Effects of Plant Essential Oils on *Ralstonia solanacearum* Population Density and Bacterial Wilt Incidence in Tomato

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ABSTRACT

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Greenhouse experiments were conducted to determine the effectiveness of plant essential oils as soil fumigants to manage bacterial wilt (caused by *Ralstonia solanacearum*) in tomato. Potting mixture ("soil") infested with *R. solanacearum* was treated with the essential oils at 400 mg or µl and 700 mg or µl per liter of soil in greenhouse experiments. *R. solanacearum* population densities were determined just before and 7 days after treatment. Populations declined to undetectable levels in thymol, palmarosa oil, and lemongrass oil treatments at both concentrations, whereas tea tree oil had no effect. Tomato seedlings transplanted in soil treated with 700 mg/liter of thymol, 700 ml/liter of palmarosa oil, and 700 ml/liter of lemongrass oil were free from bacterial wilt and 100% of plants in thymol treatments were free of *R. solanacearum*. Soil amendment with fresh leaves of essential oil-producing plants did not reduce bacterial wilt incidence compare to untreated inoculated control. Some thyme oil-producing plants such as thyme (*Thymus vulgaris*) cv. German winter, Creeping thyme (*Thymus serpyllum*), and Greek oregano (*Origanum vulgare* subsp. *hirtum*), while remaining symptomless, became systemically infected by *R. solanacearum* and were therefore identified as hosts of *R. solanacearum*.

Bacterial wilt caused by *Ralstonia solanacearum* is a serious soilborne disease of many economically important crops such as tomato, potato, tobacco, banana, eggplant, and some ornamental plants (10). This bacterium causes wilt by infecting plants through roots and colonizing stem vascular tissue. Although diseased plants can be found scattered in the field, bacterial wilt usually occurs in foci associated with water accumulation in lower areas. Under natural conditions, the initial symptom in mature plants is wilting of upper leaves during hot days followed by recovery throughout the evening and early hours of the morning. The wilted leaves maintain their green color as disease progresses. Under hot humid conditions favorable for disease, complete wilting occurs and the plant will die. The vascular tissues in the lower stem of the wilted plants usually show a brown discoloration.

Due to the limited efficacy of current integrated management strategies, bacterial wilt continues to be economically important for field grown, fresh market tomato production in the southeastern United States and many subtropical and tropical areas of the world. Cultural practices, crop rotation, and host resistance may provide limited control (10).

Many plant species produce volatile essential oil compounds. These oils are considered to play a role in host defense mechanisms against plant pathogens (12). Essential oils and their components, usually from medicinal plants, have been recognized as having fungicidal effects (23), but their efficacy as a biofumigant on *R. solanacearum* has not been studied prior to 1999. Preliminary in vitro and greenhouse experiments conducted with several plant essential oils and their components showed that some essential oils have significant efficacy against *R. solanacearum* (14) and against several soilborne fungi of tomato (13). Recently, several formulated botanical extracts including essential oils were shown to effectively reduce soil populations of *Fusarium oxysporum* and reduce Fusarium wilt incidence on muskmelon (1).

The objective of this study was to evaluate the effects of selected plant essential oils and/or their active components on population density of *R. solanacearum* in soil and on incidence of bacterial wilt in the greenhouse. We also studied whether some essential oil-producing plants can act as a host of *R. solanacearum*. A preliminary report of this study (14) has been published.

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MATERIALS AND METHODS

Bacterial culture and inoculum preparation. *R. solanacearum* (race 1, biovar 1), tomato strain Rs5 (19), isolated in Quincy, Florida was used in these studies. Pathogenicity of the strain on tomato was confirmed as described below as part of fulfilling Koch's postulates. The bacterium was grown on casamino acid peptone glucose medium (CPG) (9) for 48 h or overnight (18 h) in CPG broth on a shaker (200 RPM) at 28°C. Bacteria were suspended in sterile deionized water, and concentration of inoculum was estimated at A_{590} The viable bacterial population was determined following dilution plating on CPG agar.

Soil infestation and amendments. Terra-lite agricultural mix (Scott Sierra Horticultural Products, Co., Marysville, OH) used as plant growth medium will be referred to as "soil." One liter of soil volume weighed 425 g.

For each of experiments 1 and 2, 900 ml of soil was placed in a plastic bag. A total of 100 ml of *R. solanacearum* suspension containing 9×10^8 CFU/ml was added to each bag. Following inoculation, the soil was thoroughly mixed for uniform distribution of inoculum. Seventy-five grams each of freshly harvested leaves of thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) were homogenized separately in 100 ml of sterile tap water and added and mixed to the 900 ml of soil 2 h after infestation with *R. solanacearum*. For other amendments, infested soil was treated with a suspension of 700 µl of essential oils (wild marjoram, thyme, and palmarosa oils) dissolved in 6.3 ml of 70% ethanol and detergent at 0.1% in 56 ml of water to prepare a stable essential oil suspension. The untreated inoculated control consisted of the same amount of ethanol, detergent, and water as above. Wild marjoram and thyme oils were excluded in experiment 2. Following treatment, the potting soil in the closed bags was mixed thoroughly each day for 3 days to allow fumigation, then aerated for 4 days to vent excess volatiles. Seven days after treatment, 10-cm pots were filled with the treated soil and a tomato seedling (cv. Equinox) was transplanted into each pot. Pots were arranged in a randomized block design with five replications. Four and eight pots of each treatment comprised a replicate for experiments 1 and 2, respectively. Wilt incidence was recorded until 3 weeks after transplanting.

Thymol and palmarosa (*Cymbopogon martini*) and tea tree (*Melaleuca alternifo-*

lia) oils were evaluated in experiment 3, and in addition to these, lemongrass (*C. citratus*) oil was included in experiment 4. A reduced rate of each of the essential oils (400 µl or mg/liter of soil) was also tested against *R. solanacearum* in experiment 4. When the quantity of soil to be treated or rate of essential oil application changed, the amount of ethanol, detergent, and water were also adjusted proportionately. For greenhouse experiments 3 and 4, 200 ml of inoculum suspension was poured in a plastic bag with 1.8 liter of soil to attain a final estimated population of 10^7 to 10^8 CFU per ml of soil. Two h after inoculation, initial *R. solanacearum* population was enumerated, followed by treatment with the method described earlier. The bacterial population was also enumerated 7 days after treatment. A 10 ml soil sample from each replicated plastic bag was used to quantify bacterial populations by suspending in 100 ml of deionized water, shaking at 250 RPM for 30 min, and plating aliquots (100 µl each) of the supernatant on duplicate plates of semiselective modified SMSA medium (3,4). Typical *R. solanacearum* colonies were enumerated on SMSA 48 h after incubation at 28°C. Sample colonies from presumptive *R. solanacearum* colonies were confirmed by gas chromatographic profiling of whole-cell fatty acid methyl esters (MIDI, Newark, DE), as described by Stead (22) and polymerase chain reaction (PCR) with *R. solanacearum* specific primer (8,19). To enumerate soilborne populations as per ml of dry soil, soil moisture was recorded for each sampling date in experiments 3 and 4. Soil samples were oven dried at 110°C for 18 h to determine soil moisture. Seven days after treatment, transplants were inoculated as above. Pots were arranged in a randomized block design with five replications. Four pots of each treatment comprised a replicate. Wilt incidence was recorded until 4 weeks after transplanting. Plants that did not develop wilt symptoms within 4 weeks after transplanting were tested for *R. solanacearum* by macerating 1-cm section of the basal stem in 1 ml of sterile deionized water and streaking on SMSA agar (7,18).

For all experiments, 5-week-old tomato transplants (cv. Equinox) with 4 to 5 true leaves were inoculated and pots were placed in a saucer containing water to maintain high soil moisture to facilitate infection and wilt development. Plants were supplied with Peter's peat lite special (15:16:17 N-P-K) at 10-day intervals at the rate of 7.5 g/liter of water. Plants in pots were maintained in the greenhouse and held between 23 to 28°C (night) and 30 to 35°C (day).

Inoculation of thymol producing plants. The thymol-producing plants, thyme (*T. vulgaris*) cv. German winter, Creeping thyme (*T. serpyllum*), and Greek Oregano (*O. vulgare* subsp. *hirtum*) were used in this experiment. Seeds were sown in polystyrene flats with 2-cm^2 cells. Following germination, the plants were thinned to one plant per cell. Two months after sowing at the 10- to 15-leaf stage, plants were inoculated by pouring 5 ml of inoculum containing 1.2×10^8 CFU/ml of *R. solanacearum* (Tomato strain Rs5*)* on the soil of each cell. A total of 50 plants of each species were inoculated. Five-week-old tomato plants (cv. Equinox) with 4 to 5 true leaves were also inoculated as control plants. Three days after inoculation (in flats), plants were transplanted in 10-cm pots and maintained in the greenhouse at 28 to 32°C for 25 days. Plants were assessed for bacterial wilt, and those that had no wilt symptoms 4 weeks after inoculation were tested for latent infection by *R. solanacearum* (18). Roots were washed in running tap water and dried in tissue paper. Subsequently, pieces from the lower, middle, and upper portion of the root were cut with a flamed, sterile, scalpel blade. The sample roots from one plant were divided into two portions. One portion was macerated in 1 ml of sterile water. To detect latent infections (6), another portion was surface sterilized with 70% ethanol for 5 min (5), washed with sterile water for the same period of time to remove excess ethanol, and macerated in 1 ml of sterile water. The macerates were streaked on SMSA medium and plates were incubated at 28ºC.

To fulfill Koch's postulates, the isolates of *R. solanacearum* recovered from latently infected plants of *T. vulgaris*, *T. serpyllum*, and *O. vulgare* subsp. *hirtum* were reinoculated to the corresponding plant species (25 plants per species) with the procedure described earlier (including plant age and inoculation method). Inoculum concentrations were approximately 10^8 CFU/ml. To confirm that the *R. solanacearum* isolates recovered from symptomless plants retained their pathogenicity, each isolate, including the original Rs5 strain, was inoculated separately onto each of 10 plants of tomato cv Equinox at the four to five true-leaf stage, as a susceptible control plant.

Disease assessment and statistical analysis. Wilted plants were recorded for each treatment twice weekly as the proportion of wilted plants (bacterial wilt incidence) based on the number of plants at the beginning of each experiment. Any abnormal symptoms on leaves were observed and recorded throughout experiments. Plant height and shoot and root weight were measured to evaluate effect of treatments on plant growth in experiment 3. Roots were washed thoroughly to remove soil before measurements. Analysis of variance (ANOVA) was used to determine the effects of treatment on disease incidence and growth measurements. Means were compared using Duncan's multiple range tests. ANOVA was not carried out when there were too many zero values. Statistical analysis was performed with the SAS version 8.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Effect of soil amendments. Treatment of soil with thymol and essential oils resulted in a reduction in population density of *R. solanacearum* and in bacterial wilt incidence and an increase in plant shoot and root weight on tomato. Even though disease incidence data were collected biweekly, only final disease data were subjected to statistical analysis.

In experiment 1, none of the tomato plants wilted when grown in soil treated with thyme or palmarosa or wild marjoram oil. However, 80% or more of the plants wilted when grown in soil amended with fresh thyme or oregano leaves or the nontreated control soil (Table 1). Although 20% of tomato plants became wilted in soil treated with palmarosa oil in experiment 2, wilt incidence was significantly ($P = 0.01$) lower than in soil amended with the fresh plant materials or in untreated inoculated control soil. As a result of experiments 1 and 2, palmarosa oil and thymol were chosen for further study. Thymol is an antibacterial fraction of thyme and oregano essential oils that has contact activity against *R. solanacearum* in vitro (14).

Table 1. Effect of soil amendment with plant essential oils and fresh medicinal plant materials on bacterial wilt (caused by *Ralstonia solanacearum*) of tomato in greenhouse experiments

	Bacterial wilt incidence (%)[w]	
Treatments	**Experiment 1[x]**	**Experiment 2**
Untreated control	80	100 a
Thyme (fresh)[y]	80	100 a
Oregano (fresh)[y]	100	100 a
Wild marjoram oil[z]	0	Not tested
Thyme oil[z]	0	Not tested
Palmarosa oil[z]	0	20 b

[w] Final bacterial wilt incidence (based on four plants per replicate in experiment 1 and eight plants per replicate in experiment 2) Twenty-one days after inoculation, values followed by different letters are significantly different ($P \leq 0.01$) based on Duncan's multiple range test.

[x] Analysis of variance was not carried out due to a multitude of zero values.

[y] Seventy-five grams of freshly harvested leaves homogenized in 100 ml of water and incorporated in 900 ml of soil.

[z] Essential oil (700 µl per liter of soil).

In experiment 3, the observed initial populations recovered from the soil ranged from 9.3×10^7 to 1.2×10^8 CFU/ml. Seven days after treatment, the bacterium was not detected in soil treated with thymol or palmarosa oil, however, a population of approximately 10^8 CFU/ml was recovered in soil treated with tea tree oil or the nontreated control soil (Table 2). In this experiment, within 2 weeks after transplanting, 100% of tomato seedlings grown in pots with tea tree oil and untreated inoculated control soil wilted. Plants in soil treated with thymol or palmarosa oil were not wilted through the entire experiment (28 days after inoculation). Moreover, all plants in thymol and palmarosa oil treatments were free from any detectable *R. solanacearum* (Table 2). Some plants in soil treated with palmarosa oil developed chlorotic and necrotic leaf margins. However, new leaves that were produced following fertilizer application were apparently unaffected.

The effect of thymol and essential oils on root and shoot growth was not significant at the end of experiment 3 in noninoculated but treated pots. Thymol application significantly ($P \leq 0.05$) reduced shoot weight in the noninoculated treatment compared to the nontreated controls (Table 3). Root and shoot weights and plant height were reduced when plants were wilted. Plants grown in inoculated soil treated with thymol or palmarosa oil produced more root and shoot tissues and were taller than those of inoculated untreated control or tea tree oil treatment (Table 3).

In experiment 4, plants grown in soil treated with 700 mg/liter of thymol were free from wilt and *R. solanacearum*, whereas only 10% of plants in soil treated with palmarosa or lemongrass oil harbored the bacteria without wilt (Table 2). The disease incidence was 75% in soil treated with tea tree oil. Although the pathogen was not detected on SMSA in soil treated with 400 mg/liter (low dose) of thymol or 400 ml/liter of plant oils, bacterial wilt was observed (Table 2). In this experiment, the observed initial populations recovered from the soil ranged from 2.0×10^6 to 4.5×10^7 CFU/ml. Seven days after treatment, the bacterium was not detected in soil treated with thymol or palmarosa or lemongrass oil, whereas a large population was recovered in soil treated with tea tree oil or the nontreated control soil (Table 2).

Inoculation of thymol producing plants. Tomato plants started wilting 7 days after inoculation (DAI) and 100% wilted 15 DAI, whereas none of the thyme or oregano plants wilted (Table 4). However, 40, 20, and 60% of German thyme, Creeping thyme, and Greek oregano, respectively, were systemically infected 25 DAI. *R. solanacearum* was recovered from 40 to 60% of nonsterilized roots of these plant species (Table 4).

To confirm that *R. solanacearum* isolates, recovered from symptomless thymol-producing plants, had remained pathogenic, representative isolates were purified and reinoculated in healthy tomato plants. In all instances, tomato plants wilted within 20 days of inoculation irrespective of the isolate history, indicating that the bacteria (Rs5 strain) recovered from test plants was still pathogenic. As before, none of the thyme and oregano plants was wilted. Latent infection ranged from 20% in oregano to 40% in both thyme species. The bacterium was also recovered from the nonsterilized roots of the remainder of the nonsterilized plants of all three species.

DISCUSSION

Thymol and palmarosa, thyme, marjoram, and oregano (*O. vulgare*) oils have been shown to effectively reduce popula-

Table 2. Soil population densities of *Ralstonia solanacearum* (Rs), final bacterial wilt incidence, and detection of Rs as affected by soil application of essential oils and thymol in greenhouse experiments 3 and 4

Treatments	Rate /liter of soil	Population density[w] before treatment	Population density[w] 7 days after treatment	% Bacterial wilt[x]	% Plants with Rs[y]
Experiment 3					
Thymol	0.7 g	9.3×10^7	0	0	0
Palmarosa oil	0.7 ml	9.6×10^7	0	0	0
Tea tree oil	0.7 ml	1.2×10^8	1.3×10^8	100	–
UTC[z]		1.1×10^8	2.7×10^8	100	–
Experiment 4					
Thymol	0.7 g	5.0×10^6	0	0	0
Palmarosa oil	0.7 ml	3.7×10^6	0	0	10
Tea tree oil	0.7 ml	3.7×10^6	1.9×10^7	75	10
Lemongrass oil	0.7 ml	6.2×10^6	0	0	10
Thymol	0.4 g	2.0×10^6	0	10 c	0
Palmarosa oil	0.4 ml	4.1×10^6	0	40 cb	10
Tea tree oil	0.4 ml	2.9×10^6	4.5×10^7	100 a	–
Lemongrass oil	0.4 ml	6.5×10^6	0	65 b	0
UTC[z]		4.5×10^7	5.7×10^7	100 a	–

[w] Population densities for Rs (CFU/ml of soil) were estimated before transplanting by plating on SMSA media and confirmed by MIDI, Newark, DE and polymerase chain reaction. Data represents average of five replicates per treatment.
[x] Final bacterial wilt incidence (based on 20 plants) 28 days after inoculation, values followed by different letters are significantly different ($P \leq 0.05$) based on Duncan's multiple range test. Analysis of variance was not carried out due to multitude of zero values.
[y] Isolation of Rs on apparently healthy plants was conducted 28 days after inoculation, (–) isolation of Rs was not conducted on wilted plants. Percent plants with Rs indicate isolation of Rs from basal stem tissue on SMSA medium.
[z] UTC = Untreated control.

Table 3. Effect of biofumigants on root and shoot growth and plant height of tomato cv Equinox 25 days after transplanting into *Ralstonia solanacearum* infested and noninfested soil

	Root weight (g) per plant		Shoot weight (g) per plant		Plant height (cm)	
Treatments[x] (% Wilt)	Noninoculated	Inoculated[y]	Noninoculated	Inoculated[y]	Noninoculated	Inoculated[y]
Thymol (0)	12.18 a	14.42 a	46.14 b	59.07 a	19.82 a	21.87 a
Palmarosa oil (0)	11.11 a	13.01 a	48.78 ab	52.96 b	20.20 a	20.70 a
Tea tree oil (100)	11.58 a	0.50 b	51.01 ab	2.50 c	20.32 a	14.65 b
UTC[z] (100)	12.27 a	0.39 b	54.78 a	2.15 c	21.15 a	11.65 c

[x] Products were amended at the rate of 700 mg (thymol) or 700 µl (essential oils) per liter of soil (Experiment 3).
[y] Values followed by different letters within each column are significantly different ($P \leq 0.05$) based on Duncan's multiple range test.
[z] UTC = Untreated control.

tions of *R. solanacearum* in in vitro tests and to reduce bacterial wilt incidence on tomato in greenhouse experiments (14). On the basis of the results of in vitro tests (14) and experiments 1 and 2, palmarosa oil and thymol were selected for further study. The price of extracted thyme, wild marjoram, and oregano oils is prohibitive to commercial agriculture. Instead, thymol, which is the main and active component of these essential oils, was included in this study. Thymol is produced synthetically.

In the current study, thymol and palmarosa and lemongrass oil were found to be effective in reducing *R. solanacearum* populations and bacterial wilt incidence of tomato grown in infested soil. Efficacy of several plant essential oils on fungal plant pathogens was previously reported. For example, fumigation of apricots with thymol reduced the germination of *Monilinia fructicola* conidia and retarded mycelial growth (11). Palmarosa and lemongrass oils have also exhibited antifungal activity against *Botrytis cinerea* (23). In addition, extracts and/or essential oils from pepper, mustard, cassia tree, and clove suppressed disease development caused by *Fusarium oxysporum* f. sp. *melonis* on muskmelon and reduced population density of *F. oxysporum* f. sp. *chrysanthemi* in greenhouse experiments (1). Lack of effectiveness of tea tree oil in the current study shows that all essential oils will not be bactericidal to *R. solanacearum* even though this essential oil in high concentrations is effective in vitro against *B. cinerea* (23).

R. solanacearum was neither recovered from the soil treated with 700 mg of thymol nor did the tomato plants grown in infested soil develop bacterial wilt symptoms or harbor *R. solanacearum*. This indicates that 700 mg of thymol per liter of soil eliminated viable *R. solanacearum* under the conditions of our experiments. The antifungal activity of thyme oils is well established against fungi such as *B. cinerea* (23), *Rhizopus stolonifer* (20), *Aspergillus* spp. (16), *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium solani*, *and Colletotrichum lindemuthianum* (24), and thymol appears to be one of the predominant active components of these oils (2,20,24). Essential oil from *O. vulgare* and its main component thymol has also shown nematicidal activity against the root-knot nematode on cucumbers (15). Volatile compounds from plants, especially essential oils, have also been shown to have insecticidal activities against four major stored-product insects (21).

R. solanacearum was not detected in infested soils 7 days after application of thymol or palmarosa or lemongrass oils. However, 10% of plants grown in soil treated with 700 µl/liter of palmarosa and lemongrass oils harbored *R. solancearum*, and 40 to 65% of plants were wilted when grown in soil treated with 400 µl/liter of each of these oils. It is important to note that effective rates of essential oils against plant pathogenic microorganisms might change based on soil type. These results indicate that essential oil screening against the bacterial wilt pathogen should be based collectively on population density and wilt incidence. Growth of sufficient numbers of background bacterial populations on SMSA indicate that these essential oils might not reduce some of the soilborne microorganisms present in the soil. Semiselective medium such as SMSA cannot detect *R. solanacearum* from soil in the presence of relatively high background bacterial population (17).

Application of essential oils or their main components as a biorational alternative to conventional fumigants in field conditions will require further experimentation. In our study, soil is mixed thoroughly with amended materials. In field production of tomato, biorational materials could be applied through regular fumigation methods or drip irrigation under the polyethylene mulch to achieve uniform distribution in soil. Aeration time would need to be determined during which excess volatiles are dispersed before transplanting. If soil is not aerated well, a concentration over 800 mg of thymol per liter of soil showed phytotoxicity on leaves in tomato (T. Momol, *personal observation*). This property of thymol could be explored by studying its effect on weed seed germination as a possible preemergent herbicide. Thymol or palmarosa oil did not reduce most plant growth parameters, although a reduction in shoot weight was observed in noninoculated thymol-treated plants.

Our results suggest that German thyme, Creeping thyme, or Greek oregano can be latently infected under artificial conditions and therefore, can be potential hosts of *R. solanacearum* under natural conditions. Soil amendment with nonhost plants is one of the cultural methods of reducing *R. solanacearum* populations in soil. However, the lack of effectiveness of soil amendment with thymol-producing plants in reducing *R. solanacearum* populations indicates that fresh plant material might not contain enough essential oil to be active against the pathogen. Moreover, these plant species cannot be used as rotation crops against *R. solanacearum* as they could serve as a host plant without expressing the typical wilt symptoms.

The U.S. Food and Drug Administration lists thymol as a food additive, and the U.S. Environmental Protection Agency (EPA) lists it as a "Generally Recognized as Safe (GRAS)" compound. The first thymol product registered as a pesticide in the United States was in 1964. The EPA is not aware of any adverse effects of thymol to humans or the environment when it is used in a manner consistent with the label (11).

Our results indicate that thymol and palmarosa and lemongrass oil have the potential to suppress *R. solanacearum* populations in soil and reduce bacterial wilt incidence in greenhouse pot experiments. Since these essential oils, or their components (i.e., thymol), have been reported to have fungicidal, nematicidal, and antibacterial activities (1,13,14,15), they could be used in integrated management of soilborne disease in tomato. Soil treatment with these types of compounds can be an alternative to the use of methyl bromide. However, further research is required to determine the effectiveness and economics of natural plant products under field conditions to manage bacterial wilt of tomato. In addition, the mode of action of the essential oils used in these experiments against *R. solanacearum* needs to be studied.

Table 4. Bacterial wilt incidence and fate of *Ralstonia solanacearum* (Rs) in surface sterilized and nonsterilized roots of several plant species[x]

Plant species	% Wilt	% Latent infection[y]	% Plants with Rs on or in roots[z]	% Total plants with Rs
Thymus vulgaris (German thyme)	0	40	40	80
Thymus serpyllum (Creeping thyme)	0	20	60	80
Origanum vulgare subsp. *hirtum* (Greek oregano)	0	60	40	100
Lycopersicon esculentum (Tomato)	100	…	…	100

[x] Fifty plants of each species were inoculated with Rs. The sample roots from each plant was divided in two groups. One half of the plants were macerated in 1 ml sterile water and the other half was surface sterilized with 70% ethanol for 5 min and washed with sterile water for the same period of time to remove excess ethanol. The macerates were streaked on SMSA medium and plates were incubated at 28°C.

[y] Rs recovered from surface sterilized roots of apparently healthy plants.

[z] Rs recovered from nonsterilized roots.

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