض النبات
(أمراض بكتيرية)

تمت الموافقة على هذه الرسالة من قبل لجنة الفحص والمناقشة:

اللجنـــة:

أ.د./ نـــــــوال عبد المنعم عيسى.....
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أستاذ أمراض النبات – كليه الزراعة - جامعه الإسكندرية – ممتحناً

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أستاذ أمراض النبات - كلية الزراعة بمشتهر - جامعة بنها- مشرفاً – ممتحناً

تاريخ المناقشة: الخميس ٢٠١١/٥/١٢م

لـجنـة الإشـراف

الـمقـاومـة الـبيـولـوجيـة والـكيـمـاويـة لأمـراض الـتـبقـع الـبـكـتـيـري فـي الـطـمـاطـم

رسالة مقدمة من رسالة مقدمة من

إيـمـان عـثـمـان حسن عـلـي

بكالوريوس أمراض النبات
كلية الزراعة بمشتهر – جامعة الزقازيق/فرع بنها (١٩٩٨)
ماجستير أمراض النبات/جامعة الزقازيق/فرع بنها (٢٠٠٤)

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أ.د./ نـــــــوال عبد الـمنعم عيسى .....

أستاذ أمراض النبات - كلية الزراعة بمشتهر - جامعة بنها

أ. د./ محمد هـــــارون عبد الـمجيد .....

أستاذ أمراض النبات - كلية الزراعة بمشتهر - جامعة بنها

أ. د./ نـاجي يـاسين عبد الـغفـــــار .....

أستاذ أمراض النبات - كلية الزراعة - جامعة عين شمس

أ.د./ فـتحي جـاد محمد عبد الـجواد .....

أستاذ أمراض النبات - كلية الزراعة بمشتهر - جامعة بنها

قـسم الـنبـات الـزراعي
كـلـيـة الـزراعـــــة
جـامـعـة بـنـهـا

المقاومة البيولوجية والكيماوية لأمراض التبقع البكتيري في الطماطم

رسالة مقدمة من

إيمان عثمان حسن على

بكالوريوس أمراض النبات

كلية الزراعة بمشتهر – جامعة الزقازيق/ فرع بنها (١٩٩٨)

ماجستير أمراض النبات / جامعة الزقازيق/ فرع بنها (٢٠٠٤)

للحصول على درجة الدكتوراه في الفلسفه العلوم الزراعية
في أمراض النبات

قسم النبات الزراعي
(فرع الفطر و أمراض النبات)

كلية الزراعة
جامعة بنها

٢٠١١