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# Plant Extract Treatments Induce Resistance to Bacterial Spot by Tomato Plants for a Sustainable System

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**Abstract:** The aim of this study is to assess the effect of extracts of *Nerium oleander*, *Eucalyptus chamadulonsis* and *Citrullus colocynthis* against bacterial spot disease of tomato and to investigate the induction of resistance by tomato (*Solanum lycopersicum*) in order to promote a sustainable management system. The antibacterial activity of aqueous and ethanol plant extracts was tested against *Xanthomonas axonopodis* pv. *vesicatoria*, isolate PHYXV3, in vitro and in vivo. The highest antibacterial activity in vitro was obtained with *C. colocynthis*, *N. oleander* and *E. chamadulonsis*, respectively. In vivo, ethanol extracts of *N. oleander* and *E. chamadulonsis* were more effective than aqueous extracts in reducing pathogen populations on tomato leaves. Under greenhouse conditions, application of the plant extracts at 15% (v/v) to tomato plants significantly reduced disease severity and increased the shoot weight of ‘Super Marmande’ tomato. In most cases, plant extracts significantly increased total phenol and salicylic acid content of tomato plants compared to either healthy or infected ones. In addition, *C. colocynthis* and *E. chamadulonsis* extracts significantly increased peroxidase activity while only *E. chamadulonsis* increased polyphenol oxidase after infection with the causal agent. The results indicated that the plant extracts showed promising antibacterial activity and could be considered an effective tool in integrated management programs for a sustainable system of tomato bacterial spot control.

**Keywords:** Induced resistance; polyphenol oxidase; peroxidase; plant extract; bacterial spot

## 1. Introduction

Worldwide tomato (*Solanum lycopersicum*) production is 182,256,458 MT with a harvested area around 4762,457 ha [1]. Tomato crops are facing many bacterial diseases including bacterial spot caused by *Xanthomonas axonopodis* pv. *vesicatoria* [2,3]. The application of copper bactericides, alone or in combination with other pesticides and antibiotics (especially streptomycin), is the primary tool to control such bacterial diseases [4]. However, several limitations have restricted their use including bactericide resistance, market demands related to residues and worries of human health [5]. Thus, safe alternative control methods need to be tested and developed [6–8]. Among the alternative means,

many studies have proven the antimicrobial activity of various plant species against bacterial spot diseases [9,10]. In addition, several studies have screened extracts of various higher plant leaves, fruit and seeds for controlling phytopathogenic bacteria [11,12]. Basically, higher plants are considered to be one of the major sources of bioactive, economically significant natural compounds, pesticides and pharmaceuticals [13].

Numerous studies were mentioned that plant extracts such as *Cesalpinia coriaria* and essential oils have shown satisfactory antifungal and antibacterial properties in vivo [14,15]. Recently, many higher plant products have attracted the attention of researchers seeking phytochemicals to evaluate for their antimicrobial properties [16–18].

Leaf extracts of several species, e.g., *Eucalyptus globules*, *Datura stramonium*, *Ocimum* spp., *Salix* spp., *Rosmarinus officinalis*, *Cydonia oblonga* and *Foeniculum vulgare*, have been used successfully to control diverse plant diseases [12,19,20]. Application of some chemicals and plant extracts have induced resistance in plants against many causal pathogens including bacteria, fungi, viruses and nematodes [21,22]. In addition, plant extracts have been found to induce a defense response in infected plants [23]. Geetha and Shetty [24] mentioned that the mode of action of plant extracts against bacterial pathogens may enhance natural host defense mechanisms by increasing the activity of some antioxidant enzymes, e.g., peroxidases, polyphenol oxidase or the accumulation of phenolic compounds [25]. This may directly affect the survival of the pathogens or act indirectly on plant metabolism [26]. In addition, polyphenol oxidases (PPOs) catalyze the oxidation of several phenols to o-quinones [27].

The present study aimed to assess the effectiveness of extracts of three plant species against *X. axonopodis* pv. *vesicatoria* in vitro and the suppression of bacterial spot disease of tomato plants under greenhouse conditions. Furthermore, the potential change of total phenols, salicylic acid content, peroxidase and polyphenoloxidase activity in response to application of the extracts was investigated.

## 2. Materials and Methods

### 2.1. Seeds, Growth of Seedlings and Bacterial Isolates

‘Super Marmande’ tomato seeds used in this study were kindly provided by the Vegetable Dept. Faculty of Agriculture, Assiut University, Egypt. Pots of 30 cm diameter and containing sand (3 kg/pot) were used to grow two seedlings. The pots were kept on a greenhouse bench at  $30 \pm 5$  °C and 68%–80% RH, irrigated as required and fertilized with 30 mL of 0.01g of NPK formulation (12:4:6). *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye isolate PHYXV3 was provided from the collection of the Department of Plant Pathology, Faculty of Agriculture, Assiut University, Egypt. This isolate was identified, and its pathogenicity was confirmed according to Abo-Elyousr and El-Hendawy [3].

### 2.2. Preparation of Leaf Extracts

Methods described by Abo-Elyousr and Asran [12] were followed to prepare the aqueous and 70% ethanol extracts. Briefly, 10 g of fresh leaves of *Nerium oleander*, *Eucalyptus chamadulonsis* and *Citrullus colocynthis* were collected from a private farm belonging to Assiut Governorate and washed several times with distilled water. They were crushed in 100 mL of sterile water or ethanol (1:10 w/v) using a mortar and pestle and filtered through double-layered cheesecloth. The ethanolic filtrate was concentrated by exposition to 60 °C in a water bath for 30 min to evaporate ethanol and kept in dark glass bottles. The final extracts were diluted with distilled water to reach a 10% and 15% v/v concentration. These concentrations were sterilized by 0.2 m disposable syringe filters and kept in bottles in the dark until used [28].

### 2.3. Effect of Plant Extracts on Pathogen Growth In Vitro

To test the effect of three plant extracts on the growth of the bacterial pathogen, the paper disc diffusion method was used [29]. Sterilized filter paper discs (5 mm) were placed in Petri plates containing nutrient sucrose agar (NSA) medium and inoculated with 100 µL of the bacterial suspension

of PHYXV3 ( $5 \times 10^6$  CFU mL<sup>-1</sup>). Sterilized filter paper discs were moistened with 5  $\mu$ L of each concentration (10% and 15%) of plant extract. As check treatments, water and ethanol served as controls. After 48 h of incubation at 27 °C, the inhibition zones around the paper disc were measured. Four plates were used for each treatment, as replicates, and the whole trial was repeated twice.

#### 2.4. Effect of Plant Extracts on Disease Severity and Pathogen Population

##### 2.4.1. Preparation of Inoculum and Inoculation Methods

Inoculum of PHYXV3 was prepared from cultures shaken for 48 h and incubated at  $28 \pm 2$  °C for 48 h. The cultures were centrifuged at  $6000 \times g$  at room temperature for 20 min and were then suspended in sterile distilled water. The bacterial concentration was photometrically adjusted using a spectrophotometer (Spectronic® 20 Genesys, Schutt Labortechnik, Cambridge CB5 8HY, UK) to  $5 \times 10^6$  CFU mL<sup>-1</sup>.

Four-week-old tomato seedlings were inoculated with PHYXV3 by spraying each plant with 20 mL of the pathogen solution using small hand sprayer. This volume was enough to cover plant leaves with the pathogen solution. Inoculated plants were covered by polyethylene bags for 48 h at 25–27 °C in the greenhouse to maintain the humidity and to prevent inoculation desiccation and inoculum establishment on the plant. Two days after inoculation, tomato seedlings were treated with a 15% v/v concentration of each plant extract and were kept under greenhouse conditions. Fourteen days after inoculation, disease severity was recorded based on the scale of Abbasi et al. [30]. Four replicates (two seedlings each) were used per each treatment and greenhouse experiments were repeated twice.

##### 2.4.2. Determination of Pathogen Population on Tomato Leaves

The number of colonies of PHYXV3 ( $5 \times 10^6$  CFU mL<sup>-1</sup>) was estimated one week after the time the tomato plants were treated with the plant extracts. About 5 mm of tomato leaf was excised and homogenized in 1 mL of sterile distilled water. Several dilutions were made and then 0.1 mL of each dilution was spread on plates containing nutrient sucrose agar (NSA) medium. Petri dishes were incubated for 72 h at 26 °C, and then recovered colonies were counted. Four replicates were used from each treatment and the results were expressed as CFU/g [3].

##### 2.4.3. Determination of Fresh and Dry Weight

At the end of experiment (90 days), the seedlings from each treatment were removed, washed with distilled water to eliminate the soil, blotted with tissue paper, and then dried at 60 °C for 72 h when shoot dry weight was recorded.

#### 2.5. Determination of Total Phenols and Salicylic Acid Contents

##### 2.5.1. Preparation of Samples

Eight leaves were collected from eight seedlings per treatment. One gram of tomato leaves was ground in liquid nitrogen and homogenized in 10 mL of 80% methanol, and the homogenate was centrifuged at  $10,000 \times g$  for 30 min at 4 °C. The pellet was discarded after addition of ascorbic acid ( $0.02$  g·mL<sup>-1</sup>). A rotary evaporator was used to evaporate the supernatant at 65 °C and the process was repeated three times, each for 5 min. The residues were dissolved in 5 mL of 80% methanol. For each treatment four replicates were used [31].

##### 2.5.2. Total Phenol Content

The methods described by Sahinet al. [32] were followed to determine the total phenol content in leaves. Total phenol content was determined by spectrophotometer (Spectronic® 20 Genesys, Schutt Labortechnik, Cambridge CB5 8HY, UK) at 767 nm as mg·g<sup>-1</sup> plant fresh weight using gallic acid as standard. Total phenol content was expressed as mg of gallic acid per g plant material.

### 2.5.3. Salicylic Acid Content

Salicylic acid (SA) content was estimated using a method modified from Datet al [33]. A 500  $\mu$ L sample of leaf homogenate was mixed with 250  $\mu$ L of 10-N HCl and 1 mL of methanol. Samples were incubated in a water bath at 80 °C for 2 h. One mL of methanol was added to the mixture and each sample was neutralized with 4–5 drops of 1-M NaHCO<sub>3</sub>. The optical density (OD) was measured at 254 nm, and SA content was calculated and expressed as  $\mu$ g of SA per g plant material.

## 2.6. Enzymatic Activities

To determine peroxidase (PO) and polyphenol oxidase (PPO) activities, 1 g of fresh tomato leaf tissue was homogenized in liquid nitrogen with 10 mL of 0.1-M sodium acetate buffer (pH 5.2) and the mixture was centrifuged at 1000 $\times$  g for 30 min at 4°C. For each treatment, four replicates were used. Using Bradford reagent, the total protein content of the supernatant was determined [34].

### 2.6.1. Peroxidase Activity (PO)

Activity of peroxidase was determined using guaiacol as a substrate according to the method described by Putter [35]. The reaction mixture was composed of 0.2 mL supernatant, 1 mL 0.1-M sodium acetate buffer (pH 5.2), 0.2 mL 1% w/v guaiacol and 0.2 mL of 1% H<sub>2</sub>O<sub>2</sub>. The mixture was incubated for 5 min at 25 °C then measured at 436 nm by spectrophotometer (Spectronic® 20 Genesys, Schutt Labortechnik, Cambridge CB5 8HY, UK). Peroxidase activity was calculated as the change in absorbance units (Au) and expressed as change in Au per mg protein. Extraction buffer served as a blank reference.

### 2.6.2. Polyphenol Oxidase (PPO) Activity

Polyphenol oxidase activity was determined according to the method described by Batra and Kuhn [36]. The reaction mixture was 0.5 mL of supernatant, 2 mL 50-mM Sorensen phosphate buffer (pH 6.5) and 0.5 mL of the substrate pyrocatechol (10%, Sigma Aldrich, Missouri, USA). The reaction mixture was incubated in water bath for 2 h at 37 °C and measured at 410 nm. The activity of PPO was calculated as OD at 410 nm and expressed as OD·mg protein<sup>-1</sup>.

## 2.7. Statistical Analysis

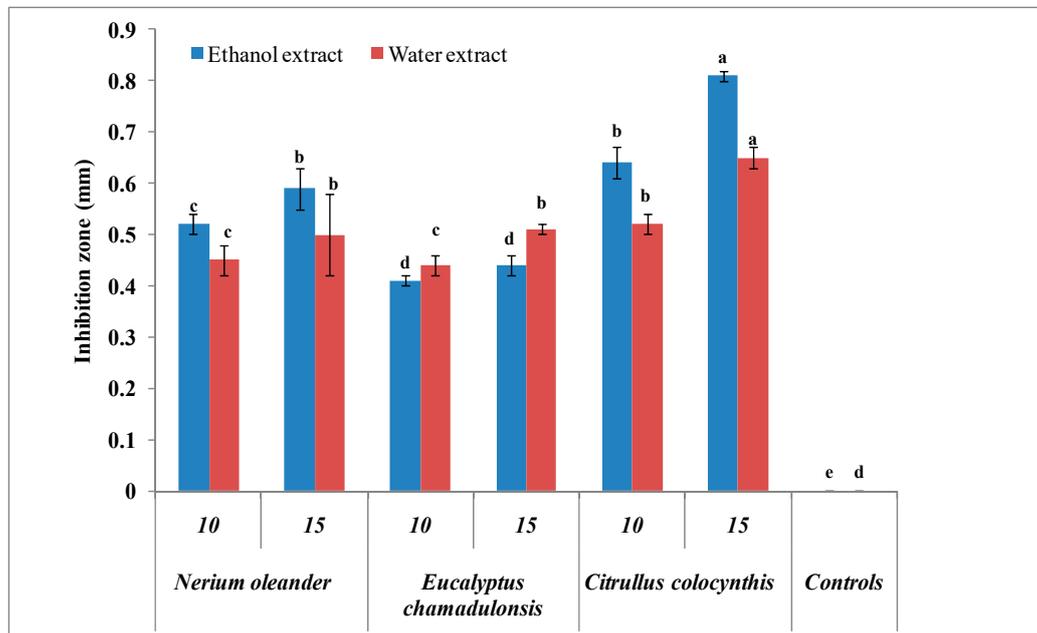
All experiments were repeated twice, and percentage data were arcsine transformed before analyses to normalize variance. Data were subjected to one-way analysis of variance (ANOVA) using Statistica Software Ver. 6.0 (Stat Soft, Inc., Tulsa, OK, USA). Fisher's protected least significant difference was used at  $p \leq 0.05$  to distinguish the differences among various treatments [37]. When applicable, error bars are shown.

## 3. Results and Discussion

### 3.1. Effect of Plant Extracts on Pathogen Growth In Vitro

The plant extracts had variable effect on the growth of the pathogen in vitro (Figure 1). The greatest reduction was obtained with both extracts of *C. colocynthis* at 15% (ethanol (0.81 mm) and water (0.65)) followed by the ethanol extract of *N. oleander* (ethanol (0.59 mm) and water (0.50)), while the least reduction was obtained from *E. chamadulonsis* (ethanol (0.44 mm) and water (0.51)). Overall, the inhibition of pathogen growth increased as the concentration increased from 10% to 15%. Thus, 15% was used for further experiments. The results herein agree with those reported by Abo-Elyousr and Asran [12], in which garlic (*Allium sativum*) extract had a strong antibacterial activity against bacterial wilt in vitro followed by *Datura* spp and then *N. oleander*. Our previous study reported that gas chromatography mass spectrometry (GC-MS) analysis of the aqueous extract of *C. colocynthis* contained 37 compounds and their derivatives, including imidazole. In addition, Rahman and

Gray [38] mentioned that dimericcarbazole was the most effective compound against *Escherichia coli* and *Proteus vulgaris*, both Gram-negative bacteria.



**Figure 1.** Antibacterial activity of 10% and 15% concentrations of plant extracts of *Nerium oleander*, *Eucalyptus chamadulensis* and *Citrullus colocynthis* against *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3 in vitro after 48 h of incubation at 27 °C. Four replicate plates were used for each treatment and the experiment was repeated twice. Columns with the same letters are not significantly different according to Fisher's protected least significant difference at  $p \leq 0.05$ .

### 3.2. Determination of Pathogen Population on the Tomato Leaves

Application of the plant extracts at the 15% concentration reduced the number of bacteria in tomato leaves compared to the infected control (Table 1). The greatest reduction was achieved by *C. colocynthis* [ethanol (3.0 CFU/g) and water (4 CFU/g)] followed by ethanol extract of *N. oleander* (5.0 CFU/g) and *E. chamadulensis* (5.1 CFU/g), with no difference between the latter two. The lowest reduction was with water extracts of *E. chamadulensis* (7.7 CFU/g) and *Nerium oleander* (6.2 CFU/g), respectively, which did not differ. The results assumed that the application of the plant extracts reduced the number of the bacterial pathogens by toxicity within the cytoplasm to the pathogen. These results are in agreement with Draz et al. [39] who revealed the bioactivity of certain components of plant extracts.

**Table 1.** Effect of 15% concentrations of water and ethanol plant extracts of *Nerium oleander*, *Eucalyptus chamadulensis* and *Citrullus colocynthis* on the population of *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3 in 'Super Marmande' tomato leaves after seven days of inoculation.

Plant Extracts	Method of Extract	CFU/g <sup>z</sup>
<i>Nerium oleander</i>	Water extract	6.2 ± 0.15 b <sup>y</sup>
	Ethanol extract	5.0 ± 0.72 c
<i>Eucalyptus chamadulensis</i>	Water extract	7.7 ± 0.38 b
	Ethanol extract	5.1 ± 0.23 c
<i>Citrullus colocynthis</i>	Water extract	4.0 ± 0.15 d
	Ethanol extract	3.0 ± 0.45 d
Controls	Infected	9.7 ± 0.53 a
	Healthy	0 e

<sup>z</sup> Bacterial population was recorded one week after inoculation with the isolate PHYXV3 and is expressed as log 10<sup>8</sup> colony forming units (CFU) g<sup>-1</sup> leaf tissue. Four replicates were used from each treatment. <sup>y</sup> Values in the column followed by the same letters are not significantly different according to Fisher's protected least significant difference at  $p \leq 0.05$  level.

### 3.3. Effect of Plant Extracts on Disease Severity and Dry Weight of Shoots

Treatment with extracts of *C. colocynthis* gave the greatest reduction of disease severity, 20.0% and 21.2% for ethanol and water extracts, respectively, with no significant difference between them, followed by *N. oleander* and *E. chamadulonsis*, respectively (Table 2). Furthermore, *C. colocynthis* gave the greatest shoot dry weight (44.7 and 43.0 for ethanol and water, respectively), while the other treatments also increased shoot weight compared to the infected control, but without statistical difference between them. These results agree with Hassan et al. [40] who mentioned that the application of certain plant extracts reduced wilt diseases of potato plants and increased the yield s of tubers compared to an infected control. This may be due to the lower numbers of the pathogen in treated plants and the induction of some antioxidant enzymes that can reduce pathogens in the tissues [29]. Similarly, Drazet et al. [39] used five plant extracts (pomegranate (*Punica granatum*), acalypha (*Acalypha wilkesiana*), henna (*Lawsonia inermis*), lantana (*Lantana camara*) and chinaberry (*Melia azedarach*) to induce resistance in wheat (*Triticum aestivum*) against rust disease and all plant extracts reduced disease severity and increased yield components.

**Table 2.** Disease severity (%) and shoot dry weight of ‘Super Marmande’ tomato treated with extracts of *Nerium oleander*, *Eucalyptus chamadulonsis* and *Citrullus colocynthis* after inoculation with *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3.

Plant Extracts	Method of Extract	Disease Severity (%)	Shoot Weight (gm)
<i>Nerium oleander</i>	Water extract	29.13 ± 0.10 <sup>z</sup> c <sup>y</sup>	25.2 ± 0.30 c
	Ethanol extract	30.00 ± 1.51 c	25.5 ± 0.38 c
<i>Eucalyptus chamadulonsis</i>	Water extract	39.90 ± 0.68 b	26.8 ± 0.60 c
	Ethanol extract	33.13 ± 0.10 cd	24.2 ± 0.30 c
<i>Citrullus colocynthis</i>	Water extract	21.23 ± 0.17 e	43.0 ± 1.51 ab
	Ethanol extract	20.00 ± 0.76 e	44.7 ± 0.45 ab
Controls	Infected	45.23 ± 0.98 a	13.2 ± 0.30 d
	Healthy	0 f	45.2 ± 0.91 a

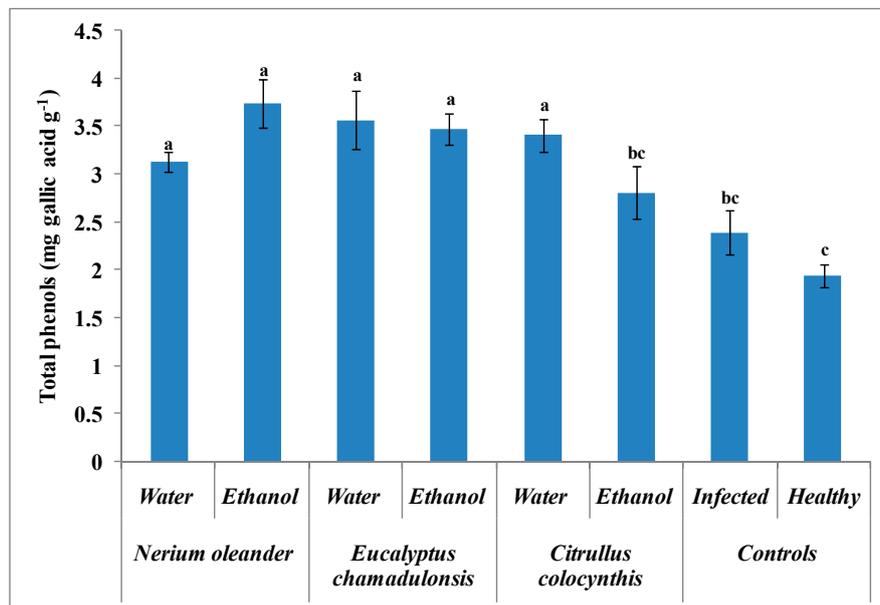
<sup>z</sup> Mean ± SE of 4 replicates (two seedlings each) for each treatment. <sup>y</sup> Values in the columns followed by the same letters are not significantly different according to Fisher’s protected least significant difference at  $p \leq 0.05$  level.

### 3.4. Effect of Plant Extracts on Total Phenol and Salicylic Acid Contents

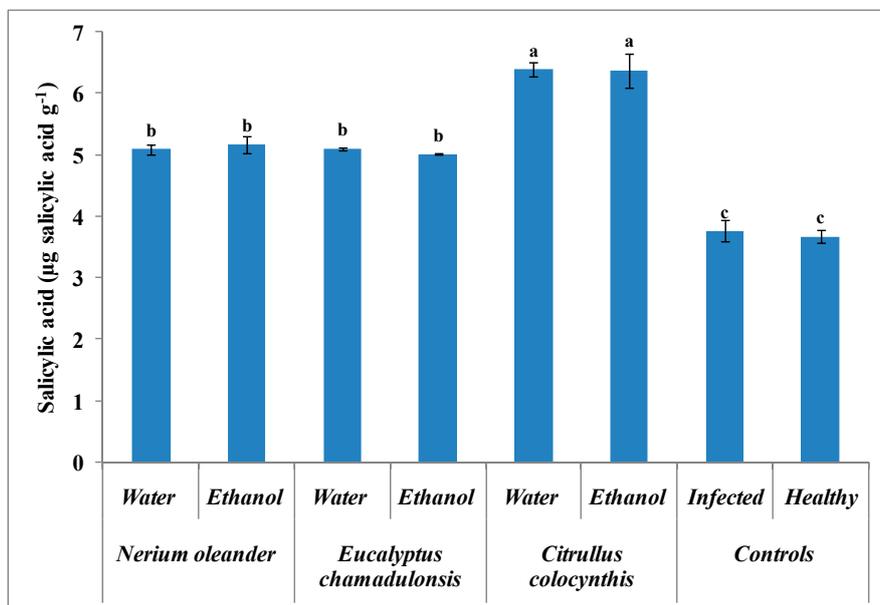
Overall, total phenol content was significantly higher in treated versus infected or healthy control plants with the exception of the ethanol extract of *C. colocynthis*. In the present study, treatment with the plant extracts increased the accumulation of phenolic substances in response to pathogen infection (Figure 2). Values of phenol content ranged from 3.13 to 3.57 mg gallic acid g<sup>-1</sup> for the water extraction method and from 2.81 to 3.74 mg gallic acid g<sup>-1</sup> for the ethanol extraction method for the three species. Accumulation of phenolic compounds at the infection site was correlated with the suppression of pathogen development since these compounds are toxic to phytopathogenic bacteria. Resistance maybe also improved by increasing the pH of the plant cell cytoplasm due to an increase in phenolic acid substances which prevents the development of the pathogen [41].

Salicylic acid (SA) content significantly increased with application of *C. colocynthis* aqueous (6.4 µg salicylic acid g<sup>-1</sup>) and ethanol (6.37 µg salicylic acid g<sup>-1</sup>) extracts after 7 days of treatment, though there was no significance difference between them (Figure 3). The results showed statistically significant increases in SA levels for all treatments versus control. However, it is also clear that ethanolic and aqueous extracts showed similar SA levels for all treatments within species and that *C. colocynthis* extracts induced the largest SA increases among the treatments. De Meyer et al. [42] suggested that SA accumulation is important for expression of several modes of plant disease resistance. In addition, SA mediates plant defense against pathogens, accumulating in both infected and distal leaves in response to pathogen infection [29]. The enhanced SA contents are a prerequisite for expression of systemic acquired resistance against *R. solanacearum* in potato (*Solanum tuberosum*) plants [43,44], and these results may also indicate that applied plant extracts induce pathogen resistance in plants either via the

activation of a signaling pathway that is dependent on SA or via the activation of a novel signaling cascade that is not dependent on SA, jasmonic acid or ethylene signaling.



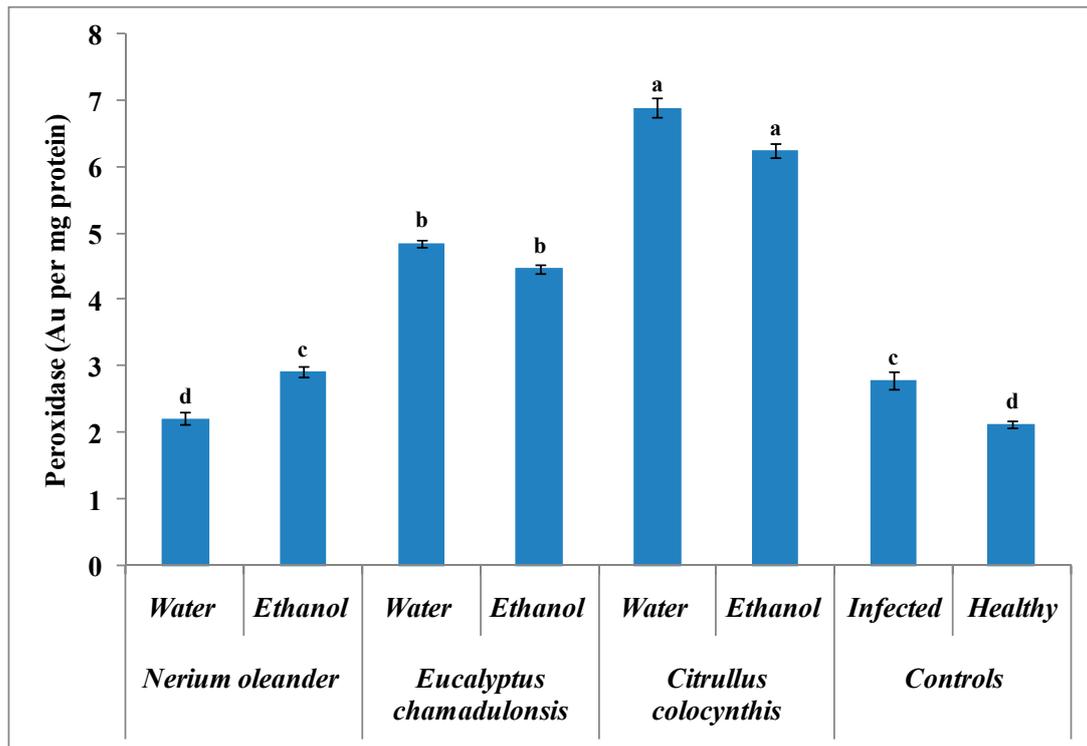
**Figure 2.** Effect of 15% concentrations of plant extracts of *Nerium oleander*, *Eucalyptus chamadulensis* and *Citrullus colocynthis* on total phenols of ‘Super Marmande’ tomato after 7 days of treatment with the extracts and inoculated with *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3. Four replicates were used for each treatment. Columns with the same letters are not significantly different according to Fisher’s protected least significant difference at  $p \leq 0.05$ .



**Figure 3.** Effect of 15% concentrations of plant extracts of *Nerium oleander*, *Eucalyptus chamadulensis* and *Citrullus colocynthis* on salicylic content of ‘Super Marmande’ tomato after 7 days of treatment with the extracts and inoculated with *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3. Four replicates were used for each treatment. Columns with the same letters are not significantly different according to Fisher’s protected least significant difference at  $p \leq 0.05$ .

### 3.5. Effect of Plant Extracts on Peroxidase (PO)

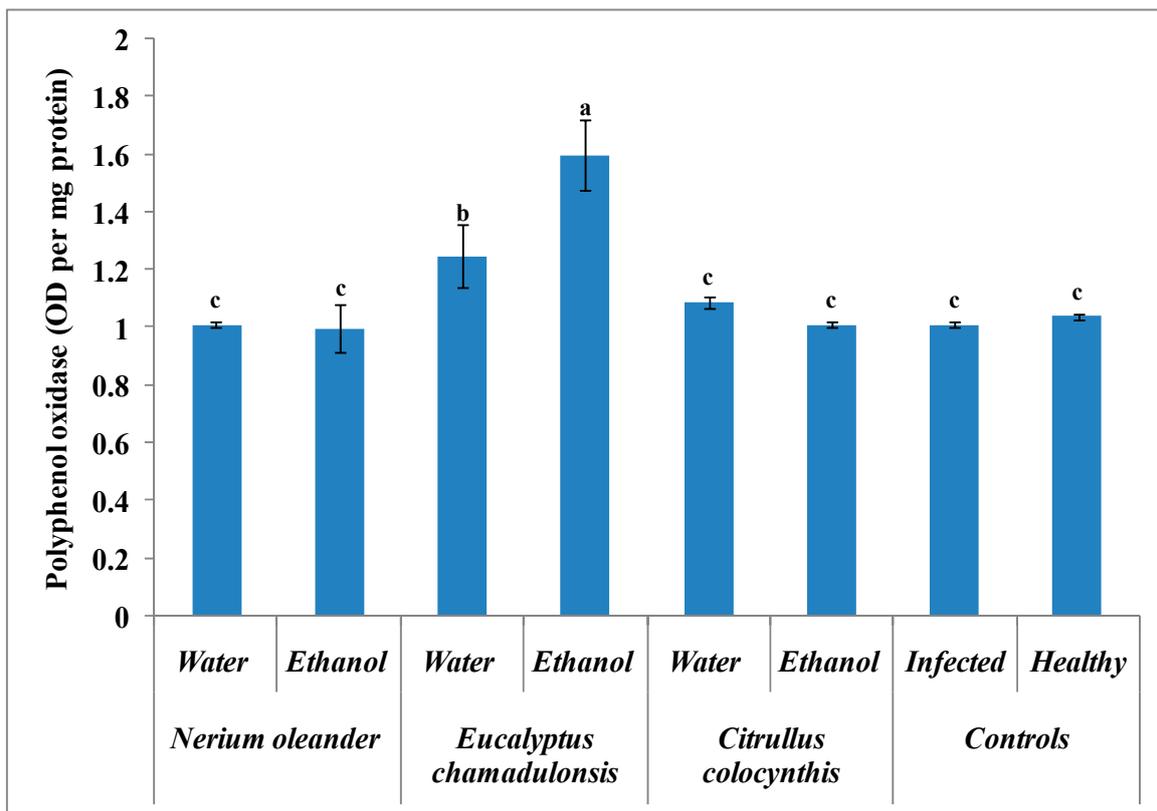
Peroxidase (PO) activity significantly increased in infected plants treated with extracts of all species except *Nerium oleander* (Figure 4). *C. colocynthis* extract caused the most increase in PO activity followed by *E. chamadulonsis*. Values for *C. colocynthis* were 6.9 and 6.25 Au per mg protein for water and ethanol extraction, respectively. The results agree with prior studies [45]. The results suggested that the plant extracts promoted an increase in defense-related peroxidase enzyme activity. Several investigators have reported that enhanced peroxidase activity was associated with plant defense against fungal, bacterial and viral pathogens [46].



**Figure 4.** Effect of 15% concentrations of plant extracts of *Nerium oleander*, *Eucalyptus chamadulonsis* and *Citrullus colocynthis* on peroxidase activity as absorbance units (Au) per mg protein of ‘Super Marmande’ tomato after 7 days of treatment with the extracts and inoculated with *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3. Four replicates were used for each treatment. Columns with the same letters are not significantly different according to Fisher’s protected least significant difference at  $p \leq 0.05$ .

### 3.6. Effect of Plant Extract on Polyphenol Oxidase (PPO)

In most cases, PPO activity showed no significant difference between infected tomato plants versus extract-treated plants using *N. oleander* and *C. colocynthis* extracts (Figure 5). The highest PPO activity was obtained following applications of *E. chamadulonsis* extracts 7 days post-application (1.25 and 1.6 nm mg<sup>-1</sup> protein for water and ethanol, respectively). Such results agree with other investigations [40,46]. The importance of PPO activity in disease resistance probably stems from its property to oxidize phenolic compounds to quinines, which are frequently more toxic to pathogens than the original phenol [46]. In addition, Draz et al. [39] mentioned that biochemical analyses proved a significant increase in the plant contents of total phenolics and oxidative enzymes activities (PPO and POX) after treated with certain plant extracts.



**Figure 5.** Effect of 15% concentrations of plant extracts of *Nerium oleander*, *Eucalyptus chamadulonsis* and *Citrullus colocynthis* on polyphenol oxidase activity as optical density (OD) per mg protein of ‘Super Marmande’ tomato after 7 days of treatment with the extracts and inoculated with *Xanthomonas axonopodis* pv. *vesicatoria* PHYXV3. Four replicates were used for each treatment. Columns with the same letters are not significantly different according to Fisher’s protected least significant difference at  $p \leq 0.05$ .

#### 4. Conclusions

The antibacterial activity of aqueous and ethanol extracts of *N. oleander*, *E. chamadulonsis* and *C. colocynthis* plants were tested against *Xanthomonas axonopodis* pv. *vesicatoria* in vitro and inoculated plants in vivo. In in vitro tests, the highest antibacterial activity was achieved by extracts of *C. colocynthis*, followed by *N. oleander* and then *E. chamadulonsis*. Application of the extracts to tomato plants significantly reduced disease severity and increased shoot weight of ‘Super Marmande’ tomato plants. Overall, plant extracts significantly increased total phenol and salicylic acid content treated plants compared to healthy or infected ones. In addition, *C. colocynthis* and *E. chamadulonsis* significantly increased peroxidase activity while only *E. chamadulonsis* increased polyphenol oxidase. Thus, alternative means for controlling plant disease—such as plant extracts—may be able to replace or reduce the use of bactericides. Leaf extracts of these species showed promising antibacterial activity against the causal agent of tomato bacterial spot and could become a part of an integrated pest management program for controlling the disease.

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