

Effect of Some Plant Extracts as Seed Treatments on Bacterial Spot Disease of Tomato and Pepper

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ABSTRACT

Bacterial spot disease caused by *Xanthomonas axonopodis* pv. *vesicatoria* is an important seed-borne disease of pepper and tomato. Antibacterial effects of aqueous extracts of radish (*Raphanus raphanistrum*), garlic (*Allium sativum*), mint (*Mentha piperita*), onion (*Allium cepa*), marata (*Clematis marata*), oleander (*Nerium oleander*), rosemary (*Rosmarinus officinalis*), eucalyptus (*Eucalyptus* sp.), pepper tree (*Schinus* sp.), terebinth tree (*Pistacia terebinthus*) and pine tree (*Pinus sylvestris*) were investigated *in-vitro* and *in-vivo* experiments for the bacterial disease agent *X. a.* pv. *vesicatoria*. Two different extract sterilization methods, autoclaving and filtering, were used *in-vitro* experiments. No growth inhibitions were recorded with the aqueous extracts of radish, mint, onion, maratha, oleander, rosemary, peppertree, terebinth tree and pine tree. Garlic and eucalyptus extracts, however, showed antibacterial activity *in vitro* experiments. Although filtered-sterilized garlic extract was effective on the growth of pathogen, autoclaved-sterilized garlic extract did not show antibacterial effect. This result clearly indicates that inhibitory compound(s) of garlic was thermolabile. Aqueous extracts of eucalyptus and garlic were very effective to reduce the seed-borne inoculum source of the disease *in-vivo* conditions. Eucalyptus extract was found to the best seed treatment. Symptom development on pepper and tomato seedlings was completely inhibited by eucalyptus extract as a seed treatment. Disease incidence was reduced at ratios of 95 and 86% by filtered-sterilized garlic extract on tomato and pepper seedlings, respectively. Similarly, disease severity on tomato and pepper seedlings was reduced between 77 and 96% at ratios by garlic and eucalyptus extracts, respectively. This study clearly demonstrated that aqueous garlic and eucalyptus extracts are effective seed treatments against seed-borne infections of bacterial spot disease of tomato and pepper for traditional and organic seedling production.

Keywords: *Xanthomonas axonopodis* pv. *vesicatoria*, extract, antibacterial activity, seed treatment, garlic and eucalyptus

INTRODUCTION

Bacterial spot, caused by *X. a.* pv. *vesicatoria*, is a destructive seed-borne disease of pepper and tomato (Buonaurio et al., 1994). The disease is a major problem for

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processing pepper production in the eastern Mediterranean region of Turkey (Aysan and Sahin, 2003) with 52-100 % prevalence in the pepper fields of Adana and Osmaniye provinces (Mirik et al., 2005). Copper containing compounds have not been effective in pepper fields in the region because of resistance to copper pesticides (Mirik et al., 2005). A local cultivar, Karaisali-Salcalik is generally grown in the pepper fields. The pathogen can survive in/on tomato and pepper seeds for 10 and 20 years, respectively (Bashan et al, 1982). Seed treatments are the best control strategies for seed-borne infections of the bacterial disease. However, the high cost of pesticides, development of pesticides/antibiotic resistant pathogenic isolates, governmental restriction on the use of antibiotics against plant pathogen in European countries, including Turkey, and the interest of environmental consideration raise the need to find alternative control methods (Soylu et al., 2003).

The objective of the study was to determine the effectiveness of some plant extracts in the decrease of seed borne inoculum. There are several studies on effects of plant extracts and essential oils for soil-borne or foliar plant pathogens (Basim and Basim, 2003; Boyraz and Ozcan, 2005; Soylu et al., 2005; Talas-Ograş et al., 2005). But there are few reports on effects of plant extracts against bacterial spot disease, *X. a. pv. vesicatoria*, as seed treatment *in vivo* experiments.

MATERIAL and METHODS

Plant Materials

The bulbs of radish (*Raphanus raphanistrum*) and garlic (*Allium sativum*), fresh leaves of mint (*Mentha piperita*), onion (*Allium cepa*), marata (*Clematis marata*), oleander (*Nerium oleander*), rosemary (*Rosmarinus officinalis*), eucalyptus (*Eucalyptus* sp.), peppertree (*Schinus* sp.), terebinth tree (*Pistacia terebinthus*) and pine tree (*Pinus sylvestris*) were collected in May 2004 from Adana, Turkey. Plant species were identified in the Field Crops and Landscape Architecture Departments of Cukurova University.

Used Microorganism

X. a. pv. vesicatoria causes bacterial spot disease on tomato and pepper. *X. a. pv. vesicatoria* strain XV-K1 strain was isolated from diseased pepper plants in Karaisali province of Adana (Aysan and Sahin, 2003). For *in-vitro* studies, bacterial suspension of the pathogen was prepared in saline buffer (0.85% NaCl). Absorbance of XV-K1 strain was measured spectrophotometrically at 600 nm and approximately diluted to 0.3 OD (10^8 cfu/ml).

Preparation of Plant Extracts

Aqueous extracts of plants were prepared by grinding 50 g of fresh leaves/bulbs with 50 ml of sterile distilled water and filtered through two layers of muslin cloths. The filtrates were centrifuged at 10.000 g for 10 min and the supernatant was used for *in-vitro* and *in-vivo* studies. Half of extracts was then autoclaved at 121°C for 15 min and

the rest of the extracts was filter sterilized by passing it through a 0.2 µm pore-size filter (Millipore, Bedford, MA, USA). Each filtrate was collected in a sterile bottle and considered as the aqueous extract with concentration 1:1.

Determination of Antibacterial Effects of Plant Extracts

***In-vitro* experiments:** Petri experiments were conducted based on paper disc method according to Mangamma and Speeramulu (1981) and Kim et al. (1995). 100 µl of the pathogen bacteria suspension (10^8 cfu/ml) was spread on 9 cm diameter petri dishes containing yeast dextrose chalk agar medium. Sterile paper discs (Wathman No.1, diameter 5 mm) were put on the medium and 10 µl of each extracts was dropped on the discs. Three discs per plate and three plates were used for each plant extracts. Sterile distilled water was used for negative control. All plates were incubated at 25°C for three days. The diameter of the clear zone around the disc was measured as millimeters. The data were subjected to analysis of variance (ANOVA) by using SPSS statistic program (Version 11.5) and the significance between treatments was determined by means of Duncan's Multiple Range Test ($P \leq 0.05$).

***In-vivo* experiments:** Selected two plant extracts (garlic and eucalyptus) according to their *in vitro* antibacterial effects to bacterial spot pathogen were used *in-vivo* experiments as seed treatments. *In-vivo* experiments were conducted in a climatized room conditions (70% relative humidity, 25±2°C temperature and 16/8 h day/night illumination) with naturally infested pepper seeds local cultivar "Karaisali Salcalik", and artificially infected tomato seeds cultivar "H-2274". Tomato seeds were artificially infected with *X. a. pv. vesicatoria* strain according to minor modified vacuum method of Bashan and Assouline (1983). Naturally infested pepper and infected tomato seeds were soaked aqueous garlic and eucalyptus extracts for 30 minutes. Treated seeds were planted in trays (10 x 25 cm) containing the same amount of soil, sand and manure, as six replicates consisting of 100 seeds per tray. Experiment was repeated two times. Untreated seeds by plant extracts were used for control. After seed germination, seedlings were checked daily for disease development. When the spot lesions appeared on the cotyledon leaves of the control plants, the experiments were evaluated. The percentage of diseased seedlings was noted in each treatment for disease incidence. Disease severity was also evaluated by 0-5 scale as follow: **0**: no symptom or disease; **1**: 1-3 spots; **2**: 4-7 spots; **3**: 8-11 spots; **4**: 12-15 spots on cotyledon; and **5**: > 15 spots on stems with dried out cotyledons due to the disease. The percentage of the efficiency was calculated by ABBOTT formula. Analysis of variance (ANOVA) was used for mean separation.

RESULTS

***In-vitro* experiments:** Determination of antibacterial activity of aqueous plant extracts by different sterilization methods were performed *in-vitro* conditions against *X. a. pv. vesicatoria*. As seen in Table 1, aqueous extracts of radish, mint, onion, maratha, oleander, rosemary, peppertree, terebinth tree and pine tree did not inhibit growth of *X. a. pv. vesicatoria* on petri dishes. Antibacterial activity on growth of the pathogen was

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only found with garlic and eucalyptus extracts. The diameters of the clear zone around the disc were ranged between 5.0 and 10.0 mm. Inhibition zones were recorded as 5.0 and 5.1 mm by eucalyptus extracts sterilized by filter and autoclave, respectively. Although filtered-sterilized garlic extract caused inhibition zone (10.0 mm), no inhibition zone was produced by autoclaved-sterilized garlic extract in petri plates. Autoclaved aqueous garlic extract was not found as an effective treatment on inhibition of the pathogen in petri dishes.

Table 1. Antibacterial activity of some plant extracts by different sterilization methods against *Xanthomonas axanopodis* pv. *vesicatoria* in Petri dishes

Extracts	Average inhibition zones (mm)	
	Autoclaved	Filtered
Eucalyptus	5.1	5.0
Garlic	0.0	10.0
Marata	0.0	0.0
Pine tree	0.0	0.0
Oleander	0.0	0.0
Terebinth tree	0.0	0.0
Onion	0.0	0.0
Mint	0.0	0.0
Radish	0.0	0.0
Rosmary	0.0	0.0
Peppertree	0.0	0.0
SDW as Negative Control	0.0	0.0

Table 2. Effects of garlic and eucalyptus extracts on bacterial spot disease incidence and severity on tomato and pepper seedlings

Extracts	Tomato				
	Disease incidence	Efficacy (%)	Avarage of Scale	Disease Severity	Efficacy (%)
Autoclaved eucalyptus extract	0.0 ^{c,*}	100.0	0.3 ^c	0.1	91.5
Filtered eucalyptus extract	0.0 ^c	100.0	0.1 ^c	0.0	95.6
Filtered garlic extract	3.8 ^b	94.8	2.3 ^b	23.4	76.6
Positive control (XCV-K1)	72.9 ^a	-	3.0 ^a	100	-
Negative control	0.0	-	0.0	0.0	-
Extracts	Pepper				
	Disease incidence	Efficacy (%)	Avarage of Scale	Disease Severity	Efficacy (%)
Autoclaved eucalyptus extract	0.0 ^c	100.0	0.1 ^b	0.0	95.8
Filtered eucalyptus extract	0.0 ^c	100.0	0.1 ^b	0.0	95.8
Filtered garlic extract	9.8 ^b	86.3	0.6 ^b	0.2	80.0
Positive control (XCV-K1)	71.4 ^a	-	3.1 ^a	100.0	-
Negative control	-	-	0.0	0.0	-

*Means with the same letter are not significantly different by Duncan multiple range test at $p < 0.05$

***In-vivo* experiments:** As seen in Table 2, both aqueous extract of eucalyptus sterilized by filter and autoclave, and filter-sterilized garlic extract were effective in reducing disease incidence and disease severity of bacterial spot on tomato and pepper

seedlings compared to the control plants. Symptom development on tomato and pepper seedlings was completely inhibited by eucalyptus extracts both filtered and autoclaved as seed treatments. Disease incidences were recorded as 73 and 71% on untreated tomato and pepper seedlings, respectively. Filtered-sterilized garlic extract suppressed disease incidence by 95 and 86% on tomato and pepper plants, respectively. Furthermore, disease severity on tomato seedlings was reduced by 96, 92 and 77% by filtered-sterilized eucalyptus, autoclaved-sterilized eucalyptus and filtered-sterilized garlic extracts, respectively. Similarly, disease severity on pepper seedlings was reduced by 96, 96 and 80% by filtered-sterilized eucalyptus, autoclaved-sterilized eucalyptus and filtered-sterilized garlic extracts, respectively.

DISCUSSION

Bacterial spot disease of pepper, caused by *X. axonopodis* pv. *vesicatoria*, is a serious problem in the processing pepper fields in the eastern Mediterranean region. Contaminated seeds by the pathogen have a significant role in disease outbreak in this region. Since it is difficult to control bacterial spot disease in the field, further study is necessary for development of effective management strategies that is considered contaminated seeds as the primary inoculum source of the disease. Use of pathogen free seeds and/or seed treatments can be recommended for effective management of the disease. One of the alternative control methods of the disease is use of effective plant extracts as seed treatments in conventional and organic agriculture. Using of plant extracts as natural products is a popular trend towards environmental friendly organic production methods in agriculture. There are many reports of the inhibitory effects of plant extracts such as garlic and eucalyptus on plant-associated bacteria (Curtis et al., 2004) and fungi (Arya et al., 1995; Bianchi et al., 1997; Curtis et al., 2004) *in-vitro* conditions. However, there are a few reports on using of plant extracts as seed treatments for fungal pathogens, not bacterial pathogens. Mishra et al., (2000) demonstrated that seed treatment with isoalantolactone, a sesquiterpene lactone from the root of *Inula racemosa* effectively inhibited take all disease development of wheat caused by pathogenic fungi *Gaeumannomyces graminis* var. *tritici*.

Our results clearly demonstrated that aqueous garlic (filtered-sterilized) and eucalyptus (filtered- and autoclaved-sterilized) extracts were effective against seed-borne infections of bacterial spot disease on tomato and pepper. The method of extract sterilization is important on its effectiveness. Autoclaved garlic extract is not effective on the growth of pathogen. Similar results have also reported by Mangamma and Speeramulu (1981) and El Astal (2004). Inhibitory component(s) of garlic was destroyed or inactivated by autoclaving or heating the extract. Because of that, antimicrobial efficacy of garlic are reduced or destroyed by heating (El Astal, 2004). The antibacterial property of garlic was identified as allicin in 1944 by Cavallito (Curtis et al., 2004). This indicates that allicin in garlic is sensitive to the heat treatment. Allicin in garlic has a wide spectrum of antimicrobial activity not only plant pathogens but also human associated microbes (Ankri and Mirelman, 1999). Tannins, flavonoids, terpenes, polyphenols and eucalyptol as main metabolites are previously isolated constituents of many eucalyptus species (Santos and Waterman 2001a, Santos and Waterman 2001b).

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Eucalyptus has also anti-inflammatory, antitumor and anti-mutagenic activities for human pathogens (Santos and Waterman 2001b) and antimicrobial activity for plant pathogens (Maji et al., 2005).

Many organic solvents such as benzene, chloroform, ethyl acetate or methanol can be used for extraction of medicinal and aromatic plants. In the study, we used only water for extraction as garlic juice and eucalyptus juice. Advantages of water extraction are their easily preparation and low cost for farmers.

In conclusion, our results suggest that garlic and eucalyptus extracts could be used as seed treatment to reduce disease incidence and severity. Further studies on the natural products in successful plant protection strategies are needed for commercial usage in conventional and organic agriculture.

ÖZET

**TOHUM UYGULAMASI OLARAK BAZI BİTKİ EKSTRAKTLARININ
DOMATES VE BİBER BAKTERİYEL LEKE HASTALIĞINA KARŞI ETKİSİ**

Xanthomonas axonopodis pv. *vesicatoria* tarafından oluşturulan bakteriyel leke hastalığı domates ve biber bitkisinin tohum kökenli önemli bir hastalığıdır. Turp (*Raphanus raphanistrum*), sarımsak (*Allium sativum*), nane (*Mentha piperita*), soğan (*Allium cepa*), marata (*Clematis marata*), zakkum (*Nerium oleander*), biberiye (*Rosmarinus officinalis*), okaliptüs (*Eucalyptus* sp.), yalancı karabiber (*Schinus* sp.), menengiç (*Pistacia terebinthus*) ve çam (*Pinus sylvestris*) bitkilerinin sulu ekstraktlarının antibakteriyel etkileri *X. axonopodis* pv. *vesicatoria* karşı *in-vitro* ve *in-vivo* denemelerle araştırılmıştır. *In-vitro* denemelerde, ekstraktlar otoklav edilerek ve filtreye olmak üzere iki farklı şekilde sterilize edilmiştir. Turp, nane, soğan, marata, zakkum, biberiye, yalancı karabiber, menengiç ve çam bitkilerden elde edilen sulu ekstraktlar herhangi bir şekilde antibakteriyel etkinlik göstermemiştir. Sarımsak ve okaliptüs ekstraktları ise *in vitro*'da antibakteriyel etkinlik göstermiştir. Filtre edilerek sterilize edilen sarımsak ekstraktı patojenin gelişimi üzerine etkiliyken, otoklavda sterilize edilmiş sarımsağın etkili maddesinin sıcaktan etkilenmesinden dolayı etkili olmamıştır. Bu sonuçlar sarımsağın engelleyici bileşik(ler)inin sıcaklığa duyarlı olduğunu açıkça göstermiştir. Sulu okaliptüs ve sarımsak ekstraktları *in-vivo* koşullarda hastalığın tohum kökenli inokulumunu azaltmada çok etkili olmuştur. Okaliptüs ekstraktı en iyi tohum uygulaması olmuştur. Tohum uygulaması olarak sulu okaliptüs ekstraktı, domates ve biber fidelerindeki hastalık gelişimini tamamen engellemiştir. Hastalık oluşumu domates ve biber fidelerinde sırasıyla % 95 ve 86 oranında filtrasyon ile sterilize edilen sarımsak ekstraktıyla azaltılmıştır. Benzer şekilde, domates ve biber fidelerindeki hastalık şiddeti sarımsak ve okaliptüs ekstraktlarıyla % 77 ve 96 arasındaki oranlarda azaltılmıştır. Bu çalışma geleneksel ve organik fide üretimi için domates ve biberin bakteriyel leke hastalığının tohum kökenli enfeksiyonlara karşı sulu okaliptüs ve sarımsak ekstraktlarının etkisini ortaya koymuştur.

Anahtar kelimeler: *Xanthomonas axonopodis* pv. *vesicatoria*, ekstrakt, antibakterial aktivite, tohum uygulaması, sarımsak and okaliptüs

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