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Effect of Some Commercial Bacteriotoxicants on Development of Bacterial Spot Disease in Tomato Caused by *Xanthomonas vesicatoria*

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ABSTRACT

Antibacterial activity of some commercial bacteriotoxicants against tomato bacterial spot disease caused by the bacterium, *Xanthomonas vesicatoria* was screened *in vitro* and *in vivo*. Antagonistic effect of tested bacteriotoxicants *i.e.* Bio arc "*Bacillus megaterium* 2.5x10^7 CFU/g" (at conc. 1.5, 2.5 and 3.5 g/L), garlic oil (at conc. 0.3, 0.5 and 1.0%), oxytetracycline (at conc. 12.0, 12.5 and 13.0 µg/ml) and copper oxychloride (at conc. 1.0, 1.5, 2.0 g/L) was achieved. All tested treatments had clear significant inhibitory effects *in vitro* on growth of *X. vesicatoria* with increasing concentration of each bacteriotoxicant in comparing with control treatment. Maximum inhibitory effect was attained by copper oxychloride at 2.0 g/L (6.87 mm) followed by oxytetracycline at 13.0 µg/ml (4.70 mm), garlic oil at 1.0% (3.90 mm) and Bio arc at 3.5 g/L (2.83 mm). Applying of median concentration of each tested commercial bacteriotoxicant under open field condition individually in regular or in un-regular succession (in program form) as foliar spray considerably and significantly reduced bacterial spot disease development (disease % and disease severity %). Maximum reduction in disease development was achieved by applying prog-4 which served as treated control (sprayed with copper oxychloride) followed by prog-8 and prog-10, respectively. All tested treatments (programs) were significantly increased total tomato yield. Moreover, all tested programs increased clearly total phenol content, peroxidase, polyphenoloxidase and chitinase activity as well as, increased vitamin C content.

Key words: Tomato.bacterial.spot, *X. vesicatoria*, Bacteriotoxicants, Control

Introduction

Bacterial spot disease of tomato plant caused by *X. vesicatoria* is one of most deleterious disease affecting tomato culturing wherever tomato being cultivate causing significant losses when environmental conditions are ideal for the pathogen (Pohronezny and Volin, 1983). Chemical control by using copper and relying on antibiotic sprays has been screened for control bacterial diseases (Thayer and Stall, 1961; Conover and Gerhold, 1981; Jones and Jones, 1985; Obradovic *et al.,* 2008). Excess copper can be enormously toxic, due in part to redox-activity where copper ions can catalyze the production of deleterious reactive oxygen species (ROS), and also because of its propensity to create extremely stable complexes with cellular components (Macomber *et al.,* 2007; Macomber and Imlay, 2009). Antibiotics are broad-spectrum agents, exhibiting activity against a wide selection of gram-positive and gram-negative bacteria. It's well established that tetracyclines inhibit bacterial protein synthesis by steering clear of the association of aminoacyl-tRNA with the bacterial ribosome (Chopra *et al.,* 1992; Schnappinger and Hillen, 1996). On the other hand, since 1980s, as a result of the excessive application of such chemicals (copper-containing bactericides) and antibiotics, copper-tolerant and antibiotic-tolerant strains of *X. vesicatoria* became quite prevalent (Obradovic *et al.,* 2008).

Recently and commercially, ecofriendly biocontrol agents; antibiotics, *Bacillus megaterium* and garlic oil has been marketed as biopesticides, biofertilizers and soil amendments (George *et al.,* 2004; Harman *et al.,* 2004). *B. megaterium*, belonging to plant growth promoting rhizobacteria (PGPR) is considering common soil beneficial bio-fertilizer and have been used as biocontrol agents against soil borne, foliar and post-harvest pathogens (Abou-Zeid and Zaid, 2006; Padgham and Sikora, 2007; Oliveira *et al.,* 2009). Egamberdiyeva, (2007) hypothesized that there are several mechanisms where rhizospheric bacteria may stimulate plant growth, such as production of plant growth

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substances, phytohormones, vitamins, solubilizing minerals and nitrogen fixation, besides their role in direct inhibition of pathogen growth, suppression of diseases and improved of plant growth and yield.

The antimicrobial activities of garlic and other plant alliums are primarily predicated on allicin, a thiosulphinate contained in crushed garlic bulbs which, accounts for approximately 75% of garlic-derived sulphinates (Block 2010, Fujisawa *et al.,* 2008, Waag *et al.,* 2010). The mechanisms by which allicin and other garlic compounds inhibit or kill bacteria proposed that inhibition of RNA synthesis is a crucial target of allicin action (Feldberg *et al.,* 1988), destruction of the microbial cells, decreasing the oxygen uptake, reducing cellular growth, inhibiting the synthesis of lipids, proteins and nucleic acids, changing the lipid profile of the cell membrane and inhibiting the synthesis of the microbial cell wall (Gupta and Porter, 2001), furthermore, allicin and other thiosulphinates are also proven to react with cysteine to get rid of antimicrobial activity (Fujisawa *et al.,* 2009) and to obstruct acetyl-CoA synthases from plants, yeasts and mammals (Focke *et al.,* 1990). Essential oils were also suggested for controlling of several tomato diseases (Soylu *et al.,* 2006, Soylu *et al.,* 2010).These reports declare that treatment of tomato plants with various essential oils from diverse plant origins could be the way to get proper disease control methods for bacterial wilt disease.

The management of plant diseases in the field includes cultural practices, crop rotation, fungicide applications, soil solarization and using of resistant or tolerant varieties of plants. At present, just one or an individual method of each of pre-mentioned practice does not provide acceptable level in controlling of plant diseases. On the other hand, there is a comprehensive array of (bio) cides showing different levels of antimicrobial activity. In this respect, it is generally accepted that, (bio) cides have one or multiple target sites within the microbial cell and the entire damage to these target sites results in the bactericidal effect. Hence, this research aimed to throw a light on the possibility of using different successions (in a program form) of some commercially bacteriotoxicants *i.e.* copper oxichloride, Bio arc (*B. megaterium*), garlic oil and oxytetracyclin as foliar spray. Information regarding the antimicrobial mechanisms and efficacy of each of tested bacteriotoxicants individually might give some useful indications about reducing of diseases assessments. Meanwhile, there is still a lack of understanding of the mode of action of those bacteriotoxicants in the tested succession.

Materials and Methods

Isolation and purification of causal bacterium:

Isolation of the causal bacterium was done from leaves of naturally infected tomato (*Lycopersicon esculentum* Mill.) plants, cultivated in El-gharbia, El-sharqia, El-qalubia and El-giza governorates. The infected leaves samples with bacterial spot lesions were picked and surface sterilized for 30 sec in 1% sodium hypochlorite, removed, then rinsed 3 times in sterile distilled water and then, dried in-between two sterilized filter papers. The samples were cut into small patch specimens, with 2-3 mm of each, each one was macerated in 0.5 ml sterilized distilled water using sterile pestle. Then, each sample suspension was streaked onto nutrient agar medium plates. The inoculated plates were incubated at 28°C for 48h. Randomly, initiated bacterial colonies were picked and transferred to new nutrient agar medium plates for purification. The resulting bacterial colonies were further purified and sub-cultured repeatedly, until pure cultures were obtained.

Identification of causal bacterium:

The resulted bacterial isolates were identified on the basis of physiological, morphological and biochemical characteristics in accordance with Parry *et al.,* (1983) and Holt and Krieg (1984).The identification of *X. vesicatoria* was confirmed by testing for xanthomonad determinative characteristics (Table, 3) using of standard bacteriological methods (Schaad *et al.,* 2001).

Pathogenicity and host range test:

Pots with 10-cm in diameter were filled with formalin-sterilized clay soil. Under laboratory conditions, aforementioned purefied *X. vesicatoria* isolates was tested for its pathogenicity using four crop species *i.e.* tomato (super strain B hybrid), eggplant (balady cv.), pepper (balady cv.) and bean (bolista cv.). Each of tested *X. vesicatoria* isolate was grown in nutrient agar medium for 48h at 28°C,

and then suspended in sterile distilled water and centrifuged at 3000 rpm/min for 30 min. The pellets were re-suspended in distilled water and adjusted turbidimetrically (O.D. at 660 = 0.06) to approximately density of 10^8 CFU/ml. Thirty days old plants belonging to each crop were artificially inoculated with 1×10^8 CFU/ml separately using low pressure hand atomizer. Before inoculation, plants were sprayed with water to make a thin film of water on leaf surface. Tween 80 was added to the bacterial suspension by a rate of 0.1 ml per liter. Plants sprayed with only water served as check (control). Inoculated plants were kept in moist hyaline polyethylene sacs for 2 days as a moist chamber. Disease severity percentage was recorded two weeks post inoculation according to Abbasi *et al.,* 2002. This laboratory experiment laid out in randomized complete design (RCD) with four replicates (pots), each pot compromising one plant.

Antibacterial assay of isolated bacterium X. vesicatoria:

The antibacterial activity of four commercial bactericides *i.e.* Bio arc (*Bacillus megaterium* $2.5x10^7$ CFU/g) at concentrations 1.5, 2.5, 3.5 g/L; garlic oil at 0.3, 0.5, 1.0%, oxytetracycline at 12.0, 12.5, 13.0 µg/ml and copper oxychloride at 1.0, 1.5, 2.0 g/L were screened against highest virulent *X. vesicatoria* isolate *in vitro*. The antibacterial activities of tested commercial bactericides were accomplished on nutrient agar plates (90 mm in diameter). The bacterial suspension was adjusted turbidimetrically to approximately 10^8 CFU/ml. Plates containing nutrient agar medium. (15 ml/plate) were seeded with 0.1 ml of prepared bacterial suspension that has been evenly spread on the surface of medium by means of a glass rod spreader. And then, four wells (5 mm in diameters) were made by means of cork borer close to the edge of each plate and 0.1 ml of any tested bactericides preparations was put into the resulting wells (Gould and Bowie, 1952). After incubation for 48h at 28°C, mean of inhibition zone for each treatment was determined. This laboratory experiment laid out in randomized complete design (RCD) with three replicates (each plate represented replicate).

Field experiment:

The current study was conducted out at the Experimental Station, Moshtohor, Faculty of Agriculture, Benha University, Egypt during the two winter growing seasons 2013/14 and 2014/15. This experiment was carried out on tomato plants (super strain B hybrid) naturally infected with bacterial leaf spot under open field condition to evaluate the efficacy of four commercial bactericides *i.e.* Bio arc (*Bacillus megaterium* $2.5x10^7$ CFU/g), garlic oil, oxytetracycline and copper oxychloride. The median concentration of each tested bactericide which has inhibitory effect on the tomato leaf spot pathogen (*X. vesicatoria*) *in vitro* tests were subjected to *in vivo* test to ascertain its efficacy against bacterial leaf spot disease development. The tested bacteriotoxicants were applied individually in regular or in un-regular succession (Table, 1) in form of control programs as foliar spray.

Table 1: Schematic diagram illustrates main treatments applied as foliar spray individually in regular or in un-regular succession

Tested Program		1st spray (at 30-DPT)	2nd spray (at 50-DPT)	3rd spray (at 70-DPT)
Regular succession	Prog-1	Bio arc	Bio arc	Bio arc
	Prog-2	Garlic oil	Garlic oil	Garlic oil
	Prog-3	Oxytetracycline	Oxytetracycline	Oxytetracycline
	Prog-4	Copper oxychloride	Copper oxychloride	Copper oxychloride
Un-regular succession	Prog-5	Bio arc	Garlic oil	Oxytetracycline
	Prog-6	Bio arc	Oxytetracycline	Garlic oil
	Prog-7	Garlic oil	Bio arc	Oxytetracycline
	Prog-8	Garlic oil	Oxytetracycline	Bio arc
	Prog-9	Oxytetracycline	Bio arc	Garlic oil
	Prog-10	Oxytetracycline	Garlic oil	Bio arc
Control		Water	Water	Water

The experimental treatments were organized in randomized complete block design with three replicates (plots). Each experimental plot included 4 ridges, every one of 70 cm wide and 3.75 m long.

Plot area was 10.5 m^2.On the 15th of October, transplanting of thirty-days old of Super Strain B hybrid tomato seedlings took place in one side of the ridge in the presence of water at 30 cm apart and each plot contained 48 plants. All recommended agronomic practices were carried out for cultivation of tomato plants, except application of fungicide practices. All treatments (programs) were conducted three times as foliar spray, first at 30-days post transplanting (DPT) as a protective treatment before initiation of the disease symptom, second at 50-DPT and finally third at 70-DPT. Prog-4 served as treated control where sprayed with copper oxychloride. Plants which sprayed with water only served as check (control) treatment. Each spray treatment supplemented with tween 80 by a rate of 0.1 ml/L. This trail was designed as a randomized complete plot design (RCPD) with three replicates for each treatment.

Disease and total yield assessments:

The disease percentage and disease severity percentage of bacterial leaf spot was recorded at 90-days post transplanting. Disease percentage (Disease %) was calculated and expressed in percentage scale by using the following formula: Disease %= (D/T) X 100, where, (I) = Number of diseased plants; (T) = Total observed plants. Disease severity percentage was calculated using the disease scale of Abbasi *et al.,* (2002) as follows: 1 =no disease symptoms, 2 = a few necrotic spots on a few leaflets, 3 = a few necrotic spots on many leaflets, 4 = many spots with coalescence on few leaflets, 5 = many spots with coalescence on many leaflets, 6 = severe disease and leaf defoliation, and 7 = plant dead. Bacterial leaf spot disease severity % was assessed according to the following formula: Disease severity % = Σ(n X v)/7N) X 100, Where: (n) = Number of plants in each category; (v) = Numerical values of symptoms category; (N) = Total number of plants; (7) = Maximum numerical value of symptom category.

As well as, total tomato yield (ton/feddan) for each treatment (program) was recorded for each growing season.

Biochemical assessments:

Samples representing the tenth plant leaf were taken apically from each particular treatment (program) for determining of total phenols contents, oxidative enzymes assessments and vitamin C content.

- *Total phenol contents determination:*

For total phenol contents determination, leaves samples were extracted separately by using the method suggested by kâhkônen *et al.,* (1999). The total phenol contents in extracts was determined by Folin – Ciocateu method as modified by Singleton and Rossi (I965), and were calculated for each treatment as milligrams of gallic acid per one gram dry weight (mg GA/DW) according to standard curve of gallic acid.

- *Oxidative enzymes activity:*

The crude leaf enzyme extract was prepared as recommended by Ni *et al.,* (2001). The activity of peroxidase enzyme was measured as described by Vetter (I958), and was calculated for each treatment as the change in absorbance at 430 nm per minute per gram fresh weight (Δ_{430}/min/g FW). Polyphenoloxidase activity was determined according to a modification of Ishaaya (I971), and was calculated Δ_{405}/min/g FW. Chitinase activity was determined by the sensitive method of Waterhouse *et al*., (1961), and was expressed as µg N-acetylglucoseamine × 10^3 /min/g fresh weight (µg NAGA X 10^3/g FW).

- *Vitamin C determination:*

Determination of vitamin c in tomato was done calorimetrically by using 2,6-dichlorophenol-indophenol dye method outlined by (A.O.A.C., 1975), and was expressed as µg ascorbic acid per gram fresh weight (µg A.A./g FW).

As well as, increase percentage of all determined enzymes and vitamin C were calculated using the following formula:

Increase (%) = (value of treatment - value of control / value of control) × 100%

Statistical analysis:

The presented data were laid out in triplicates and were statistically analyzed for the least significant difference (L.S.D.) according to Gomez and Gomez (1984).

Results and Discussion

Isolation of tomato leaf spot pathogen and frequency of isolated bacteria:

Isolation trials which were carried out from different localities during tomato growing season associated with bacterial leaf spot disease symptoms resulted 45 bacterial isolates (Table, 2) identified as *Bacillus sp.* (13 isolates), *Pseusomonas sp*. (12 isolates), *P. flurescens* (10 isolates) and *X. vesicatoria* (10 isolates). Data illustrated in Table (2) indicate that *Bacillus* sp. and *Pseudomonas* sp. had superior frequency (28.5 and 26.7%, respectively) than *P. flurescens* and *X. vesicatoria* (22.15 and 22.58%, respectively).

Table 2: Isolation localities of tomato leaf spot pathogen and frequency of isolated bacteria

Isolated bacteria	Isolation localities							
	El-Garbia		El-Sharqia		El-Qalubia		El-Giza	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Bacillus sp	4	33.3	3	30.0	4	28.5	2	22.2
Pseudomonas sp	3	25.0	2	20.0	4	28.5	3	33.3
P.fluorescens	3	25.0	2	20.0	3	21.4	2	22.2
X.vesicatoria	2	16.7	3	30.0	3	21.4	2	22.2
Total	12		10		14		9	

Confirmation the identification of isolated bacterium *X. vesicatoria*:

Ten bacterial isolates identified previously (data in Table, 2) as *X. vesicatoria* were subjected to further physiological, morphological and biochemical characteristics to confirm the identification. Data in Table (3) confirm that tested bacterial isolates scored positive reaction for growth on common media, starch hydrolysis, yeast extract dextrose $CaCO_3$, yellow pigment, growth on peptone yeast extract agar (PYEA), H_2S production, motility, catalase activity, utilization of glucose, acid from glucose and relation to O_2, but negative to gram reaction, pigment on K.B., gelatin liquefaction, spore production, utilization of sorbitol and acid from Sorbitol.

Pathogenicity and host range test:

Illustrated data in Table (4) prove that all tested *X. vesicatoria* isolates recorded positive pathogenicity on tomato plants. Meanwhile, only I_9 recorded low pathogenicity index only on pepper plants. The exact same table reveals that I_2 recorded highest pathogenicity index on tomato plants. No infection symptoms were observed on the un-inoculated tomato plants. It would appear that all isolates had a narrow host range and specific to tomato and pepper plants. These results could possibly be discussed in light that some *X. vesicatoria* isolates have the capability to infect leaves of tomato, cowpea and bean showing hypersensitive reaction when sprayed with the bacterial inoculum (El-Sadek *et al.,* 2001).

Table 3: Physiological, morphological, biochemical and xanthomonad reaction characteristics for *X. vesicatoria*

Identification Tests	No. of isolates									
	1	2	3	4	5	6	7	8	9	10
Gram reaction	-	-	-	-	-	-	-	-	-	-
Growth on common media	+	+	+	+	+	+	+	+	+	+
Size	Short									
KOH 3%,	+	+	+	+	+	+	+	+	+	+
Pigment on K.B.	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	-	-	-	-	-	-	-	-	-	-
Yeast extract dextrose $CaCO_3$	+	+	+	+	+	+	+	+	+	+
Spore production	-	-	-	-	-	-	-	-	-	-
Yellow pigment	+	+	+	+	+	+	+	+	+	+
Growth on peptone yeast extract agar (PYEA)	+	+	+	+	+	+	+	+	+	+
H_2S production	+	+	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+	+
Utilization of Glucose	+	+	+	+	+	+	+	+	+	+
Utilization of Sorbitol	-	-	-	-	-	-	-	-	-	-
Acid from Glucose	+	+	+	+	+	+	+	+	+	+
Acid from Sorbitol	-	-	-	-	-	-	-	-	-	-
Relation to O_2	+	+	+	+	+	+	+	+	+	+
Bacterial genera	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*	*X.*

Table 4: Pathogenicity and host range test of isolated bacterium *X. vesicatoria*

Common name	Scientific name	Tested *X. vesicatoria* isolates									
		*I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}
Tomato	*Lycopersicon esculentum*	+	+++	+	+	+	+	+	+	++	+
Eggplant	*Solanum melongena*	-	-	-	-	-	-	-	-	-	-
Pepper	*Capsicum annum* L.	-	-	-	-	-	-	-	-	+	-
Bean	*Phaseolus vulgaris*	-	-	-	-	-	-	-	-	-	-

** I_1, I_2= ElGharbia; I_3,I_4,I_5= El-Sharqia; I_6, I_7, I_8= Elqalubia; I_9, I_{10}= El-Giza isolates*

** + = low pathogenic; ++ = moderately pathogenic; +++ = highly pathogenic*

Inhibiting effect of tested commercial bacteriotoxicants against isolated bacterium *X. vesicatoria* in vitro:

As seen in Table (5), four different commercial bacteritoxicants were tested against *X. vesicatoria* for their antibacterial properties. Data in the same table reveal that all tested treatments had clear significant inhibitory effects on *X. vesicatoria* in comparing with control treatment. In this respect, copper oxychloride followed by oxytetracycline, garlic oil and bio arc treatments recorded highest significant increase in inhibition zone (6.53, 4.13, 3.54 and 1.74 mm, respectively). On the other hand, the exact same table clearly implies that inhibition zone increased by increasing concentration per each main treatment in comparison with control treatment. These results could possibly be discussed in light that the inhibitory effect of copper oxychloride might be as a result of copper toxicity. It is well established that copper affect bacteria in two sequential steps; the first step is a direct interaction involving the copper and the bacterial outer membrane, evoking the membrane to rupture. The second is linked to the holes in the outer membrane, through which the cell loses vital nutrients and water, causing an over-all weakening of the cell (Macomber and Imlay, 2009). Chemical control of plant diseases by using copper and antibiotic sprays has also been screened (Thayer and Stall, 1961; Conover and Gerhold, 1981; Jones and Jones, 1985). Also, it is well established that tetracyclines inhibit bacterial protein synthesis by avoiding the association of aminoacyl-tRNA with the bacterial ribosome (Chopra *et al.,* 1992; Schnappinger and Hillen, 1996). The antimicrobial activities of garlic and other plant alliums are primarily centered on allicin, a thiosulphinate within crushed garlic bulbs. More recent analyses revealed that allicin accounts for approximately 75% of garlic-derived sulphinates (Block, 2010; Fujisawa *et al.,* 2008; Waag *et al.,* 2010). Studies on

inhibition of some pathogenic bacteria using allicin prepared from reacting allicin with allicin lyase suggested that inhibition of RNA synthesis is really a primary target of allicin action (Feldberg *et al.,* 1988). Allicin and other thiosulphinates are also recognized to react with cysteine to abolish antimicrobial activity (Fujisawa *et al.,* 2009) and to inhibit acetyl-CoA synthases from plants, yeasts and mammals (Focke *et al.,* 1990). *B. megaterium* is common soil beneficial bio-fertilizer belonging to plant growth promoting rhizobacteria (PGPR) have also been used as biocontrol agents against soil borne, foliar and post-harvest pathogens, (Abou-Zeid and Zaid, 2006; Padgham and Sikora, 2007; Oliveira *et al.,* 2009). The inhibitory effect of most Bacilli genera could be in form of a competitor for nutrient and space, as well as the production of hydrolytic enzymes, *i.e.* protease, glucanase (Cazorla *et al.,* 2007), chitinase (Manjula *et al.,* 2004), lipase (Detry *et al.,* 2006) and amylase (Konsoula and Liakopoulou-Kyriakides, 2006) which are capable to degrade the cell wall of broad spectral range of pathogens (Saha *et al.,* 2012), along with, production of several ribosomal and non-ribosomal. peptides that behave as antibiotics such as for example iturins, surfactins.and zwittermycin (Asaka and Shoda, 1996; Stein, 2005).

Table 5: Inhibiting effect of tested commercial bacteritoxicants against isolated bacterium *X. vesicatoria in vitro*

Treatment	Conc.		Inhibition zone (mm)
Bio arc	1.5 g/L		0.17
	2.5 g/L		2.23
	3.5 g/L		2.83
	Mean		1.74
Garlic oil	0.3%		3.20
	0.5%		3.53
	1.0%		3.90
	Mean		3.54
Oxytetracycline	12 µg/ml		3.57
	12.5 µg/ml		4.13
	13 µg/ml		4.70
	Mean		4.13
Copper oxychloride	1.0 g/L		6.23
	1.5 g/L		6.50
	2.0 g/L		6.87
	Mean		6.53
Control			0.00
L.S.D at 1% for		Materials	0.014
		Conc.	0.023
		Interaction	0.070

Effect of tested programmed commercial bacteriotoxicants on tomato bacterial spot disease assessment *in vivo*:

Data illustrated in Table (6) demonstrate that applying of median concentration of each tested commercial bacteriotoxicant (Bio arc, garlic oil, oxytetracycline and copper oxychloride) individually in regular or in un-regular succession (in program form) as foliar spray (Table 1) considerably and significantly reduced bacterial leaf spot disease development (disease % and disease severity %) in comparison to control treatment during the tow growing seasons 2013/14 and 2014/2015. All tested treatments (programs) were far better in decreasing bacterial leaf spot disease assessment than control treatment. Also, it clear that results of the second season 2014/2015 of the tested programs were more effective in decreasing diseases % and disease severity % of bacterial spot disease than the first season 2013/2014. Data in the table reveal that prog-4 (copper oxychloride; considered as treated control) recorded highest significant decrease % and disease severity % during the both successive growing seasons (by mean 0.00% and 0.00%) comparing with un-treated control (by mean 71.67% and 61.19%, respectively). In general, the tested programs (prog-8, prog-10 and prog-6, respectively) scored highest significant reduction in disease % during the two seasons without any significant differences in-between them comparing with control. Considering effect of tested programs on bacterial leaf spot disease severity %, the same trend was observed with some modifications, where,

prog-4 scored highest significant reduction, while, prog-8 and prog-10 recorded the highest significant reduction in disease severity % with no significant differences in-between them comparing with control. It would appear that prog-3 had a substantial effect on bacterial leaf spot disease severity reduction, where recorded significant reduction (by mean 3.81%) comparing with control treatment. Agricultural practices for management of diseases in the field includes cultural practices, crop rotation, fungicide applications, methyl bromide fumigation, soil solarization and utilization of resistant or tolerant plant varieties. At present, no single method provides adequate control of those diseases. On the other hand, there is a wide range of (bio) cides showing different degrees of antimicrobial activity. Hence, this experiment aimed to evaluate different successions (programing) of those (bio) cides as foliar spray which could supply a sufficient protection for infecting plants. Chemical control by using copper and antibiotic sprays has been screened by many researchers (Thayer and Stall, 1961; Conover and Gerhold, 1981; Jones and Jones, 1985).The role of copper in decreasing disease assessments (disease% and disease severity%) could possibly be interpreted in light that copper toxicity for bacterial pathogen where, excess copper can be hugely toxic, due in part to redox-activity by which copper ions can catalyze the production of deleterious reactive oxygen species (ROS), and also due to its propensity to form extremely stable complexes with cellular components (Macomber *et al.,* 2007; Macomber and Imlay, 2009).

Table 6: Effect of tested programmed commercial bacteriotoxicants on tomato bacterial spot disease assessment under field condition during growing seasons 2013/14 and 2014/15

Tested Program*	Disease %			Disease Severity %		
	2013/14	2014/15	Mean	2013/14	2014/15	Mean
Prog-1	50.00	43.33	46.67	13.81	10.48	12.145
Prog-2	40.00	36.67	38.34	12.38	9.52	10.950
Prog-3	23.33	16.67	20.00	4.76	2.86	3.810
Prog-4	0.00	0.00	0.00	0.00	0.00	0.000
Prog-5	26.67	23.33	25.00	6.19	4.76	5.475
Prog-6	16.67	13.33	15.00	4.76	4.29	4.525
Prog-7	36.67	33.33	35.00	7.62	7.14	7.380
Prog-8	13.33	7.13	10.23	3.33	1.90	2.615
Prog-9	46.67	43.33	45.00	10.95	7.62	9.285
Prog-10	13.33	10.00	11.67	2.86	2.38	2.620
Control	70.00	73.33	71.67	62.86	59.52	61.190
L.S.D at 5%	8.996	6.154	-	2.403	1.946	-

** Prog-1,2,3,4,5,6,7,8,9 and10 ; see table (1)*

Tetracyclines are broad-spectrum agents, exhibiting activity against a wide variety of gram-positive and gram-negative bacteria. In this respect, tetracyclines could reduce disease assessments via affecting on pathogen where, tetracyclines inhibit microbial protein synthesis by avoiding the attachment of aminoacyl-tRNA to the ribosomal acceptor (A) site (Chopra *et al.,* 1992; Schnappinger and Hillen, 1996). Allicin and other thiosulphinates exists in alliums are also proven to react with microbial cysteine to abolish antimicrobial activity (Fujisawa *et al.,* 2009) and to inhibit acetyl-CoA synthases (Focke *et al.,* 1990). Applying *B. megaterium* as biocontrol agents against foliar phytopathogenics reduced disease severity (Abou-Zeid and Zaid, 2006; Padgham and Sikora, 2007; Oliveira *et al.,* 2009). The role of Bacillus in decreasing disease severity could be form of a competitor for nutrient and space, in addition to the production of hydrolytic enzymes, *i.e.* protease, glucanase (Cazorla *et al.,* 2007), chitinase (Manjula *et al.,* 2004), lipase (Detry *et al.,* 2006) and amylase (Konsoula and Liakopoulou-Kyriakides, 2006) which are capable to degrade the cell wall of broad spectrum of phytopathogens (Saha *et al.,* 2012), along with, production of several ribosomal and non-ribosomal peptides that act as antibiotics such as for instance iturins, surfactins and.zwittermycin (Asaka and Shoda, 1996; Stein, 2005). Wilson *et al.,* (2006) mentioned that foliar bacterial biological control agents and plant growth promoting rhizobacteria (PGPR) have been tested for control of bacterial spot of tomato. In field trials foliar biological control agents and PGPR strains controlled bacterial spot while they provided variable results. PGPR strains may induce plant resistance under field conditions, providing effective suppression of bacterial spot of tomato.

Effect of tested programmed commercial bacteriotoxicants on tomato yield:

As for the effect tested control programs on total yield weight (ton/feddan) of tomato, data in Table (7) reveal that all tested programs had a great effect in increasing total yield of tomato plants during both growing seasons (2013/14 & 2014/15) comparing with control treatment. All tested programs recorded significant increase on total tomato yield with no significant differences between them except a few treatments (prog-8, prog-7 and prog-2, respectively) in comparing with control treatment during the two growing seasons. Data in the table reveal that prog-4 (Copper-treated control) scored highest significant increase in total yield (by mean 50.66 ton/feddan) comparing with un-treated control treatment followed by prog-10, prog-1and prog-3 (by mean 48.52, 46.50 and 45.38 ton/feddan, respectively) during both successive growing seasons. These results could be interpreted in light that, bacteriocides predicated on copper are good effective resource for controlling bacterial leaf spot brought on by *X. campestris* pv. *vesicatoria* on pepper plants with significant increase in fruit yield weighed against the un-treated control (Ju-Hee *et al.,* 2015; Mirik *et al.,* 2007; Mc Carter, 1992; Marco and Stall,1983). On the other hand, *B. megaterium* is common soil beneficial bio-fertilizer belonging to plant growth promoting rhizobacteria have also been used as biocontrol agents against foliar phytopathogens (Abou-Zeid and Zaid, 2006; Padgham and Sikora, 2007; Oliveira *et al.,* 2009). Wilson *et al.,* (2006) mentioned that foliar bacterial biological control agents and plant growth promoting rhizobacteria (PGPR) have been tested for control of bacterial spot of tomato. In field trials foliar biological control agents and PGPR strains controlled bacterial spot although they provided variable results. PGPR strains may induce plant resistance under field conditions, providing effective suppression of bacterial spot of tomato and increased tomato yield. Antimicrobial essential oils, such as garlic, thymol, palmarosa oil descends from plant materials have been suggested as among the promising eco-friendly alternatives of chemical pesticides and enhances plant yield (Gupta and Porter, 2001; Ji *et al.,* 2005; Pradhanang *et al.,* 2003). Various combined and/or mixed methods including bactericide treatments have been requested applications for the bacterial disease management in tomato plant fields. Two antibiotics, streptomycin and oxytetracyclin were effective to shield tomato fruits against bacterial speck disease by *Pseudomonas syringae* pv. *tomato* and increase yield (Jardine and Stephens, 1987).

Table 7: Effect of tested programmed commercial bacteriotoxicants on tomato yield weight (ton/feddan) under field condition during growing seasons 2013/14 and 2014/15

Tested Program*	2013/14	2014/15	Mean
Prog-1	45.06	47.94	46.50
Prog-2	35.46	35.84	35.65
Prog-3	46.78	43.97	45.38
Prog-4	49.60	51.71	50.66
Prog-5	43.26	40.83	42.05
Prog-6	44.48	41.92	43.20
Prog-7	31.10	33.09	32.10
Prog-8	30.59	35.78	33.19
Prog-9	42.82	44.93	43.88
Prog-10	49.09	47.94	48.52
Control	25.15	23.87	24.51
L.S.D. at 5%	7.496	7.385	

** Prog-1,2,3,4,5,6,7,8,9 and10 ; see table (1)*

Effect of tested programmed commercial bacteriotoxicants on biochemical assessments:

Using the tested bacteriotoxicants in program form increased the total phenol content, activities of oxidative enzymes (peroxidase, polyphenoloxidase and chitinase) in tomato leaves comparing to treated-copper control (prog-4) and un-treated control treatment (Fig. 1). In this respect, prog-1 raised the total phenol content comparing to control treatment where, prog-1 followed by prog-2 and prog-10 scored the highest increase% (273.3, 242.1 and 208.8%, respectively). In addition, all tested bacteriotoxicants raised the activities of peroxidase enzyme in tomato leaves in comparing with control treatment (Fig. 2). In this respect, prog-6 followed by prog-2 and prog-10 gave the highest

increase (226.8, 199.7 and 158.6%, respectively). Concerning polyphenoloxidase activity (Fig. 3), prog-6 followed by prog-7 caused the highest increase in activities of polyphenoloxidase enzyme (162.3 and 116.7%, respectively) comparing to control treatment. Also, data in fig. (4) show increase of chitinase activity considerably by using prog-6 (230.2%) when compared with control treatment. Considering vitamin C increase% (Fig. 5), all tested bacteriotoxicants increased vitamin C content comparing with control treatment. In this respect, prog-6 followed by prog-10 and prog-5 caused the highest increase (324.0, 297.5 and 217.2%, respectively). Among the potential plant disease management strategies is the employment of systemic acquired resistance (SAR) to activate host defense mechanisms (Ryals *et al*., 1994). Successful disease management and control practices greatly are determined by a comprehension of the ecology of the pathogenic organism in the environmental surroundings (Pradhanang *et al.,* 2000). On the other hand, Hoffland *et al.,* (1996) demonstrated that the plant growth promoting rhizobacterium (PGPR) *Pseudomonas fluorescens* strain WCS417 has been shown to induce systemic resistance against *Fusarium oxysporum* in a number of plant species. These mechanisms could possibly be: altered ion fluxes throughout the plant cell membrane, generation of active oxygen species, changes in the phosphorylation state of regulatory proteins and transcriptional activation of plant defense systems culminate in cell death at the location of infection, local accumulation of phytoalexins and cell wall deterioration as a result of callose, lignin and suberin deposition (Hammond-Kosack and Jones, 1996; Yang *et al.,* 1997). Also, SAR mechanisms might be as a result of induce plant resistance (De Meyer *et al.,* 1998), produce extracellular enzymes and antifungal or antibiotics, which decrease biotic stress on plant, and produce growth promoters substances (Szczech and Shoda, 2004). Also, these results could be discussed in light the findings of Chérif *et al.,* (2007) and Mohamed *et al.,* (2007) who reported that, resistance could be exhibited on treated plants as a result of accumulation of vitamin C (George *et al*., 2004) and various phenolic compounds and the activation of chitinases, ß-1,3-glucanases, peroxidases and polyphenoloxidases and key enzymes in the phenylpropanoid and isoflavonoid pathways may play an essential role in the biological control of diseases and resistance to pathogenic attack in plants.

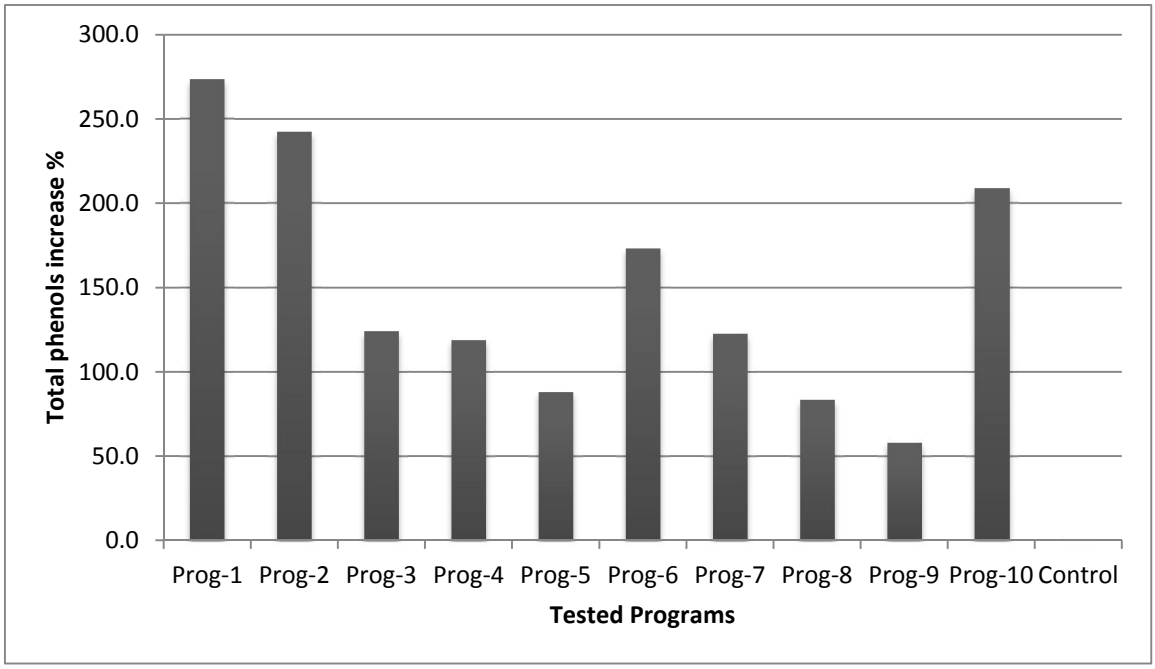

Fig. 1: Effect of tested programmed commercial bacteriotoxicants on total phenol increase %

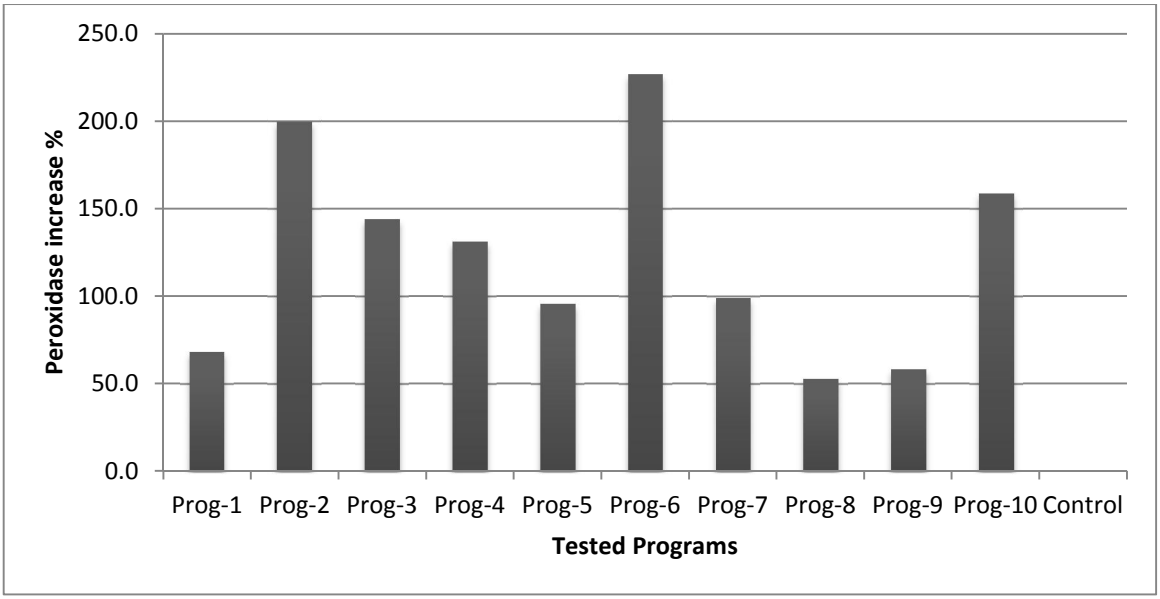

Fig. 2: Effect of tested programmed commercial bacteriotoxicants on peroxidase increase %

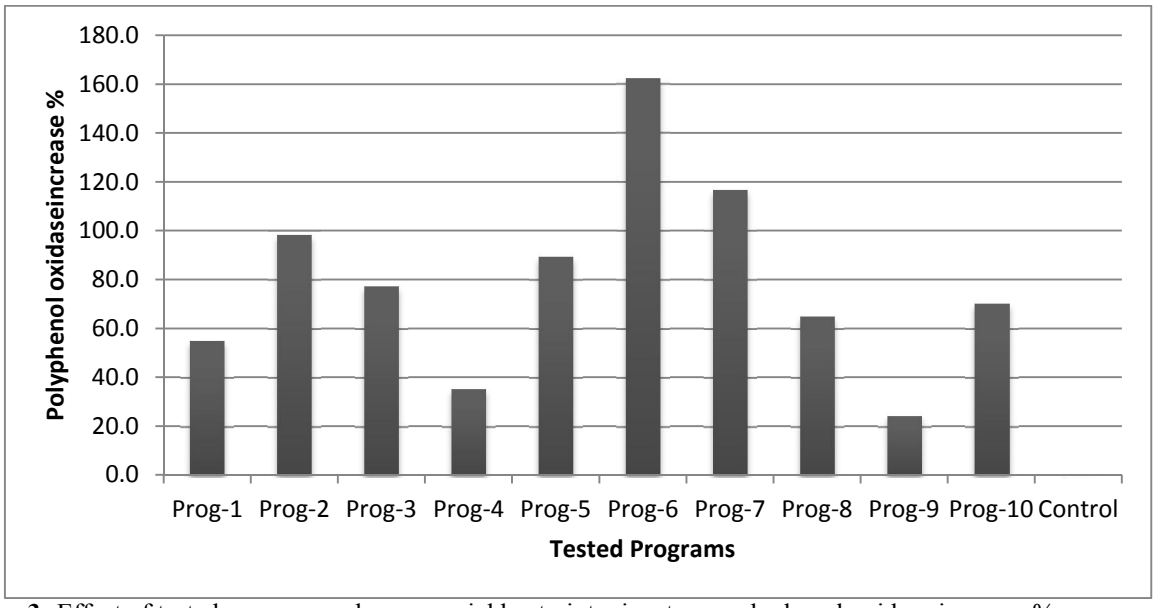

Fig. 3: Effect of tested programmed commercial bacteriotoxicants on polyphenol oxidase increase %

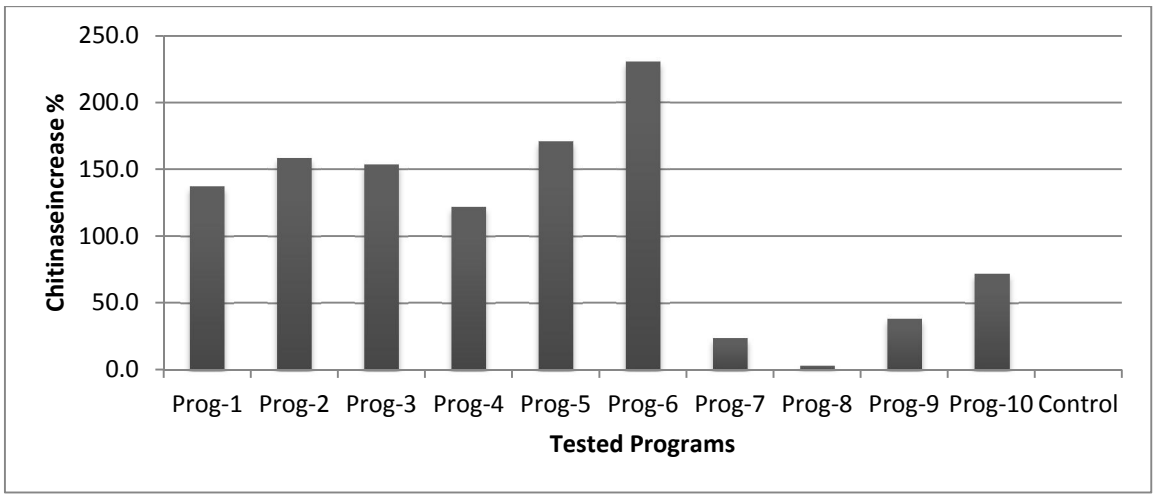

Fig. 4: Effect of tested programmed commercial bacteriotoxicants on chitinase increase %

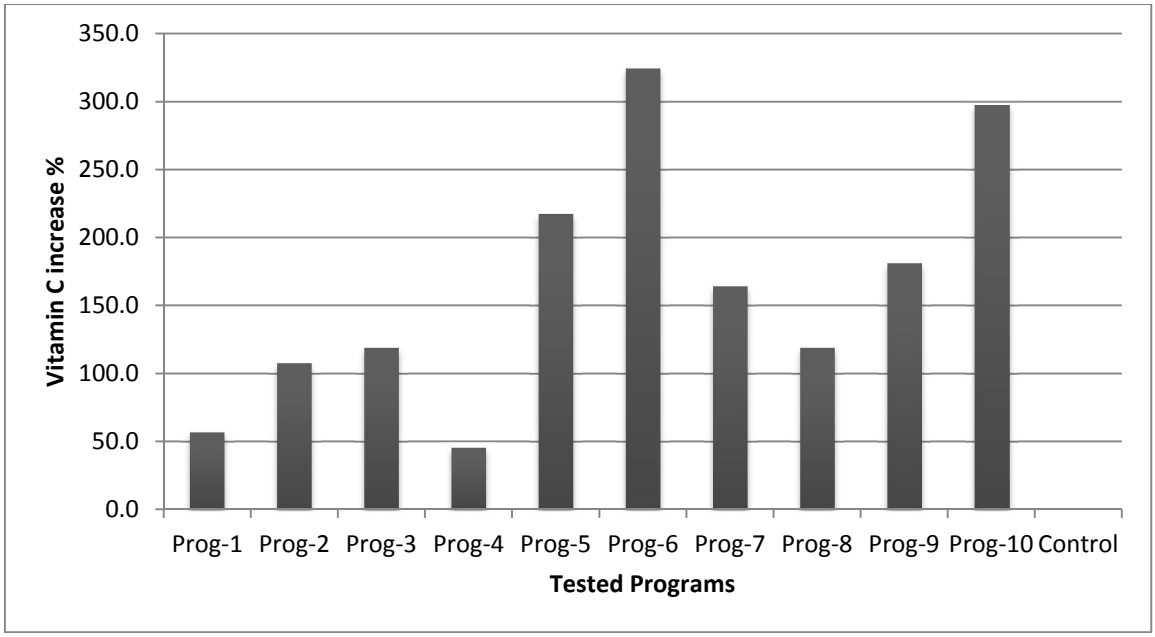

Fig. 5: Effect of tested programmed commercial bacteriotoxicants on vitamin C increase %

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