Determination of Antibacterial Efficacies of Plant Extracts on Tomato Bacterial Speck Disease

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Accepted for publication June 25, 2015

ABSTRACT

Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* is an economically important disease of tomato. The aim of this study was to evaluate the effectiveness of some medicinal and aromatic plant extracts on tomato seeds (cv. H-2274) and plants against *P. s.* pv. *tomato in vitro* and *in vivo*. The extracts obtained from leaves, stems, seeds and fruits of *Juglans regia, Lavandula officinalis, Eucalyptus globulus, Rosmarinus officinalis, Olea europaea, Tilia tomentosa, Jatropha curcas, Humulus lupulus, Nigella sativa, Trigonella foenum-graecum, Cuminum cyminum, Rhus coriaria, Daucus carota, Rosa canina* and *Moringa oleifera.* Amongs the 15 different plants, three plant extracts inhibited the growth of the pathogen with inhibition zone diameter ranging from 13 to 22 mm at 20% (w/v) in absolute methanol. Bacterial population on tomato seeds after applications of the plant extracts at 5 doses was also determined. *Rhus coriaria* and *Eucalyptus globulus* extracts at 20% concentration completely inhibited the *P. s.* pv. *tomato* on tomato seeds. In spite of the highest antibacterial effects of sumac and eucalyptus to the pathogen, there was no a negative effect on tomato seed germination. The effects of *E. globules, R. coriaria* and *R. officinalis* extracts against the pathogen were examined in greenhose conditions and, the highest efficiency was achieved by *E. globulus* (65.86%) on tomato. The peroxidase enzyme and total protein values have shown an increase in the leaves following application of sumac, rosemary and eucalyptus extracts before the inoculation with *P. s.* pv. *tomato.* This study indicated that some plant extracts may be used in prevention programs to combat the bacterial speck disease on tomato.

Key words: *Pseudomonas syringae* pv. *tomato,* tomato, plant extracts, biological control, peroxidase, protein

ÖZET

Bitki Ekstratlarının Domates Bakteriyel Benek Hastalığı Üzerinde Antibakteriyel Etkililiğinin Belirlenmesi

Bakteriyel benek, *Pseudomonas syringae* pv. *tomato*'un sebep olduğu, ekonomik olarak domatesin önemli bir hastalığıdır. Bu çalışmanın amacı, bazı tıbbi ve aromatik bitki ekstraklarının, H-2274 domates çeşidi tohumları ve bitkilerinde *in vivo* ve *in vitro*' da *P. s.* pv. *tomato*' ya karşı etkilerini değerlendirmektir. Ekstraktlar, *Juglans regia, Lavandula officinalis, Eucalyptus globulus, Rosmarinus officinalis, Olea europaea, Tilia tomentosa, Jatropha curcas, Humulus lupulus, Nigella sativa, Trigonella foenum-graecum, Cuminum cyminum, Rhus coriaria, Daucus carota, Rosa canina* and *Moringa oleifera*' nın yaprak, sap, tohum ve meyvelerinden elde edilmiştir. Elde edilen sonuçlar göstermiştir ki %20 (w/v) mutlak metanolde ekstrekte edilen ve test edilen 15 bitki ekstraktından üçü, 13-22 mm engelleme çapı oranı ile patojenin gelişimini engellemiştir. Bitki ekstraktlarının 5 farklı dozunun uygulamasından sonra domates tohumlarındaki bakteriyel populasyon da belirlenmiştir. *R. coriaria* ve *E. globulus* ekstraklarının %20'lik konsantrasyonları domates tohumlarında *P. s.* pv. *tomato* 'yu tamamıyla engellemiştir. Sumak ve okaliptüsün patojene karşı yüksek antibakteriyel etkilerine rağmen domates çimlenmesi üzerine olumsuz bir etkiye sahip olmamıştır. *E. globules, R. coriaria* ve *R. officinalis* ekstraktlarının patojene karşı etkileri sera koşulları araştırılmış, en yüksek etkinliğe *E. globulus* (65.86%) ekstraktı ile ulaşılmıştır. Sumak, okaliptüs ve biberiye ekstraktlarının, *P. s.* pv. *tomato* inokulasyonundan önce uygulamalarıyla, peroksidaz enzimi ve toplam

protein miktarlarında önemli düzeyde artış görülmüştür. Bu çalışma, domates bakteriyel benek hastalığı mücadelesi için koruma programlarında bazı bitki ekstraktlarının kullanılabileceğini göstermektedir.

Anahtar kelimeler: *Pseudomonas syringae* pv. *tomato*, domates, bitki ekstraktları, biyolojik kontrol, peroksidaz, protein

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill.) is one of the most important greenhouse and field-grown vegetables in Turkey, and in terms of 328.000 ha of area and 11.003.433 tons of production, in the 4th place of the world tomato production after China, USA and India (FAO, 2011). Since environmental factors and variable colonization strategies play an important role in phytobacteria spread on tomato crops, without effective preventive measures it is difficult to reduce their damage (Beattie and Lindow, 1999; Pietrarelli et al., 2006).

Pseudomonas syringae pv. *tomato*, causal agent of tomato speck, is one of the most spread, in greenhouse and in open field tomato crops in the world. Bacterial speck is a significant source of economic loss in the tomato industry. Lesions may make fruit unfit for fresh market. On tomatoes for processing, lesions may be deep enough to cause considerable of grading or loss in quality (Goode and Sasser, 1980). *P. s.* pv. *tomato* can survive in the seeds, crop residues and multiple alternative hosts (McCarter et al., 1983).

The first determination of this disease in Turkey was made by Saygili (1975) in the Aegean region and Cinar (1977) in the east Mediterranean region. In the spring of 1999 and 2000, approximately 20% yield loss occurred by *P. s.* pv. tomato in commercial fields in the eastern Anatolia region (Sahin, 2001). In commercial seedling companies of Antalya province, the disease determined 20 and 25% seedling losses in 2002 and 2003, respectively. In addition, in 2003, the disease incidence was approximately 5% in 142 commercial greenhouses (Basim et al., 2004).

Its control is mainly related to appropriate cultural methods, such as seeds certification, irrigation and fertilization, and preventive copper treatments (Balestra et al., 1998). Copper compounds represent a problem due to their phytotoxicity, their accumulation in soil and the necessity of frequent applications (Balestra et al., 1999). Antibiotics are questionable for various reasons and therefore forbidden in many countries, and are not effective against several pathogens (Jones and Jones, 1989; Loper et al., 1991; Pohronezny et al., 1994; Spotts and Cervantes, 1995; Schnabel and Jones, 1999; Mc Manus et al., 2002). *P. s.* pv. *tomato* control strategies, characterized by a low environmental impact and able to substitute copper compounds result particularly requested because copper use restriction and the importance of tomato crop also in organic agriculture.

Many plants and plant products have been reported to possess antimicrobial properties (Grange and Ahmed, 1988). The demand for medicinal plants by the modern pharmaceuticals industries has increased considerably. Plant extracts and essential oils are shown to have antimicrobial effects on bacterial pathogens and the presence of antibacterial compounds in higher plants has long been recognized as an important factor in disease control. They have also shown their potential in agriculture, and their plant toxicity has yet to be occurred (Balestra et al., 1998; De Castro, 2001; Varvaro et al., 2001, 2002; Lo Cantore et al., 2004; Iacobellis et al., 2005).

Peroxidase enzyme is present in many higher plants and it is part of the multi-component defense system and has been implicated in defense reactions of plants against pathogens. Peroxidase is widely distributed in plant (Lagrimini and Rothstein, 1987) and induction of new cell wall biosynthesis (Gasper et.al, 1982). Levels of peroxidase expression and its isozyme patterns have been shown in several plant systems to be altered by stress chemicals and infection (Gasper et al., 1982; Dixon et al., 1994).

The objective of this study was to evaluate the *in vitro* and *in vivo* antibacterial effect of some medicinal and aromatic plant extracts on *P. s.* pv. *tomato* and to determine accumulation of peroxidase enzyme and total protein amounts as host defence reactions.

MATERIALS AND METHODS

Plant Material

The leaves of walnut (*Juglans regia*), lavender (*Lavandula officinalis*), eucalyptus (*Eucalyptus globulus*), rosemary (*Rosmarinus officinalis*), olive (*Olea europaea*) and lime (*Tilia tomentosa*); seeds of jatropha (*Jatropha curcas*), hop (*Humulus lupulus*), nigella (*Nigella sativa*), fenugreek (*Trigonella foenum-graecum*), cumin (*Cuminum cyminum*); fruits of sumac (*Rhus coriaria*), black carrot (*Daucus carota*) rosehip (*Rosa canina*) and stems of moringa (*Moringa oleifera*) were collected between May and August, 2013 from Konya province (except jatropha and moringa, these plants were obtained from Sudan, Dr. M. I. Akdag, in the same dates). Plant species were identified by Prof. Dr. Y. Kan and Assoc. Prof. Dr. A.T. Polat from departments of Field Crops and Landscape Architecture, Selcuk University.

Vegetal materials for 500 g for each plant were washed under sterile distilled water and blotted with paper towels. Samples were then sliced into small pieces and blended using a twister blender (Hamilton Beach, 16-speed Turbo-Twister blender) for 10 min at room temperature. In each case, powdered air-dried plant material was extracted with methanol. The crude methanol extract was prepared by maceration of plant material (100 g) with methanol (300 ml) (Alanis et al., 2005; Ojo, 2007). The extract was filtered and evaporated to dryness in vacuum. The methanol extracts were dissolved in dimethyl sulfoxide (DMSO, Merck).

Pseudomonas syringae pv. *tomato* strains

P. s. pv. *tomato* str. PstKKB12 was isolated from diseased tomato plants in Konya, Central Anatolia in 2012. *P. s.* pv. *tomato* str. AY1 obtained from Prof. Dr. Yesim Aysan (Department of Plant Protection, Cukurova University/Adana / Turkey). The strains, PstKKB12 and AY1, were used because they represent Central Anatolia and Medirrenean Regions. They were stored at -30 ^{0}C for long-term storage in the phytobacterial collection of the Department of Plant Protection, Selcuk University, Konya, Turkey. The subcultures of the pathogen were obtained by growing bacteria for 48–72 h at 25± 2 ^{0}C on NSA (nutrient broth 8 g l^{-1}, sucrose 50 g l^{-1} and agar 18 g l^{-1}). The bacterial isolates were stored in Luria-Berthani (LB) medium plus 30% glycerol at -70°C.

In vitro Antibacterial Assays

The antibacterial *in vitro* assays were carried out by paper disc tests according to Mangamma and Speeramulu (1981). The suspensions of the bacteria (100 µl) were spread on Nutrient Agar (NA) in Petri dishes with 9 cm diameter. Sterilised paper discs (Whatmann no: 3 and 5 mm in diameter) were put on the medium and 10 µl of each extracts was dropped on the discs. Absolute methanol was used as negative control. After the discs were placed on the surface of the agar medium, the compound was allowed to diffuse for 5 min, and the plates incubated at 25°C. After 48 h, the inhibition zones around the discs were measured using Vernier calibers. Each experiment was repeated three times with three replicates each.

In vivo Antibacterial Assays

One thousand seeds of tomato (*Solanum lycopersicum* L.) cultivar, H-2274 F1 which were free for bacterial infection, were surface-disinfected in 1% sodium hypochlorite for 3 min and rinsed three times in sterile distilled water. The seeds were then transferred to Petri dishes containing sterile filter papers and allowed to air-dry overnight in a laminar flow chamber and stored at 4°C until used. Surface-disinfected seeds of tomato were inoculated with *P. s.* pv. *tomato*. Inoculum was prepared from 48-h-old bacterial cultures grown on nutrient agar (NA) medium at 28°C. Bacterial cultures were flooded with 10 ml of sterile distilled water and scraped with flamed Drigalski spatula. The inoculum suspension was homogenized and suspended in sterile distilled water to adjust 10^8 CFU ml^{-1} (OD600=0.01) (NanoDrop, Thermo Fisher Scientific, USA). The seeds were vacuum-infiltrated for 30 min with 10 ml of the bacterial suspension (Bashan and Assouline, 1983), and seeds were air-dried in laminar air flow chamber and stored at 4°C until used.

To determine antibacterial activity of plant extracts on bacterial population on tomato seeds, twenty five infected tomato seeds with *P. s.* pv. *tomato* were treated with 1 ml of 20, 10 and 5% the extracts in methanol. Eppendorf tube and placed on agitation table at 200 *rpm*, for one hour at 25°C. Streptomycin (100 µg/ml) and sterile distilled water (SDW) were included as controls. After overnight incubation, treated seeds were allowed to air-dry for 1 h in laminar air flow cabinet. Washing samples (100 µl) from treated seeds were collected using sterile pipettes and serially diluted to 10^{-2} with sterile distilled water in Eppendorf tubes. An aliquout of 100 ml from each dilution was spreaded onto NA medium using Drigalski spatula. The plates were incubated at 28°C. A pure culture of the bacteria was included as controls. The identity of suspected colonies from each plate was confirmed by Polymerase Chain Reaction (PCR) using spesific PST1/PST2 primers according to Bereswill et al (1994).

The effect of the extracts on seed germination was also evaluated in bacteria free tomato seeds. Bacteria free seeds were treated with plant extracts as explained above. Seed germination tests were conducted using 500 tomato seeds per treatment. The standard International Seed Testing Association top of paper method ISTA (ISTA, 2005) was used. The seeds were plated uniformly (50 seeds per replicate) onto three layers of moist blotter paper in a plastic container kept at 26±2°C and RH>85% for 14 days. Normal and dead seeds were counted for germination tests.

In greenhouse experiments, two different study were conducted to determine the effects of plant extracts on disease severity.

First, seeds inoculated with the bacteria and treated by the extracts were planted in trays (10x25 cm) containing the same amount of soil, sand and manure, as six replicates consisting of 100 seeds per tray. The seedlings were grown under greenhouse conditions (25±2 °C, 8/16 photoperiod and relative humidity between 70 and 80%).

Secondly, six-week-old tomato plants were grown in a greenhouse in 15-cm-diameter pots containing a sterilized mix of soil–sand–peat (2:1:1 by volume) and watered daily by drip-irrigation. A mineral solution (NPK 20–20–20) at 2 g l^{-1} was distributed weekly into the pots to maintain optimum nutritional conditions. Heating and drip-irrigation data were recorded by logger at 60-min intervals. In the greenhouse, 2 h before and 2 h after bacterial inoculation, the relative humidity was maintained at 90% to favour stomata opening. The plants were inoculated by spraying of *P. s.* pv. *tomato* PstKKB12 and YA-1 strains (10^8 CFU ml^{-1}) with an airbrush until leaf surfaces were uniformly wet. Selected three plant extract (*Rosmarinus officinalis*, *Rhus coriaria*, *Eucalyptus globulus*) were used at a concentration of 200 g l^{-1}. For each combination (bacterial strain/the extract) 60 tomato plants were used, subjected to the following treatments: 60 inoculated plants treated with the extract; 60 inoculated, non-treated plants as negative control; 15 inoculated plants treated with copper oxychloride (357,5 g l^{-1} ai; SC formulation, commercial name: Calleon, Hektas) as positive control. Each plant extract was repeated three times, once before bacterial inoculation to activate the plant defence system and twice after the inoculations with 5 days intervals, and experiments were observed over the 15 days after treatments. Negative control plants were sprayed with sterile distilled water. The experiments were set in greenhouse with randomized design blocs. Disease severity was also evaluated by modified 0-4 scale (Chambers and Merriman scale, 1975) as follow: 0: no symptom, 1: 1-5 spots; 2: 6-12 spots; 3: 13-20 spots; 4: 21-30 spots on the leaves. The percentage of the efficiency was calculated by ABBOTT formula.

Determination of Peroxidase Enzyme and Total Protein Amounts

Leaf samples were taken at 36 hours after applications of the extracts for peroxidase enzyme and protein analysis. For enzyme analyses, the leaves were harvested, freeze-dried in liquid nitrogen, lyophilized. Crude extracts (0.2 g) were homogenized with 2 mL sodium phosphate buffer (0.05 M, pH 6.5) and centrifuged. The supernatants were collected and their protein concentrations were determined by the Bradford method (Bradford, 1976) using bovine serum albumin (BSA) as a standard. The peroxidase enzyme activity was assayed spectrophotometrically (Kanner and Kinsella, 1983).

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) by using SPSS statistic program (Version 11.5) and the significance of the treatments was determined by means of Duncan's Multiple Range Test ($P < 0.05$).

RESULTS

Antibacterial efficacy of different medicinal and aromatic plant extracts on *P. s.* pv. *tomato* strains was evaluated using the disc diffusion method to measure the surrounding inhibition zones at 5 doses (Table 1). The mean values of the inhibition zones caused by extract to the bacterial agent ranged from 0 to 22 mm. The inhibition zone increased in a dose-dependent manner for all extracts. The maximum inhibition zone for the pathogen was obtained for the 20% concentration of *Rhus coriaria* extract (22 mm) comparison with streptomycin (34 mm). The lowest inhibition zones of 5, 6 and 8 mm were obtained from *Lavandula officinalis, Nigella sativa* and *Daucus carota* at the same concentration dose of extracts, respectively.

Table 1. Antimicrobial activities of selected plants extracts against *Pseudomonas syringae* pv. *tomato*

	Extract Concentration (%)*				
Plant extracts	**20**	**10**	**5**	**2,5**	**1**
Cuminum cyminum	[a]11.0±1.76 f**	5.0±1.18 g	1.0±1.19 h	-	[b]-
Daucus carota	8.0±1.63 k	3.0±1.35 k	1.0±1.10 h	-	-
Eucalyptus globulus	**19.0±1.90 c**	11.0±2,17 c	7.0±1.17 b	1.0±0.72 b	-
Humulus lupulus	10.0±2.69 g	4.0±1.45 h	0.5±2.10 lm	-	-
Jatropha curcas	10.0±1.24 g	6.0±1.19 f	3.0±0.78 f	-	-
Juglans regia	11.0±1.03 f	6.0±1.91 f	3.0±1.14 f	-	-
Lavandula officinalis	5.0±1.12 m	1.0±0.18 m	-	-	-
Moringa oleifera	10.0±1.62 g	6.0±0.14 f	3.0±0.97 f	1.0±1.89 b	-
Nigella sativa	6.0±1.91 l	1.0±0.84 m	0.3±0.79 m	-	-
Olea europaea	9.0±2.87 h	2.0±0.13 l	0.6.0±0.90 klm	-	-
Rhus coriaria	**22.0±2.69 b**	14.0±1.97 b	6.0±0.61 c	1.0±1.75 b	-
Rosa canina	12.0±1.59 e	7.0±0.96 e	4.0±0.89 e	1.0±0.91 b	-
Rosmarinus officinalis	**13.9±1.84 d**	8.0±0.16 d	5.0±0.79 d	1.0±0.15 b	-
Tilia tomentosa	9.0±1.73 h	5.0±1.78 g	1.0±1.06 h	-	-
Trigonella foenum-graecum	10.0±1.06 g	4.0±0.13 h	1.5±0.92 gh	-	-
Streptomycin (control)	34.0±3.9 a	25.0±2.00 a	19.0±4.24 a	13.0±3.10 a	7.0±3.64

[a] Values expressed are mean standard deviation of each three replicates for two *P. s.* pv. *tomato* strains (PstKKB12 and AY1),
[b] (-): no inhibition zone
* Zones of growth inhibition (mm) showing antibacterial activity of plant extracts as determined by paper disc diffusion techniques
** In a column, values followed by the same letter are not significantly different ($p < 0.01$) as determined by Duncan's New Multiple Range Test.

Bacterial population on tomato seeds after applications of the plant extracts at 5 doses was determined comparison with streptomycin and SDW as positive and negative controls. The concentration, 20%, of *Rhus coriaria* and *Eucalyptus globulus* completely inhibited the *P. s.* pv. *tomato* on tomato seeds. The lowest antibacterial effects were obtained by *Daucus carota* and *Nigella sativa* extracts as bacterial populations $1.4x10^6$ and $0.9x10^6$ CFU ml^{-1}, respectively.

In accordance with Koch postulates, the identity of suspected colonies from each plate was confirmed by PCR using specific PST1/PST2 primers in *in vivo* and *in vitro* experiments.

Although the highest antibacterial effects of sumac (*R. coriaria*), eucalyptus (*E. globulus*) and rosemary (*R. officinalis*) were caused against the pathogen, these extracts did not negative effect on tomato seed germination (98%) compared to control treatment (98,5%) at the highest dose used (20%). The results showed that the

percentage of germinated seeds treated with the all plant extracts, except the extracts of *Juglans regia* (92%) and *Cuminum cyminum* (94%), was not significantly higher than the untreated.

According to the results of disc diffusion test, the most successful three extracts (*Rhus coriaria, Eucalyptus globulus* and *Rosmarinus officinalis*) were selected for greenhouse experiments. The selected plant extracts and bacterial strains were applied three times (to activate the plant defense system and their bactericidal effects) and experiments were observed over the 15 days after treatments. *E. globules, R. coriaria* and *R. officinalis* treatments resulted in a significant reduction in disease severities compared to controls (91.4%) for *P. s.* pv. *tomato* strains at the ratios of 31.2%, 36.8% and 58.7%, respectively (Table 2). The most effiency was achieved by *E. globulus* (65.86%) on tomato cv. H-2274 in the greenhouse conditions. No negative (phytotoxic) effects were recorded on any of the tomato plants. There was no symptom on negative control plants as in the other experiments.

Table 2. The efficacy of plant extracts on disease severity caused by *P. s.* pv. *tomato*

Applications	**Avarage of Scale**	**Disease severity %**	**Efficacy %**
Control (negative control)	3.67	91.4 ± 2.40	-
Rosmarinus officinalis	1.94	58.7 ± 3.20	35.77c*
Rhus coriaria	2.80	36.8 ± 1.16	59.73b
Eucalyptus globulus	1,35	31.2 ± 3.52	65.86b
Streptomycin (positive control)	3.82	0.3±2.11	99.63a

*The values shown are the mean averages of ten replications for each *P. s.* pv. *tomato* strain (PstKKB12). The average values in a row followed by different letters are significantly different at $p < 0.05$ according to the Duncan's Multiple Range Test.

The peroxidase enzyme and total protein have shown an increase on tomato plants cv. H-2274 by application of plant extracts. While peroxidase activity on control plants was 156 mg/mL/min, the highest peroxidase activity were determined as 313 mg/mL/min on treated plants with *Moringa oleifera* compared to controls. Total protein and peroxidase enzyme results were shown in Table 3.

Table 3. Total protein and peroxidase enzyme activity in tomato plant (cv. H-2274) leaves treated with different plant extracts

Application	**Toplam protein (mg/mL)**	**Peroxidase Enzimi mg/mL/min**
Control	0.59 ± 0.01b*	156 ± 2.84c
Rhus coriaria	0.79 ± 0.02a	220 ± 3.15b
Rosmarinus officinalis	0.69 ± 0.03a	304 ± 2.69a
Eucalyptus globulus	0.72 ± 0.03a	313 ± 1.69a

*The values shown are the mean averages for three replications. The average values in a row followed by different letters are significantly different at $P < 0.05$ according to the Duncan's Multiple Range Test.

DISCUSSION

The control of bacterial diseases in plants is mainly achieved by the use of antibiotics and copper compounds which can result in toxicity to animals and humans as well as accumulation in living systems (Ansari and Malik, 2008). In addition, plant pathogens commonly acquire resistance to chemical pesticides as a result of their continual uses (Jones et al., 1991; McManus et al., 2002).

The possibility of controlling tomato bacterial diseases with plant extracts appears of particular interest considering the lack of valid alternatives to copper compounds and the non-availability of commercial *P. s.* pv. *tomato* resistant cultivars, especially in organic agriculture. Plant-derived compounds have the advantage of limited negative impact on human health and on the environment. A very diverse range of plant natural products have been used, either directly, or as lead molecules, for the development of new agrochemicals. These products can inspire modern agrochemical research enormously (Cho et al., 2007a,b).

Contaminated seeds by the pathogen have a significant role in disease outbreaks. Since it is difficult to control of bacterial speck disease in the field, further study are necessary for development of effective management strategies. Use of pathogen free seeds or seed treatments is recommended for effective control of the disease. One of the alternative control methods is use of effective plant extracts in conventional and organic agriculture. These approaches are a popular trend towards environmental friendly methods in agriculture.

Extracts of some plant have been shown to be effective against *P. s.* pv. *tomato*, and some important bacterial pathogens, thus suggesting their potential use in agriculture as alternatives to or in combination with a reduced amount of copper compounds (Mirik and Aysan, 2005; Balestra et al., 2009; Basım and Basım, 2013).

Three plant extracts tested in this study proved useful for effective biocontrol of *P. s.* pv. *tomato* on tomato plants. The *Rhus coriaria* extract successfully reduced the disease severity caused by virulent strains of *P. s.* pv. *tomato* (PstKKB12 and AY1). No negative (phytotoxic) effects were recorded on the tomato plants tested.

The *in vivo* antibacterial activity of this natural substance continued to be effective for at least 15 days, offering the possibility of replacing, reducing or even alternating treatments involving copper compounds. The identification and characterization of the *Rhus coriaria* active compounds and a better understanding of their active mechanisms might contribute to a better utilization of them either alone or in combination with other natural extracts. *Rhus coriaria* (Anacardiaceae) commonly known as *sumac* is a wild bush that grows in all Mediterranean areas. Phytochemicals in *Rhus coriaria* are being used as antibacterial, antidiarrhoea, antidysenteric, antihepatoxic, antiseptic, antispasmodic and antiviral due to their contents of ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid (Abu-Shanab et al., 2005; Mahmood et al., 2010).

Eucalyptus is native to Australia, and the genus Eucalyptus contains about 600 species. Of all the species, *Eucalyptus globulus* is the most widely cultivated in subtropical and Mediterranean regions (Takahashi et al., 2004). It has been reported that trichlosan interacted with an enzyme in the fatty acid biosynthetic pathway and exhibited inhibitory activities against both Gram-positive and Gram-negative bacteria (Heath et al., 1998 and 2000). The main difference between Gram-positive bacteria and Gram-negative bacteria is the structure of their cell walls. Unlike Gram-positive bacteria, Gram-negative bacteria have a high content of lipopolysaccharide layer in the cell wall. Therefore, it is postulated that the three flavonoids isolated from *Eucalyptus* spp. are unable to pass through the lipopolysaccharide layer of Gram-negative bacteria (Takahashi et al., 2004).

The chemical composition of rosemary has components were mostly monoterpenes, the major ones being pinene, 1,8-cineole and camphor (camphene, limonene, borneol, verbenone, bornyl acetate, etc.). These contents supply a moderate antibacterial activity to *R. officinalis*. The Gram-positive strains are more sensitive than the Gram-negative bacteria for rosemary treatments (Pintore et al., 2002). In our studies, we obtained moderate antibacterial efficiency from rosemary treatments as a similar result. In further researches, it should be investigated differences of Gram negative and positive both rosemary and eucalptus treatments and, interactions between host and peroxidase and total protein in the extract treatments.

To our knowledge, this study is the first research on controlling of tomato bacterial speck disease by using sumac, rosemary and eucalyptus extracts.

Many organic solvents such as benzene, chloroform, ethyl acetate or methanole can be used for extraction of medicinal and aromatic plants. Comparing with water experiments, methanol extractions of these plants are highly successful (pre-experiments in this study) for this reason we used these method. However, advantages of water extraction are their easily preparation, eco-friendly method and low cost (Mirik and Aysan, 2005).

In conclusion, the study suggests that medicinal plant, especially sumac, eucalyptus and rosemary, extracts could be used as seed treatment to reduce disease incidence and severity. Further investigations are being carried out to understand more thoroughly their role in antibacterial efficacy against different bacterial plant pathogens, as well as their large scale use in disease management. The activity of these plant extract might provide new opportunities to improve control of different bacterial tomato diseases, including bacterial speck, spot, canker and pith necrosis. Preservation of environmental quality and slowing the rate of development of pesticide-resistant strains are some of the benefits that the use of plant extracts can have on IPM and on sustainable agriculture.

LITERATURE CITED

Abu-Shanab, B., Adwan, G., Abu-Safiya, D., Adwan, K., Abu-Shanab, M., 2005. Antibacterial activity of *Rhus coriaria*. L extracts growing in palestine. J. Is. University of Gaza, Natural Sciences Series, 13(2): 147-153.

Alanis, A.D., Glazada, F., Cervantes, J.A., Tarres, J., Ceballas, G.M., 2005. Antibacterial properties of some plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J. Ethnopharmacol., 100 (1-2): 153-157.

Ansari, M.I. and Malik, A., 2008. Genotoxicity of wastewaters used for irrigation of food crops. Environ. Toxicol. 24, 103-115.

Balestra, G. M., Antonelli, M., Varvaro, L., 1998. Effectiveness of natural products for in vitro and in vivo control of epiphytic populations of *Pseudomonas syringae* pv. *tomato* on tomato plants. J. Plant Pathol. 80, 251.

Balestra, G.M., Antonelli, M., Fabi, A., Varvaro, L., 1999. Effect of organic cropping system on the presence of Pseudomonas syringae pv. tomato in the phyllosphere and in the rhizosphere of tomato plants. Abstract on Journal of Plant Pathology 81, 227.

Balestra, G.M., Heydari, A., Ceccarelli, D., Ovidi, E., Quattrucci, A., 2009. Antibacterial effect of *Allium sativum* and *Ficus carica* extracts on tomato bacterial pathogens. Crop Prot. 28, 807-811.

Bashan, Y. and Assouline, I., 1983. Complementary bacterial enrichment techniques for the detection of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in infested tomato and pepper seeds. *Phytoparasitica* 11: 187-93.

Basım, H., Basım, E., Yılmaz, S., Dickstein, E.R., Jones, J.B., 2004. An outbreak of bacterial speck caused by *Pseudomonas syringae* pv. *tomato* on tomato transplants grown in commercial seedling companies located in the Western Mediterranean region of Turkey. Plant Disease, 88, 1050-1050.

Basim, E. and Basim, H., 2013. Antibacterial activity of Turkish endemic sığla (*Liquidambar orientalis* Mill. var. *orientalis*) storax against agricultural plant pathogenic bacteria and its use as a seed protectant. Journal of Food Agriculture and Environment 11: 2447-2450.

Beattie, G.A. and Lindow, S.E., 1999. Bacterial colonization of leaves: a spectrum of strategies. Phytopathology 89, 353-359.

Bereswill, S., Bugert, P., Völksch, B., Ullrich, M., Bender, C.L. and Geider, K., 1994. Identification and relatedness of coronatine-producing Pseudomonas syringae pathovars by PCR analysis and sequence determination of the amplification products. Appl Environ Microbiol 60: 2924-2930.

Bradford, M. M., 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Analytical Biochemistry, 72: 248-254.

Chambers, S.C. and Merriman, P.R., 1975. Perennation and control of *Pseudomonas syringae* pv. *tomato*, Victoria. Austuralian Journal of Agricultural Research, 26, 657-663.

Cho, J.Y., Choi, G.J., Son, S.W., Jang, K.S., Lim, H.K., Lee, S.O., Sung, N.D., Cho, K.Y., Kim, J.C., 2007a. Isolation and antifungal activity of lignans from Myristica fragrans against various plant pathogenic fungi. Pest Manag. Sci. 63, 935-940.

Cho, K.M., Hong, S.Y., Lee, S.M., Kim, Y.H., Kahng, G.G., Lim, Y.P., 2007b. Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. Microb. Ecol. 54, 341-351.

Cinar, O., 1977. A bacterial disease on tomatoes in Mediterranean Region. Bitki. 4: 282-288. Saygili, H., 1975. Investigation on new bacterial disease of tomatoes in Ege. J. Turk. Phytopathol. 4, 723-727.

De Castro, S.L., 2001. Propolis: biological and pharmacological activities. Therapeutic uses of this bee-product. Annu. Rev. Biol. Sci. 3, 49-83.

Dixon, R.A., Harrison, M.J. and Lamb. C.J. 1994. Early events in the activation of plant defense responses. *Annu.Rev. Phytopathol*. 32. 479-501.

FAO, 2011. Agricultural Production Data. http://faostat3.fao.org/faostat, Date accessed: 4/6/ 2014.

Gasper, T., Penel. C. and Greppin, H., 1982. Peroxidases: A survey of their biochemical and physiological roles in higher plants, *University of Geneva Press*, Geneva.

Goode, M.J. and Sasser, M., 1980. Prevention - The key to controlling bacterial spot and speck of tomato. Plant Dis. 64: 831-34.

Grange, M. and Ahmed, S., 1988. Handbook of Plants with Pest Control Properties. John Wiley & Sons, New York.

Heath, R.J., Roland, G.E. and Rock, C.O., 2000. Inhibition of the *Staphylococcus aureus* NADPH-dependent enoyl-acyl carrier protein reductase by trichlosan and hexachlorophene. Journal of Biological Chemistry 275, 4654-4659.

Heath, R.J., Yu, Y.T., Shapiro, M.A., Olson, E. and Rock, C.O., 1998. Broad-spectrum antimicrobial biocides target the FabI component of fatty acid synthesis. Journal of Biological Chemistry 273, 30316-30320.

Iacobellis, N.S., Lo Cantore, P., Capasso, F., Senatore, F., 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J. Agric. Food Chem. 53, 57-61.

ISTA, 2005. Method Validation Reports 2, 1-17.

Jones J.P. and Jones J.B., 1989. Field control of target spot and bacterial speck of tomato. Proc. Fla. State Hortic. Soc. 101:358-361.

Jones, J.B., Woltz, S.S., Jones, J.P. and Portier, K.L., 1991. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. Phytopathology 81, 714-719.

Kanner, J and Kinsella, J.E. 1975, 1983. Lipid deterioration initiated by phagocytic cells in muscle foods: β-carotene destruction by a myeloperoxidase-hydrogen peroxide-halide system. J.Agric.Food Chem., 31:370-376. 12 nd. edn. Churchill Livingstone, Edinburgh, London, New York..

Lagrimini, L. M., Burkhart, W., Moyer, M., Rothstein, S., 1987, Molecular cloning of complementary DNA encoding the lignin-forming peroxidase from tobacco: molecular analysis and tissue-specific expression, Proc. Natl. Acad. Sci. USA, 84, 7542-7546.

Lo Cantore, P., Iacobellis, N.S., De Marco, A., Capasso, F., Senatore, F., 2004. Antibacterial activity of Coriandrum sativum L. and *Foeniculum vulgare* Miller var. vulgare (Miller) essential oils. J. Agric. Food Chem. 52, 7862-7866.

Loper, J. E., Henkels, M. D., Roberts, R. G., Grove, G. G., Willett, M. J., Smith, T. J., 1991. Evaluation of streptomycin, oxytetracycline, and copper resistance in *Erwinia amylovora* isolated from pear orchards in Washington State. Plant Dis. 75: 287-290.

Mahmood, M.A., Al-Dhaher, Z.A., AL-Mizraqchi, A.S., 2010. Antimicrobial activity of aqueous extracts of pomegranate, sumac, sage, anise, hand bull tongue, thyme, cloves, lemon and mint against some food-borne pathogens. Iraqi J. Vet. Med., 34 (2): 85-94.

Mangamma, P., and Speeramulu, A., 1991 Garlic extract inhibitory to growth of *Xanthomonas campestris* pv. *vesicatoria*. Indian Phytopasthology, 44: 372-372

McCarter, S.M., Jones, J.B., Gitatitis, R.D., and Smitley, D.R., 1983. Survival of *P. syringae pv. tomato* in Association with tomato seed, soil, host tissue, and epiphytic weed hosts in Georgio. Phytopathology 73 (10): 1393-1398.

McManus, P.S., Stockwell, V.O., Sundin, G.W. and Jones, A.L., 2002. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol. 40, 443-465.

Mirik, M. and Aysan, Y., 2005. Effect of some plant extracts as a seed treatments on bacterial spot disease of tomato and pepper. The Journal of Phytopathology 34 (1-3): 9-16.

Ojo, O.O., Ajayı, A.O., Anibijuwon, I.I., 2007. Antibacterial potency of methanol extracts of lower plants. J. Zhejiang Univ. Sci. B. 8 (3): 189-191.

Pietrarelli, L., Balestra, G.M., Varvaro, L., 2006. Effects of simulated rain on *P. syringae pv. tomato* populations on tomato plants. J. Plant Pathol. 88, 245-251.

Pintore, G., Usai, M.L., Bradesi, P., Juliano, C., Boatto, G., Tomi, F., Chessa, M., Cerri, R. and Casanova, J., 2002. Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. Flavour Fragr. J., 17: 15-19.

Pohronezny, K., Sommerfeld, M. L., Raid, R. N., 1994. Streptomycin resistance and copper tolerance among strains of *Pseudomonas cichorii* in celery seedbeds. Plant Dis. 78: 150-153.

Sahin, F., 2001. Severe outbreak of bacterial speck, caused by *Pseudomonas syringae* pv. *tomato*, on field-grown tomatoes in the eastern Anatolia region of Turkey Plant Pathology 50, 799.

Schnabel, E. L. and A. L. Jones, 1999. Distribution of tetracycline resistance genes and transposons among phylloplane bacteria in Michigan apple orchards. App. Environ. Microbiol. 65: 4898-4907.

Spotts, R. A., Cervantes, L. A., 1995. Copper, oxytetracycline and streptomycin resistance of *Pseudomonas syringae* pv. *syringae* strains form pear orchards in Oregon and Washington. Plant Dis. 79: 1132-1135.

Takahashi, T., Kokubo, R. and Sakaino, M., 2004. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculate*. Letters in Applied Microbiology, 39, 60-64.

Varvaro, L., Antonelli, M., Balestra, G.M., Fabi, A., Scermino, D., 2001. Control of phytopathogenic bacteria in organic agriculture: cases of study. J. Plant Pathol. 83, 244.

Varvaro, L., Antonelli, M., Balestra, G.M., Fabi, A., Scermino, D., Vuono, G., 2002. Investigations on the bactericidal activity of some natural products. In: Proc. Int. Cong. Biol. Products: Which Guarantees for the Consumers?, October 2002, Milan, Italy.