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Controlling The Bacterial Leaf Spot Disease in Pepper Caused by *Xanthomonas vesicatoria* Using Natural Bacteritoxicants

Ibrahim A. I. EL-Fiki [1]; Youssef, Maryan, M.[2] and Hassan O. Eman [1]

1- Plant Pathology Department, Faculty of Agriculture, Moshtoher, Benha University, Benha13511, Egypt
2- Plant Pathology Department, Faculty of Agriculture, Cairo University, 12612 Giza, Egypt.

*E-mail: ibrahim.elfiki@fagr.bu.edu.eg - maryan.youssef@agr.cu.edu.eg-Eman.ali@fagr.bu.edu.eg

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ABSTRACT

Isolation trials of bacteria associated to leaf spot collected from various locations in Egypt yielded 39 bacterial isolates. Following purification, the isolated bacteria were identified as *Bacillus* spp. (15 isolates), *Pseudomonas* spp. (12 isolates), and *Xanthomonas campestris* pv. *vesicatoria* "*Xcv*" (12 isolates). Both *Bacillus* spp. and *Pseudomonas* spp. had the higher frequency, being 38.46 and 30.77%, respectively than *Xcv* (30.77%). I_1, I_3, I_4, I_6, I_9, I_{10}, I_{11}, and I_{12} had a low pathogenicity index exclusively on pepper plants. The antibacterial capabilities of five tested bacteriotoxicants were evaluated against *Xcv in vitro*. Copper oxychloride had the greatest increase in the inhibitory zone, followed by oxytetracycline then hydrogen peroxide, and clove oil treatments, being 6.32, 5.45, 5.14, and 4.52 mm, respectively. *In vivo*, all treatments examined outperformed the control treatment in terms of reducing bacterial leaf spot disease evaluation. All evaluated treatments increased total pepper production. Copper-treated control treatment resulted in the greatest increase in total fruit yield (23.68 kg/plot) during two successive growing seasons (2019/2020 and 2020/2021). The oxytetracycline, curcumin, clove oil, and hydrogen peroxide treatments all had a great effect on increasing total fruit yield. The investigated treatments enhanced total phenols content and oxidative enzymes activity in pepper leaves as compared to the treated-copper control and untreated control treatments. Curcumin, followed by clove oil and then hydrogen peroxide, provided the greatest rise in the determined oxidative enzymes activities in leaves. In terms of vitamin-C increase percentage, all evaluated bacteriotoxicants enhanced vitamin-C content.

INTRODUCTION

The bacterial leaf spot of pepper incited by *Xanthomonas campestris* pv. *vesicatoria* "*Xcv"* is a costly disease that threatens pepper production and its industry worldwide (Potnis *et al.*, 2015). Management of the disease has been a serious issue since its discovery in 1920 due to the pathogen's seed-borne nature (Momol, *et al*., 2002). Bacterial spots on tomatoes and peppers were first seen in South Africa and the United States almost simultaneously in the early 1920s (Indiana). The causal agent was first referred to as *Bacterium vesicatorium*

(Gardner & Kendrick, 1921, 1923; Momol, *et al.*, 2002; Roach, *et al.*, 2018), but this nomenclature was later changed to *Xcv* (Dowson, 1939). Bacterial leaf spot affects peppers cultivated in greenhouses and fields in a number of other countries, with particular importance in regions with warm, humid climates (EPPO, 2013). In the case of severe infections, direct losses in fruit production of between 23 and 44 percent are conceivable, while indirect losses in severely infected plants are mostly brought on by leaf dropping and fruit exposure to sunlight, which results in sunscald (Bashan *et al.*, 1985). Symptoms of bacterial leaf spot include lesions that first resemble round, wet lesions but later develop dark brown to black and are surrounded by a chlorotic halo, without a shot-hole appearance (Osdaghi *et al.*, 2016). In the case of severe pepper infections, the necrotic patches congeal, leading to the defoliation of the infected leaves and, most likely, the sun scalding of the fruits on hot, sunny days. After an artificial inoculation for 12–15 days, early water-soaked lesions on the leaves turn into chlorotic, and then necrotic regions. Transporting contaminated seeds and transplants to growing locations is one method by which the disease bacterial leaf spot is spread (Potnis *et al.*, 2015). Critical is the pathogen's capacity to multiply during transplanting (Simonton *et al.*, 2020). The rapid growth of bacterial leaf spot during transplantation production is accelerated by high plant densities, overhead watering, and high humidity and temperatures, which can cause catastrophic outbreaks of seedlings. Based on the circumstances, the development of symptoms in newly infected transplants might take anywhere between 5 and 7 days (Abrahamian *et al.*, 2021).

The best way to manage bacterial leaf spot is to treat it early in the production cycle, starting with the use of healthy, pathogen-free seeds and transplants to keep the pathogen out, avoiding handling wet plant material and free moisture on foliage to stop disease development, and using protective chemicals or biological treatments to lessen the severity and spread of the disease during transplant production (Abrahamian *et al.*, 2019; Potnis *et al.*, 2015). The most prevalent method to bacterial leaf spot management is the use of copper-based bactericides as a prophylactic measure.

The presence of copper tolerance/resistance in populations of bacterial spot pathogens frequently contributes to poor disease control (Abbasi *et al.*, 2015; Arajo *et al.*, 2012; Khanal *et al.*, 2020; Martin *et al.*, 2004). Consistent disease control, however, is challenging when favorable environmental conditions for bacterial leaf spot development exist (Vallad *et al.*, 2010). Groundwater and the ecosystem have been contaminated by excessive use of copper-based insecticides in agriculture (Lamichhane *et al.*, 2018). Hydrogen peroxide (H_2O_2) is one of the most important reactive oxygen species (ROS) that can be used as a safe compound at low concentrations to combat plant pathogens such as barley net blotch disease (Abdelaal *et al.*, 2020; Hafez *et al.*, 2019), wheat leaf rust (Omara *et al.*, 2015; Hafez and Király, 2009), barley powdery mildew (Hafez *et al.*, 2008). *Botrytis cinerea* was controlled on white pepper fruits using a low concentration of H_2O_2 during postharvest storage (Hafez, 2010). Early H_2O_2 rise is critical in non-host resistance mechanisms in bean and cereal plants against incompatible pathogens (Hafez, 2015). H_2O_2 is, also, utilized to boost seed germination and seedling growth in cabbage and watermelon plants that grow in soil contaminated with soil-borne diseases (Hafez *et al.*, 2012). Through up-regulation of antioxidant enzymes, five to seven millimoles of H_2O_2 decrease necrotic disease signs in tobacco infected with fungal, bacterial, and viral pathogens (Hafez *et al.*, 2012). As a result, the current study intends to investigate the use of hydrogen peroxide against bacterial pepper leaf spot caused by *Xcv*, with low cost as compared to control techniques employing copper hydroxide as a bactericide, and therefore enhance production with high fruit quality.

MATERIALS AND METHODS

Isolation, Purification and Identification of Causal Bacterium:

The causative bacterium was isolated from the leaves of naturally infected pepper (*Capsicum annum* L.) plants grown in the governorates of El-Giza, El-Qalubia, El-Behera, and El-Minia. The contaminated leaf samples with bacterial spot lesions were plucked and surface sterilized in 1% sodium hypochlorite for 30 seconds before being removed by washing three times in sterile distilled water, and dried in-between two sterilized filter paper. A sterile pestle was used to macerate the infected portions, which were divided into small patch specimens of 2-3 mm each. After that, the sample suspensions were applied onto plates with nutrient agar media. The inoculation plates were incubated for 48 hours at 28±2°C. Initiated bacterial colonies were selected at random and transferred onto new nutrient agar medium plates then purified.

Pathogenicity and Host Range Test:

The aforementioned purified *Xcv* isolates were evaluated for their pathogenicity in the laboratory using four crop species: tomato (Super Strain B Hybrid), eggplant (Balady cv.), pepper (Balady cv.), and bean (Bolista cv.). Each *Xcv* isolate used in the experiment was grown for 48 hours at 28±2°C on nutrient agar before being suspended in sterile distilled water and centrifuged for 30 minutes at 3000 rpm. The pellets were re-suspended in distilled water after being turbidimetrically adjusted to around 10^8 CFU/ml of density (O.D. at 660 = 0.06). Thirty-day-old plants of each crop, grown in pots (15 cm in diameter) contained clay soil that had been formalin-sterilized, were artificially infected with 1 x 10^8 CFU/ml using a low-pressure hand atomizer. Plants were sprayed with water before inoculation to create a thin layer of water on the leaf surface. At a rate of 0.1 ml per liter, Tween 80 was added to the bacterial suspension at the rate of 0.1 ml/l water. Plants sprayed just with water served as a control. For two days, inoculated plants were housed in wet hyaline polyethylene sacs as a moist chamber. According to Horsfall and Barratt (1945), disease severity % was measured two weeks post-inoculation on randomly selected 10 leaves of pepper plants for each treatment. This laboratory experiment used a randomized complete design (RCD) block with four replicates (pots), each containing one plant.

Management of the Disease:

1.In vitro:

The antibacterial activities of five substances, namely hydrogen peroxide (at concentrations of 0.5, 1.0, 1.5 ml/L); clove oil (at concentrations of 0.05, 0.1, 0.2%); curcumin (at concentrations of 10.0, 20.0, 30.0 mg/L); oxytetracycline (at concentrations of 12.0, 12.5, 13.0 µg/ml); and copper oxychloride fungicide (at concentrations of 1.0, 1.5, 2.0 g/L) were screened against highest virulent *Xcv* isolate *in vitro*. In a 100 ml conical flask containing 40 ml of nutrient broth at 28±2°C, the pathogenic bacterium was grown for two days on a shaker at 150 rpm. Each sterile Petri plate (90 mm in diameter) was filled with 15 ml of molten nutritional agar medium and one milliliter of the bacterial suspension just before it solidified. In the preparation of any of the tested substances, a sterile filter paper disc (6 mm in diameter) was submerged. On each inoculated Petri-plate with the pathogenic bacterium, four discs saturated with any of the tested treatments were placed. For two days, the plates were incubated at 28±2°C. The inhibitory zone generated around the disc was measured in diameter. A randomized complete design (RCD) with three replicates was used for this experiment (each plate represented a replicate).

2.In vivo:

During the spring growing seasons of 2019/2020 and 2020/2021, the current study was conducted at the Moshtohor Experimental Station, Faculty of Agriculture, Benha University, Egypt. The effectiveness of five substances; hydrogen peroxide, clove oil,

curcumin, oxytetracycline, and copper oxychloride fungicide, was evaluated on pepper plants (cv. Balady) naturally infected with bacterial leaf spot in open field circumstances. Each evaluated bactericide's median concentration has an inhibitory impact on the pepper leaf spot pathogen (*Xcv*) *in vitro* tests were submitted to *in vivo* experiments to assess its efficiency against bacterial leaf spot development. Using a three-replicate, randomized in complete block design, the treatments were organized (plots). There were four ridges on each experimental plot, measuring 3.75 m long and 70 cm wide. The plot is about 10.5 m^2. Thirty-day-old pepper (Balady cv.) seedlings were planted on one side of the ridge on April 15 in the presence of water, 50 cm apart, with around 48 plants per plot. Except for the use of fungicides, all required agronomic procedures were followed for growing pepper plants. All treatments were given as foliar spray three times: first at 30-days after transplanting (DPT) as a preventative treatment before the onset of disease symptoms, again at 50-DPT, and finally at 70-DPT. The copper oxychloride treatment served as the treated control. Water-sprayed plants only acted as a check (control) treatment. Tween 80 was added to each spray treatment at a rate of 0.1 ml/l water. This study used a randomized complete plot design (RCPD) with three replicates for each treatment.

2.1. Disease and Total Fruit Yield Assessments:

The disease severity percentages of bacterial leaf spot were determined 90 days after transplantation. Horsfall and Barratt's (1945) disease scale was used to count the number of lesions on each of 50 randomly selected leaves to determine the severity of the disease 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 pepper fruit yield (kg/plot) for each treatment was recorded for each growing season.

2.1.1. Biochemical Assessments:

For the assessing total phenol level, oxidative enzyme estimation, and vitamin-C content, apical samples from the tenth plant leaf from each treatment were taken.

2.1.2. Total Phenols Content Determination:

Leaf samples were extracted separately in order to determine the total phenol content, following the recommendation of Kâhkônen et *al.,* (1999). The total phenol content was determined for each treatment as milligrams of gallic acid per gram of dry weight (mg GA/DW) by using a gallic acid standard curve using the Folin-Ciocateu technique, as modified by Singleton and Rossi (I965).

2.1.3. Oxidative enzymes activity:

According to Ni *et al.,* (2001) the crude leaf enzyme extract was prepared. According to Vetter (1958), the peroxidase enzyme activity was calculated for each treatment as the change in absorbance at 430 nm per minute per gram of fresh weight (Δ_{430}/min/g FW). A modified description of Ishaaya's (I971) technique was used to calculate the polyphenol oxidase activity at Δ_{405}/min/g FW. A technique developed by Waterhouse *et al.,* (1961) was used to assess chitinase activity, which was expressed as g N-acetylglucoseamine µg N-acetylglucoseamine × 10^3/min/g fresh weight (µg NAGA X 103/g FW).

2.1.4. Vitamin-C Determination*:*

Using a calorimetric method published by A. O. A. C. (1975) and the 2,6-dichlorophenolindophenol dye, vitamin C in pepper fruits was quantified as microgram

ascorbic acid per gram of fresh weight (µg A.A/g FW). The percentages of all identified enzymes and vitamin C were also calculated using the following formula:

Increase (percent) = (treatment value - control value / control value) / control value x 100

Statistical Analysis:

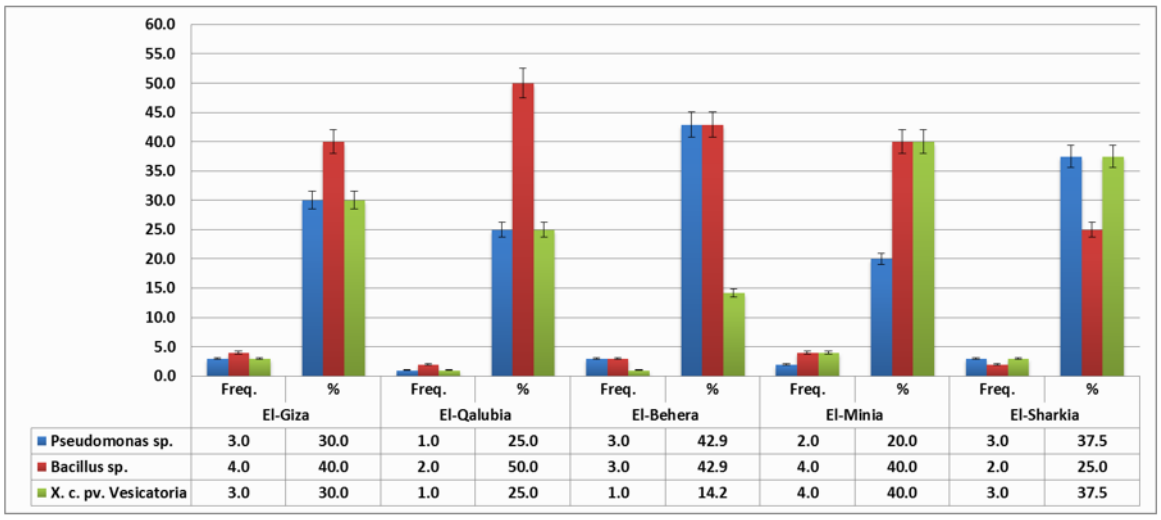
According to Gomez and Gomez, (1984) the reported data were formatted in triplicates and statistically examined for the least significant difference (L.S.D.).

RESULTS

Isolation, Purification Identification and Confirmation of The Identification of Pepper Leaf Spot Pathogen:

Isolation trials of bacteria associated to leaf spot collected from various locations in Egypt yielded 39 bacterial isolates. The isolated bacteria were purified and identified as *Bacillus* spp., *Pseudomonas* spp. and *Xanthomonas campestris* pv. *vesicatoria* "*Xcv"*. Both *Bacillus* spp. and *Pseudomonas* spp. had the higher frequency, than *Xcv* **(**Fig. 1**)**.

The identification of the twelve *Xcv* bacterial isolates (Fig. 1) was confirmed with positive reactions 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 reactions for gram reaction, pigment on K.B., gelatin liquefaction, spore production, utilization of sorbitol and acid from Sorbitol.



	El-Giza		El-Qalubia		El-Behera		El-Minia		El-Sharkia	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
Pseudomonas sp.	3.0	30.0	1.0	25.0	3.0	42.9	2.0	20.0	3.0	37.5
Bacillus sp.	4.0	40.0	2.0	50.0	3.0	42.9	4.0	40.0	2.0	25.0
X. c. pv. Vesicatoria	3.0	30.0	1.0	25.0	1.0	14.2	4.0	40.0	3.0	37.5

Fig. 1. Isolation localities of pepper leaf spot pathogen and frequency percentages of isolated bacteria.

Pathogenicity and Host Range Test:

The findings in Table (1**)** show that all *Xcv* isolates tested were positive for their pathogenicity on pepper plants. In the meanwhile, only I_1 and I_8 isolates showed low positive pathogenicity on pepper plants. On the other hand, isolates I_1, I_3, I_4, I_6, I_9, I_{10}, I_{11}, and I_{12} had a low pathogenicity index exclusively on pepper plants. The same Table shows that isolates

I_2, I_5, and I_8 had the greatest pathogenicity index on pepper plants. The uninoculated pepper plants showed no signs of infection.

Table 1: Pathogenicity and host range test of isolated bacterium *Xanthomonas campestris* pv. *Vesicatoria* "*Xcv*".

Common name	Scientific name	Tested *Xcv* isolates											
		*I_1	I_2	I_3	I_4	I_5	I_6	I_7	I_8	I_9	I_{10}	I_{11}	I_{12}
Tomato	*Lycopersicon esculentum*	+	-	-	-	-	-	-	+	-	-	-	-
Eggplant	*Solanum melongena*												
Pepper	*Capsicum annum*	+	++	+	+	+++	+	+	++	+	+	+	+
Bean	*Phaseolus vulgaris*												

* I_1, I_2, I_3= El-Giza; I_4= El-Qalubia; I_5= El-Behera; I_6, I_7, I_8, I_9= El-Minia; I_{10}, I_{11}, I_{12}= El-Sharkia isolates.
* + = low pathogenic; ++ = moderately pathogenic; +++ = highly pathogenic.

Management of the Disease:

1.1. In vitro

As shown in Figure 2, the antibacterial capabilities of five different bacteritoxicants were evaluated against *Xcv*. According to the data in the same table, all tested treatments demonstrated obvious substantial inhibitory effects on *Xcv* when compared to the control treatment. In this regard, copper oxychloride had the greatest significant increase in the inhibitory zone, followed by oxytetracycline, hydrogen peroxide, and clove oil treatments, being 6.32, 5.45, 5.14, and 4.52 mm, respectively. The same data indicates that increasing concentration per main treatment enhanced the inhibition zone in comparison to the control treatment.

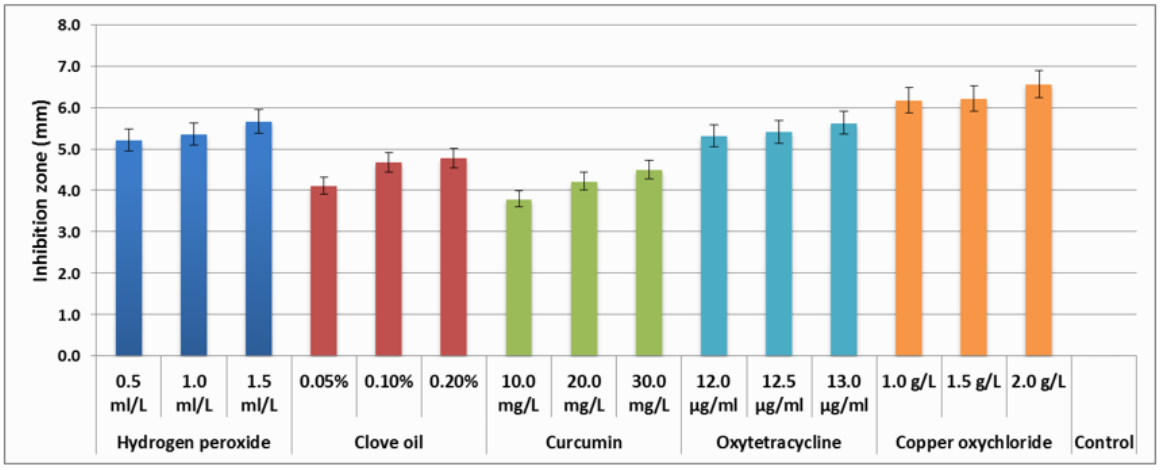


Fig. 2. The inhibiting effect of tested treatments; hydrogen peroxide, clove oil, curcumin, oxytetracycline, and copper oxychloride at different concentrations against tested bacterium *Xanthomonas. campestris* pv.*vesicatoria in vitro*.

***3.2. In vivo*:**

The data in Table (2**)** show that, applying the median concentration of each tested treatment *i.e.*, hydrogen peroxide, clove oil, curcumin, oxytetracycline, and copper oxychloride as a foliar spray significantly reduced bacterial leaf spot development (disease severity %) in comparison to the control treatment during the two growing (2019/2020 and 2020/2021) seasons. All treatments examined outperformed the control treatment in terms of reducing bacterial leaf spot evaluation. The data in the Table show, also, that copper oxychloride, when used as the treated control, resulted in the greatest significant decrease in disease severity % during both growing seasons, being 11.32 and 10.63% when compared to the untreated control (35.66 and 32.84%, respectively). When compared to the control, treatment

with copper oxychloride, oxytetracycline, and hydrogen peroxide generally produced the highest mean reduction in disease severity across the two seasons, being 10.98, 12.23, and 13.06%, respectively.

4. Effect of tested treatments on pepper fruit yield:

In terms of the effect of the tested control treatments on total fruit yield weight of pepper (kg/plot), data in Table (2) show that, when compared to the control treatment, all tested treatments significantly increased the average fruit production of pepper plants throughout both the 2019 and 2020 growing seasons. During the two growing seasons, all evaluated treatments increased total fruit production, in contrast to the control treatment. The data in the Table, also reveal that, copper-treated control treatment resulted in the greatest significant increase in total fruit yield, being of 23.68 kg/plot when compared to the un-treated control treatment (13.19 kg/plot), followed by the oxytetracycline, curcumin, clove oil, and hydrogen peroxide, being 23.20, 21.44, 18.13, and 16.35 kg/plot, respectively during both growing seasons.

Table 2: Effect of tested treatments on pepper bacterial spot disease development and pepper fruit yield assessment under field condition during growing seasons 2019 and 2020.

Treatment	Disease severity %			Pepper fruit yield weight (kg/plot)		
	2019	2020	Mean	2019	2020	Mean
Hydrogen peroxide	13.67	12.45	**13.06**	16.37	16.33	**16.35**
Clove oil	14.33	13.84	**14.09**	18.58	17.68	**18.13**
Curcumin	14.73	14.67	**14.70**	21.16	21.71	**21.44**
Oxytetracycline	12.67	11.78	**12.23**	23.68	22.71	**23.20**
Copper oxychloride	11.32	10.63	**10.98**	24.21	23.14	**23.68**
Control	35.66	32.84	**34.25**	13.82	12.55	**13.19**
Mean	**17.06**	**16.04**	---	**19.64**	**19.02**	---
LSD at 5%	**1.95**	**2.01**		**3.54**	**4.13**	

5. Effect of tested treatments on biochemical assessments:

The investigated treatments enhanced total phenols content and oxidative enzymes activity (peroxidase, polyphenoloxidase, and chitinase) in pepper leaves as compared to the treated-copper control and untreated control treatments (Fig. 3). Curcumin increased total phenols content as compared to the control treatment. Curcumin, hydrogen peroxide and clove oil scored the greatest increase percentages, being 111.68, 106.71, and 82.91%, respectively. Furthermore, compared to the control treatment, data illustrated in the same Figure reveal that all the tested treatments increased the activity of the peroxidase enzyme in pepper leaves. Curcumin, followed by clove oil and hydrogen peroxide, provided the greatest rise, being 138.77, 137.12, and 123.92%, respectively. In terms of polyphenol oxidase activity (Fig. 3), curcumin, clove oil, and hydrogen peroxide all increased polyphenol oxidase activity, being 112.92, 101.52, and 92.47%, respectively when compared to the control treatment. In addition, findings in the same Figure reveal that utilizing curcumin increased chitinase activity (75.91%) when compared to the control treatment. In terms of vitamin-C increase percentage (Fig. 3), all evaluated bacteriotoxicants enhanced vitamin-C content when compared to the control treatment. Curcumin was the most effective followed by clove oil then hydrogen peroxide, being 158.36, 136.54, and 133.61%, respectively.

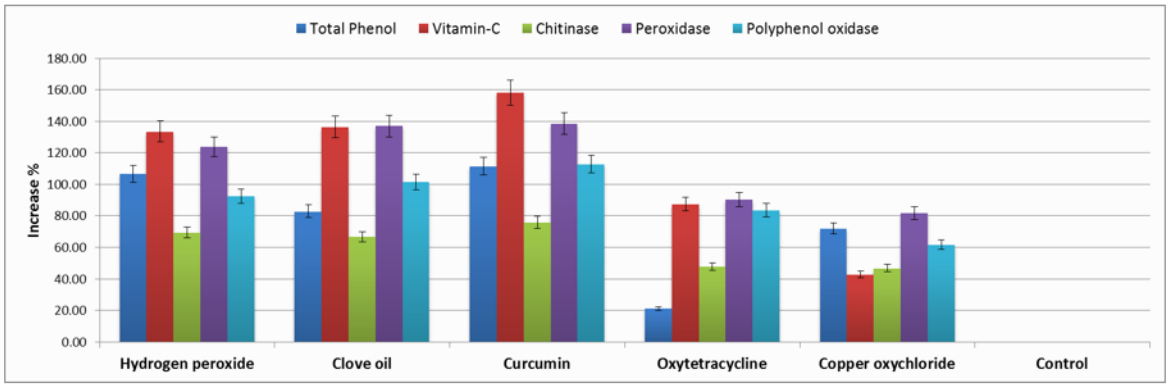


Fig 3. Effect of tested treatments on total phenols, vitamin-C, chitinase, peroxidase and polyphenol oxidase increase %.

DISCUSSION

The purpose of this research was to determine if hydrogen peroxide, clove oil, curcumin, oxytetracycline; and copper oxychloride fungicide are efficient *in vitro* against the pathogenic bacterium *Xathnsomonas campestris* pv. *vesicatoria*, as well as whether these substances might be employed *in vivo* to reduce pepper bacterial leaf spot. The results obtained showed that, hydrogen peroxide, clove oil, curcumin, oxytetracycline; and copper oxychloride fungicide can prevent *X. campestris* pv. *vesicatoria* "*Xcv*" infection *in vitro* and *in vivo*.

Isolation trials from pepper leaves with characteristic bacterial spot symptoms obtained from the governorates of El-Giza, El-Qalubia, El-Behera, and El-Minia generated 39 bacterial isolates. *Bacillus* spp., *Pseudomonas* spp., and *Xcv* were isolated and identified. The isolated 12 bacterial strains were identified as *Xcv*. According to Schaad *et al.*, (2001) the initial identification of the 12 strains was confirmed by morphological, biochemical, and physiological tests. Numerous authors determined that *Xcv* was the responsible bacteria for pepper leaf spot (Bashan *et al.*, 1985; Jones *et al.*, 1998; Mirik *et al.*, 2007 and 2008; Ju-Hee *et al.*, 2015). It appears that all isolates had a limited host range and were exclusive to pepper and tomato plants. These findings might be considered in light of the fact that some *Xcv* isolates can infect tomato, cowpea, and bean plants, causing a hypersensitive reaction (El-Sadek *et al.*, 2001). The findings of the pathogenicity test revealed that the inoculated pepper plants that received the *Xcv* isolate No. 3 from the El-Behera governorate experienced the highest disease severity. According to Mirik *et al.*, (2007), 67 bacterial strains of *Xanthomonas axonopodis* pv. *vesicatoria* were isolated and identified from pepper plants. All of the isolates infected pepper plants and the reference strain (GSPB 224) displayed the typical symptoms of bacterial spots on pepper leaves in 7 to 14 days during pathogenicity testing. The bacteria initially injected were recovered through re-isolations performed from artificially infected plants, and all strains were pathogenic on pepper plants of the cv. Balady. Copper-based compounds are, also, commonly utilized against bacteria, including Xanthomonas species (Araújo *et al.*, 2012), and their usage is permitted in the organic horticulture system (Jeyaraman & Robert, 2018). Copper bactericides have given control of copper-sensitive bacteria, but the presence of copper-tolerant pathogens makes copper compound control exceedingly challenging. When a copper bactericide is used in conjunction with mancozeb or maneb, copper-tolerant bacteria are more effectively controlled (Stall *et al.*, 1986). Fixed copper, often in combination with maneb and mancozeb, has been the primary chemical used to treat the bacterial spots caused by *Xcv* on pepper (Mirik *et al.*, 2007). Marco and Stall (1983), Mc Carter (1992), and Ju-Hee *et al.*, (2015) all

produced comparable findings. These findings might be interpreted in light of the possibility that, the inhibitory impact of copper oxychloride is due to copper poisoning. Copper has been shown to affect bacteria in two steps. The first is a direct interaction between the copper and the bacterial outer membrane, causing the membrane to rupture. The second is related to the pores in the outer membrane that allow the cell to lose important nutrients and water, leading the cell to weaken overall (Macomber and Imlay, 2009). Chemical plant disease prevention with copper and antibiotic sprays has also been investigated (Thayer and Stall, 1961; Conover and Gerhold, 1981; Jones and Jones, 1985). Furthermore, it is well recognized that, tetracyclines suppress bacterial protein production by preventing aminoacyl-tRNA from interacting with the bacterial ribosome (Oliva *et al.,* 1992; Ettner *et al.,* 1996).

Hydrogen peroxide (H_2O_2) is one of the most important reactive oxygen species (ROS) that can be used as a low-concentration safety compound against plant pathogens such as barley net blotch disease (Abdelaal *et al.,* 2020; Hafez *et al.,* 2019), wheat leaf rust (Omara *et al.,* 2015), barley powdery mildew (Hafez *et al.,* 2014; Hafez *&* El-Baghdady, 2013), Tobacco Mosaic Virus (Hafez, 2013), and cucumber powdery mildew fungus (Hafez *et al.,* 2008). The most stable reactive oxygen species is H_2O_2. Similar findings were achieved by van Doorn (2012); Macarisin *et al.,* (2010), who discovered that the application of H_2O_2 lowered the rate of bacterial growth and that it might be employed as a biocontrol agent for plant diseases (Bayoumi, 2008). H_2O_2 has recently been identified as a defensive signal molecule that plays a function in the activation of plant resistance against pathogen assaults. Plant defense responses were mediated by H_2O_2 through inducing resistance to pathogen infection (Byun and Choi, 2004; Hafez *et al.,* 2012). High doses of H_2O_2 can have direct antibacterial effects, killing microorganisms such as plant diseases. H_2O_2 has a direct antibacterial effect on bacterial blight of cowpea caused by *Xanthomonas campestris* pv. *vignicola*, and pretreatment of cowpea seeds and seedlings with H_2O_2 decreased disease severity (Kotchoni *et al.,* 2007).

Turmeric and its components curcumin extracted from turmeric (*Curcumin longa*) has been utilized in the treatment of various diseases for thousands of years due to its effectiveness, low cost, and high antioxidant content. Furthermore, toxicity tests revealed that it is rather safe even at large dosages (up to 12 g in humans). Curcumin has also been demonstrated to have anti-microbial activity *in vitro* against a variety of pathogens, including fungi (Neelofar *et al.,* 2011) and numerous Gram-positive bacteria (Lüer, 2012; Rudrappa and Bais, 1955). However, only few researches have revealed the mechanism of antibacterial action of curcumin -I which tends to change depending on the strain being investigated. Curcumin's antibacterial action against *Bacillus subtilis*, for example, has been shown to impede bacterial cell growth by disrupting the assembly dynamics of FtsZ in the Z ring (Rai *et al.,* 2008; Kaur *et al.,* 2010). Curcumin was demonstrated to have anti-infective efficacy against *Pseudomonas aeruginosa* infection via altering pathogenicity, quorum sensing, and biofilm initiation (Rudrappa and Bais, 2008). Furthermore, because these processes have not been verified in other bacterial taxa, they cannot be extended to other bacteria. As a result, a comprehensive research of curcumin's antibacterial mechanism including a large number of bacteria from various genera is required. Curcumin has been found in several studies to exhibit broad-spectrum antibacterial action as well as high biological activity against both Gram-positive and Gram-negative bacteria (Di Mario *et al.,* 2007). Curcumin can also limit bacterial growth by targeting the bacterial cell membrane, cell wall, protein, DNA, and other cellular components, or by interfering with the quorum sensing (QS) system.

Overuse of synthetic antibiotics leads to disease resistance, increased deposition of harmful residue in fruits, and affects the environment (McManus & Stockwell, 2000). One of the main techniques in integrated disease control is the creation of local and systemic

resistance in plants through the use of natural extracts (Doddaraju *et al.*, 2019; Kumar *et al.*, 2021; McManus & Stockwell, 2000; Srivastava *et al.*, 2011). The quest for plant-derived antimicrobial compounds has garnered substantial interest in recent years. Numerous organic compounds and essential oils have been demonstrated to increase resistance and stop pathogen invasion of the host system (Benagi & Ravi Kumar, 2009; Sharma & Sharma, 2011). Phenolic compounds, which are abundant in plant oils and have a wide spectrum of antibacterial properties, are among these chemicals (Chandra *et al.*, 2017). Essential oils, such as clove oil, have drawn a lot of attention in crop protection due to its antibacterial components, insect repellant characteristics, and nematicide capabilities (Deans & Ritchie, 1987; Huang & Lakshman, 2010; Lucas *et al.*, 2012; Sangwan *et al.*, 1990). Clove oil has amounts of eugenol, a phenolic component, up to 76.8%, which is known to be an effective antibacterial (Kishore *et al.*, 2007; Nurdjannah & Bermawie, 2012) and antiviral agent to prevent the yellow leaf curl virus in tomato plants (Wang & Fan, 2014). Applying the proper amount of clove oil is the most effective technique to reduce *Xcv* in tomatoes (Lucas *et al.*, 2012). Along with having a direct influence on pathogens, clove oil is recognized to increase plant competitiveness. Even though clove oil is effective against a number of plant diseases, the molecular mechanism by which clove oil and its combination with copper oxychloride (COC) cause plant defensive reactions while also preventing bacterial blight remains unknown. Clove oil had an antimicrobial effect at concentrations between 0.1 and 0.5 percent, with 0.5 percent displaying the strongest inhibition, followed by 0.3 percent and eugenol, which had the widest inhibition zone at 0.2 percent. Numerous bacteria and fungi, such as *Pseudomonas syringae* (Sukatta *et al.*, 2008), *Ralstonia solanacearum*, *X. compestris* pv. *pelargonii*, *Streptomyces* spp., *Rhodococcus fascians* (Huang & Lakshman, 2010), and *Erwinia caratovora* (Deans & Ritchie, 1987), have been proven to be sensitive to clove oil and eugenol's antibacterial activities. Similar findings were obtained when Kishore *et al.*, (2007) used clove oil as a foliar therapy to treat peanut diseases, late leaf spot, and crown rot. It has already been demonstrated that clove oil supports tomato disease resistance and protects against bacterial spots caused by *Xcv* (Lucas *et al.*, 2012). By rupturing their cytoplasmic membrane and raising non-specific cell permeability, eugenol, the main ingredient in clove oil, kills bacteria. According to recent studies, eugenol's hydroxyl group interacts with cellular proteins to stop *Enterobacter aerogenes* from building enzymes (Burt, 2004). Eugenol's hydrophobicity also allows it to pass through the gram-negative bacterial cell membrane's lipopolysaccharide, alter the cell's structure, and cause internal components to leak out, which inhibits the infection (Devi *et al.*, 2010; Sikkema *et al.*, 1994). These scenarios in other horticultural crops revealed that clove oil may significantly affect pepper resistance to *XCV* by promoting systemic resistance and obstructing the pathogen in the host system (Lucas *et al.*, 2012), validating the conclusions of the present investigation.

ROS production is a key feature of effective microbe detection and defense response activation by plants (Dat *et al.*, 2000; Demidchik, 2015). ROS burst has been linked to the overproduction of a variety of reactive chemicals, primarily hydrogen peroxide (H_2O_2) and superoxide anion (Cheng *et al.*, 2016), which can cause oxidative stress and plant cell death. Plants have a finely organized antioxidant system that keeps cells at a stable state to balance these processes of ROS formation and elimination (Dat *et al.*, 2000; Apel and Hirt, 2004), which involves the action of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Apel and Hirt, 2004; Noctor and Foyer, 1998; Noctor *et al.*, 2016). The treatments that were tested had high levels of peroxidase activity, which is known to catalyze reactive oxygen species and other lignans to strengthen the antioxidant systems by fortifying the cell wall through cross-linking with molecules that resemble glycoproteins and are rich in the amino acid hydroxyproline. Wang and Fan (2014) revealed that experimental treatment of tomato plants causes a hydrogen peroxide explosion that activates

several immune-responsive genes and gives the host systemic resistance to the virus. The treatments boosted catalase and peroxidase activity in Newhall navel oranges that were kept after harvest, increasing their shelf life (Zeng *et al.*, 2012). Callose deposition accumulates between the cell wall and plasma membrane during biotic stress and acts as a physical barrier against infections. Treatments reduced post-harvest deterioration in Newhall orange by increasing the activity of the glucanase and chitinase genes during post-harvest storage. Furthermore, it has been demonstrated that pomegranate callose deposition functions as a structural deterrent to XAP (Kumar & Mondal, 2013).

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