Development of an Integrated Approach for Managing Bacterial Wilt and Root-Knot on Tomato Under Field Conditions

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ABSTRACT

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A 2-year field study was conducted to develop a field application method using thymol as a preplant soil treatment for controlling bacterial wilt and root-knot nematode on tomato (*Lycopersicon esculentum*). In addition, acibenzolar-S-methyl (ASM), which induces plant systemic resistance, was applied in conjunction with thymol to determine whether combining these tactics could improve bacterial wilt management. The test sites were artificially infested with *Ralstonia solanacearum* and *Meloidogyne arenaria*, and thymol was applied as preplant fumigation through drip irrigation lines under polyethylene mulch at a rate of 73 kg/ha in both 2004 and 2005. ASM was applied primarily as foliar spray at a concentration of 25 mg/liter. Application of thymol significantly reduced incidence of bacterial wilt on tomato in both years of the trial. In thymol-treated plots, 26.0 and 22.6% of the plants wilted in 2004 and 2005, respectively; whereas, in untreated plots, more than 95% of the plants wilted in each year. Number of root-knot nematode juveniles was significantly reduced in field plots treated with thymol and ASM for both years. The combined use of thymol and ASM provided the greatest reduction of root galling among the treatments. Tomato yield (cv. FL47) was evaluated only in the 2005 trial; thymol-treated plots produced significantly higher marketable yield than untreated plots, and the thymol treatment in combination with ASM significantly increased tomato yield compared with thymol or ASM alone. These results indicate that use of thymol and ASM was beneficial in controlling bacterial wilt and root-knot. We developed an effective method for applying thymol through drip irrigation lines for managing these diseases in tomato production.

Additional keywords: integrated disease management, plant essential oil

Tomato is the most important vegetable crop in the southern United States, totaling more than U.S.\$1 billion in farm gate value, with Florida accounting for nearly 65% of the fresh-market production (11). Bacterial wilt and root-knot are two soil-borne diseases that cause serious damage in the production of tomato (*Lycopersicon esculentum* L.) and many other crops. Bacterial wilt caused by *Ralstonia solanacearum* and root-knot by *Meloidogyne* spp. occur worldwide and affect hundreds of plant species, which makes them among the most important plant diseases (5,13,21). In North America, bacterial wilt primarily occurs in the southeastern United States, causing significant losses on solanaceous crops such as tomato, potato (*Solanum tuberosum* L.), and flue-cured tobacco (*Nicotiana tabacum* L.) (12,20,38). Root-knot nematodes (*Meloi-*

dogyne spp.) are the major nematode taxa that cause disease in U.S. vegetable and agronomic production systems (21). In addition to direct crop damage, root-knot nematodes predispose plants to infection by bacterial and fungal pathogens, which contribute to additional yield reductions (8,26,29).

The efficacy of current strategies for management of bacterial wilt and root-knot is limited. Bacterial wilt is a soilborne and systemic disease that cannot be effectively managed with foliar application of copper pesticides (M. T. Momol, unpublished data). Soil treatments with traditional general-purpose fumigants such as methyl bromide do not provide satisfactory disease control (7,10). Some resistant tomato cultivars are available, but they have not been generally accepted by the tomato industry due to poor horticultural quality and varying levels of disease resistance resulting from pathogen and climate variation in different locations (22). In case of root knot, although the widespread use of methyl bromide has substantially reduced damaging nematode populations in many tomato production areas, this is expected to dramatically change with the phase out of methyl bromide. Hence, development of

biological soil treatments for effective management of bacterial wilt and root-knot is highly desirable.

Studies have shown that a plant-derived volatile compound, thymol, suppresses bacterial wilt of tomato (18,36). Mode of actions of the antibacterial property of thymol appeared to include disruption of bacterial cell membrane integrity by altering protein reactions (14,19,41). Thymol was shown to be effective in vitro against *R. solanacearum*, and application of thymol as a soil treatment eliminated this pathogen in the soil and provided complete protection of tomato plants against bacterial wilt under greenhouse conditions (28,36). Field experiments using thymol for preplant soil fumigation indicated that it was effective in reducing bacterial wilt incidence on tomato and resulted in higher yields (18). In those field experiments, thymol solution was drenched into individual planting holes infested with *R. solanacearum* (18), which may not be practical in large-scale commercial tomato production. Hence, although on the basis of these studies we concluded that thymol has great promise as an effective component in integrated management of bacterial wilt of tomato, further research is needed to establish practical methods for producers to apply thymol in the field. Thymol has been shown to have broad-spectrum activities against a variety of plant pathogens, including root-knot nematodes, in laboratory and greenhouse studies (6,27,30). It was unknown, however, whether thymol could provide effective suppression of root-knot nematodes under field conditions.

Previous studies have shown that foliar application of acibenzolar-S-methyl (ASM), a plant activator that enhances plant resistance, reduced root-knot nematode (*Meloidogyne javanica*) and reniform nematode (*Rotylenchulus reniformis*) in pineapple under greenhouse conditions (9). In our previous studies, application of ASM enhanced resistance of moderately resistant tomato cultivars and resulted in significant reduction in bacterial wilt incidence (34). Although ASM did not provide effective protection against bacterial wilt on commercially acceptable tomato cultivars in the presence of high inoculum pressure, it significantly reduced bacterial wilt incidence when pathogen population was relatively low in greenhouse studies (2).

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Hence, we hypothesized that ASM might provide additional disease suppression when populations of *Ralstonia solanacearum* in the soil are reduced by thymol treatment.

The main objective of this study was to evaluate the efficacy of thymol, when used for preplant soil treatment through drip irrigation lines, in suppression of bacterial wilt and root-knot on tomato. In addition, we evaluated the combination of thymol and ASM for developing an integrated approach for managing bacterial wilt and root-knot on tomato.

MATERIALS AND METHODS

Field preparation. Field experiments to evaluate the efficacy of thymol and acibenzolar-S-methyl for managing bacterial wilt and root-knot on tomato were conducted in 2004 and 2005 at the University of Florida North Florida Research and Education Center in Quincy, FL. The soil in the experimental fields was an Orangeburg loamy fine sand with 78% sand, 14% silt, 8% clay, and a pH of approximately 6.0. Raised beds (10 cm high, 91 cm wide, and centered 1.8 m apart) were prepared for tomato growth. The fields were fertilized with N-P-K at 218-67-218 kg/ha. After preparing the beds according to commercial standards, a single drip line was laid. A second additional drip line was laid in this study to facilitate the delivery and distribution of thymol. One drip tube was located at each side of the bed center, 8 cm from the center, and buried 3 to 4 cm deep. The beds were covered with polyethylene mulch and irrigated to field capacity by drip irrigation prior to pathogen inoculation in the field.

Soil infestation with *R. solanacearum* and *M. arenaria*. *R. solanacearum* strain Rs5 (race 1, biovar 1) isolated from tomato (35) was used in the studies. The bacterial strain was grown on casamino acid peptone glucose (CPG) agar plates at 28°C for 48 h, washed from the plates, and suspended in sterile deionized water. Eggs and second-stage infective juveniles (J2) of *M. arenaria* race 1 were extracted from tomato roots (cv. Rutgers) with sodium hypochlorite (15). Inoculation of the pathogens in the field was as described previously (18). Briefly, a 15-cm-deep application hole was made where tomato plants were going to be transplanted, and a volume of 50 ml of a suspension containing *M. arenaria* (eggs and J2 at 100/ml) or a mixture of *R. solanacearum* (3×10^7 CFU/ml) and *M. arenaria* (eggs and J2 at 100/ml) was applied to each application hole. In 2004, the field plots were infested with *R. solanacearum* and *M. arenaria*. In 2005, an additional main treatment was added where the field plots were infested with *M. arenaria* only. The holes on plastic mulch were sealed with duct tape after application of the pathogen suspension. These fields previously were inoculated

with the same nematode, and methyl bromide or chloropicrin was not used for the control of nematodes and weeds.

Application of thymol solutions through drip lines. Thymol (Sigma-Aldrich, St. Louis) was applied to the field through drip irrigation lines at a rate of 73 kg/ha 3 days after inoculation of the pathogens. A thymol solution was prepared using the ratio as described previously (18) with modification for water: 1.4 g of thymol, 7 ml of 70% ethanol, 0.2 ml of detergent (Dawn; Procter & Gamble, Cincinnati, OH), and 88.7 ml of water. The thymol solution for the entire field experiment (57 liters for 2004 and 47.5 liters for 2005 experiments) was placed in two spray tanks: two-thirds of the solution in a 38-liter tank and one-third in a 19-liter tank. The two tanks then were filled with water, linked in tandem, and connected to the drip lines. The thymol solution in the tanks was injected into the field through the two drip lines by a CO₂-powered tank at 10 psi while irrigation water was running through the drip lines. The flow rate was controlled using a flow adjustment screw in order to allow the injection to last about 170 min for a target concentration of thymol in the soil of approximately 700 µg/liter. In our previous study, the solution containing only ethanol, detergent, and water (without thymol) was not effective against bacterial wilt on tomato (18); hence, in this study, the control plots were not treated. The holes in the plastic mulch sealed with duct tape were cut open 3 days after thymol application for aeration.

Tomato plants and application of ASM. Susceptible tomato cvs. Solar Fire and FL47 were used in the field experiments in 2004 and 2005, respectively. Tomato plants were grown in the greenhouse in Terra-lite agricultural mix (Scott-Sierra Horticultural Products Co., Maryville, OH) in expanded polystyrene flats with 3.5-by-3.5-cm² cells. The initial foliar application of ASM (25 µg/ml) was done 2 weeks after seedling emergence. Plants were sprayed until run-off with ASM solution using a hand-held garden sprayer. A second application of ASM was made by both foliar spray (25 µg/ml) and soil drench (12.5 µg/ml) 5 days prior to transplanting into the field. For the soil drench, a volume of 5 ml of ASM solution was poured into each cell. Five-week-old tomato seedlings were transplanted 50 cm apart in the row into raised beds 4 days after thymol application holes were cut open. Experimental plots consisted of single rows which were 7 m long with 14 plants per row in both years. Buffer zones with 2-m spacing without tomato seedlings were maintained between plots. Tomato plants were staked, tied over time, and treated with foliar sprays of standard pesticides for control of insects and foliar diseases at weekly intervals (31). A split-plot design was employed for both years with thymol (or without thymol)

treatment as the main plot and ASM (or without ASM) treatment as the subplot in 2004. In 2005, the main treatments were thymol (soil infested with *R. solanacearum* and *M. arenaria*), no thymol and soil infested with *R. solanacearum* and *M. arenaria*, and no thymol and soil infested with *M. arenaria* only. ASM (or without ASM) treatment was the subplot in 2005. A randomized complete block arrangement was used for the main plots, with six replicates for the 2004 and five replicates for the 2005 experiments.

Field applications of ASM were made with a backpack CO₂-powered sprayer fitted with three nozzles and sprayed at 60 psi in a 1-m band with 330 to 600 liters of water per hectare, depending on size of plants. A total of six foliar sprays of ASM were applied in the field at a concentration of 25 µg/ml. The first four applications were made at 7-day intervals following transplanting, and two additional applications were made at 15-day intervals.

Disease and yield assessment and data analysis. Wilted tomato plants were harvested and tested for bacterial ooze and isolation of *R. solanacearum* on a previously described modified semiselective medium, South Africa (SMSA) (34). Presumptive colonies of *R. solanacearum* were confirmed by tests with immunoassay strips specific to *R. solanacearum* (Agdia, Inc., Elkhart, IN). The number of plants wilted was counted weekly after transplanting. Bacterial wilt incidence was calculated as the percentage of wilted plants and transformed into proportion of wilted plants for the statistical analysis. Root galling was evaluated at the end of the experiments. Six and eight plants were sampled from each plot in 2004 and 2005, respectively, and root galling severity on each plant was estimated (where 0 = no root galling and 100 = 100% of the root system galled; 37) and transformed into proportion data for the statistical analysis. Immediately prior to root gall severity assessments, soil samples were taken from each plot to determine root-knot nematode populations. Five soil cores (2.5 cm in diameter) to 25 cm deep were collected from each plot, soil was processed with a modified centrifugation-flotation technique, and root-knot nematode juveniles (J2) were counted (17).

To determine the effect of the treatments for main plots and subplots on bacterial wilt incidence, root gall severity, and root-knot nematode population in the soil, generalized linear mixed models in PROC GLIMMIX of SAS (SAS Institute, Cary, NC) were used. Appropriate distribution models were selected for each type of disease or pathogen populations based on the type of data collected, and *t* tests were used to compare the means.

Tomato fruit were harvested in 2005. A total of three harvests were made at weekly intervals near the end of the sea-

son, and marketable and unmarketable yields were determined. Analysis of variance was used to determine the effect of the treatments on tomato yield using the Statistical Analysis System (SAS Institute) for a split-plot randomized complete block design. Least significant difference was used to compare means.

RESULTS

Field experiment (2004). Typical bacterial wilt symptoms were observed 1 week after transplanting and disease incidence in untreated control plots progressed rapidly thereafter (Fig. 1A). Application of ASM was not effective in reducing bacterial wilt incidence. Thymol application provided effective protection against bacterial wilt. No plants wilted within 14 days of transplanting and, 21 days after transplanting, only 7.1% of plants wilted in the thymol-treated plots compared with 69% plants wilted in the untreated control plots (Fig. 1A). The final bacterial wilt incidence 66 days after transplanting in thymol-treated plots (20%) was significantly lower compared with that in the untreated plots (97%) (Table 1).

Integrated use of thymol as a soil treatment and ASM as a foliar spray numerically reduced bacterial wilt incidence compared with either thymol or ASM applied alone (Fig. 1A; Table 1); however, this reduction was not significant statistically. Root gallings in plots treated with thymol plus ASM also was reduced compared with plots treated with thymol. Soil populations of root-knot nematode J2 in plots treated with thymol versus thymol plus ASM were significantly different. Root gallings and root-knot nematode J2 populations in the soil were not determined in the untreated control plots and plots treated with ASM because most of the plants in these plots were dead due to bacterial wilt long before harvest.

Field experiment (2005). Similar to Solar Fire in the 2004 field test, tomato seedlings of FL47 showed bacterial wilt symptoms 7 days after transplanting in the field. In the untreated control plots, bacterial wilt incidence increased rapidly, with 95.8% of the plants wilting by the end of the season (Fig. 1B; Table 2). Application of ASM did not reduce the final bacterial wilt incidence, although there was a tendency to reduce disease incidence during the first 21 days after transplanting (Fig. 1B). Preplant soil treatment with thymol consistently reduced bacterial wilt incidence during the whole growing season. Only 16.2% of plants wilted in thymol-treated plots by the end of the experiment, which was significantly lower than that in the untreated plots (95.8%; Table 2).

Integrated use of thymol and ASM did not significantly reduce bacterial wilt incidence compared with the other treatments (Table 2). Root gallings in thymol-treated plots was not significantly reduced com-

pared with that in the plots inoculated with *M. arenaria* but not treated with thymol or ASM. Root gallings in plots treated with thymol plus ASM was not significantly reduced compared with those in the plots inoculated with *M. arenaria* and treated with ASM (Table 2). There was a significant ($P = 0.003$) interaction between main

plots and subplots for the soil populations of J2 root-knot nematodes. At the end of the season, soil populations of root-knot nematode J2 in the plots treated with thymol and ASM were reduced significantly when compared with other plots. Most of the plants were dead in the two treatments inoculated with *R. solanacea*-

Fig. 1. Bacterial wilt incidence on tomato in field plots treated with thymol and acibenzolar-S-methyl (ASM) in **A**, 2004 and **B**, 2005. Thymol was applied through drip irrigation lines for preplant soil treatment, and ASM was applied as both a drench to the transplants and a foliar spray. Values in the figure represent means of six replicates. Error bars indicate standard errors of the means. Tomato cultivars: Solar Fire (2004) and FL47 (2005). UTC = untreated control.

rum but not treated with thymol; hence, root galling and root-knot nematode populations in the soil in these plots could not be determined.

When the soil was infested with *R. solanacearum* and *M. arenaria*, thymol-treated plots produced 30.1 tons of marketable fruit per hectare, which was significantly higher than the untreated control, which had 1 ton/ha (Table 2). In addition, integrated use of thymol plus ASM increased tomato yield significantly compared with thymol or ASM. When the soil was infested only with *M. arenaria*, ASM application did not significantly increase tomato yield compared with the untreated control (Table 2).

DISCUSSION

The tomato production system almost universally practiced by growers in Florida is an intensively managed raised-bed system that includes soil fumigation, polyethylene mulch, drip or seepage irrigation, and trellising, which has been in use for over 25 years (33). These data show that the application of thymol through drip irrigation lines for preplant soil fumigation is practically feasible in this tomato production system. Thymol applied through drip lines for soil treatment before transplanting provided significant protection against bacterial wilt in the field experiments conducted in 2004 and 2005. This result was consistent with our previous

findings that thymol provided effective control of bacterial wilt of tomato under greenhouse (36) and field conditions (18). Foliar application of ASM did not reduce bacterial wilt incidence significantly in both years due to high inoculum applied as reported in previous studies (2,34). The integrated use of thymol plus ASM reduced bacterial wilt incidence compared with thymol or ASM, but this reduction was not statistically significant. The field study in 2005 indicated that tomato yield was significantly increased by thymol treatment compared with the untreated control, and application of thymol in conjunction with ASM further increased tomato yield compared with thymol or ASM

Table 1. Efficacy of combined use of thymol and acibenzolar-*S*-methyl (ASM) in control of *Ralstonia solanacearum* and *Meloidogyne arenaria* on tomato (cv. Solar Fire) under field conditions (fall 2004)

Treatment ^x	Bacterial wilt incidence (%) ^y			Root gall severity (%) ^{y,z}			No. of J2 per 100 cm ³ of soil ^{y,z}		
Untreated	97.0 a	97.6 a		ND			ND		
ASM		96.5 a		ND			ND		
Thymol	20.0 b	26.0 a		30.3 a			41.6 b		
Thymol + ASM		15.1 a		17.3 a			92.0 a		
T-grouping according to the model (<i>P</i> = 0.05)									
	Binomial distribution			Binomial distribution			Poisson distribution		
Source of variation	df	<i>F</i>	<i>P</i>	Effects of ASM on thymol plots,					
Thymol (main plots)	1	6.69	0.0490	no subplots					
ASM (subplots)	1	0.08	0.7793	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Interactions (thymol × ASM)	1	0.00	0.9471	1	0.27	0.6233	1	107.96	0.001

^x Thymol was used as preplant soil fumigation and ASM was applied as both a drench to the transplants and a foliar spray. Main plots were thymol versus no thymol applications, and subplots were ASM versus no ASM.

^y Final disease incidence at 66 days after transplanting. Means within a column with the same letter are not significantly different according to the *t* test at $P = 0.05$.

^z Root gall severity (where 0 = no root galling and 100 = 100% of the root system galled) and numbers of root-knot nematode juveniles (J2) were determined at 66 days after transplanting. ND = not determined.

Table 2. Efficacy of combined use of thymol and acibenzolar-*S*-methyl (ASM) in control of *Ralstonia solanacearum* and *Meloidogyne arenaria* on tomato (cv. FL47) under field conditions (fall 2005)

Treatment ^v	Soil infestation	Bacterial wilt incidence (%) ^w			Root gall severity (%) ^{w,x}			No. of J2 per 100 cm ³ of soil ^{w,x}			Marketable yield (ton/ha)		
Untreated control (I)	<i>R. solanacearum</i> and <i>M. arenaria</i>	95.8 a	95.8 a		ND		ND		ND		1.0 c		
ASM (I)	<i>R. solanacearum</i> and <i>M. arenaria</i>		95.8 a		ND		ND		1.1 c				
Thymol	<i>R. solanacearum</i> and <i>M. arenaria</i>	16.2 b	22.6 b		3.0 a		187.2 a		30.1 b				
Thymol + ASM	<i>R. solanacearum</i> and <i>M. arenaria</i>		9.8 b		1.5 a		126.8 b		38.1 a				
Untreated control (II)	<i>M. arenaria</i>	NA		5.2 a		340.8 a		36.0 ab					
ASM (II)	<i>M. arenaria</i>	NA		4.9 a		299.2 a		36.8 ab					
T-grouping according the model used or LSD (<i>P</i> = 0.05) ^y											Normal distribution		
		Binomial distribution			Binomial distribution			Poisson distribution			LSD = 7.9		
Source of variation		df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Thymol (main plots)		1	98.96	0.0006	1	1.69	0.2634	1	4.92	0.0909	1	110.20	<0.0001
ASM (I) and/or ASM II (subplots)		1	1.03	0.3404	1	0.26	0.6251	1	69.25	0.0001	1	1.93	0.18
Interactions (thymol × ASM I or II)		1	1.03	0.3404	1	0.15	0.7064	1	17.25	0.0032	ANOVA sources ^z		

^v Thymol was used as preplant soil fumigation and ASM was applied as both a drench to the transplants and a foliar spray. Main treatment was thymol application (inoculated with *R. solanacearum* and *M. arenaria*) versus no thymol application (inoculated with *R. solanacearum* and *M. arenaria* or *M. arenaria* alone). In untreated control (I), soil was infested with *R. solanacearum* and *M. arenaria*, and in untreated control (II), soil was infested with *M. arenaria* only.

^w Final disease incidence at 83 days after transplanting. Means within a column with the same letter are not significantly different according to the *t* test at $P = 0.05$.

^x Root gall severity (where 0 = no root galling and 100 = 100% of the root system galled) and numbers of root-knot nematode juveniles (J2) were determined at 105 days after transplanting. ND = not determined and NA = not applicable.

^y LSD = least significant difference.

^z ANOVA = analysis of variance.

applied alone. These results suggest that integrated use of thymol plus ASM is beneficial in tomato production. Addition of ASM treatment does not bring any extra cost to the growers, because it is already used to control bacterial spot and tomato spotted wilt in tomato production in Florida (31). With this integrated approach, it appeared that ASM applications could reduce reproduction of nematode populations and increase marketable yield significantly. Due to the high value of the tomato crop, even apparently small differences in tomato yields are important because the crop value can exceed U.S.\$25,000/ha (3).

In addition to bacterial wilt reduction, thymol application also provided a moderate level of root-knot galling suppression; however, this reduction was not significant. Root-knot nematode juvenile populations in thymol-plus-ASM-treated plots were significantly lower than those in the other plots in 2005. The effect of thymol on root-knot nematodes was not evaluated previously under field conditions. However, thymol mixed in sandy soil artificially infested with *M. javanica* reduced root galling of tomato seedlings under growth-chamber conditions (30). These studies also showed that thymol immobilized J2 of *M. javanica* and inhibited hatching in vitro, indicating nematocidal activity of this compound. It is noteworthy that application of thymol in conjunction with ASM resulted in the greatest reduction in root galling and juvenile nematode populations. Unfortunately, the effect of thymol, and thymol plus ASM, on root-knot was assessed only in the 2005 experiment. In 2004, root galling in the plots inoculated with *R. solanacearum* but not treated with thymol was not determined because most of the plants were dead in these treatments.

We determined that the use of thymol and ASM provided significant reduction of bacterial wilt incidence and root-knot nematode juvenile populations, respectively, in soil on commercially acceptable, bacterial wilt- and nematode-susceptible tomato cultivars. Both ASM and thymol are regarded as reduced-risk biorational products. ASM acts as a functional analog of salicylic acid in the systemic acquired resistance signaling pathway (23,32) and was reported to induce defense reactions in tomato plants such as the accumulation of pathogenesis-related proteins and ultrastructural changes (4,16). It is commonly used in tomato production in Florida for management of bacterial spot and speck (31). Thymol is a known antibacterial agent produced by thyme (*Thymus* spp.) or other medicinal plants and has been widely used in medical and food sciences (1,24,39,40). As a plant-derived compound, it is generally recognized as safe by the U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) and was exempted from the

requirement of a tolerance by EPA for application on edible agricultural products (40 CFR Part 180, June 6, 2003). Although pure thymol (99% purity) is not economical to use for tomato disease control, it is estimated that agricultural-grade thymol can be produced at a cost less than U.S.\$14/kg (18), which makes application of thymol economically feasible in commercial tomato production.

Development of alternatives to methyl bromide for managing soilborne diseases has been the subject of increasing interest (25). The activities of thymol against *R. solanacearum* and of ASM against *M. arenaria* as shown in this study afford promise for developing an integrated approach for the management of various tomato diseases. Although application of thymol through a drip irrigation system appeared to be a practically and economically feasible measure for control of plant diseases such as bacterial wilt, further studies are needed for a more comprehensive evaluation of the role of thymol as a biofumigant in the production of tomato. For instance, the dosage of application in the field needs to be optimized to achieve maximum disease control efficacy and economic benefit. Studies in *R. solanacearum* and *M. arenaria* naturally infested fields can be performed and the effect of thymol application on soil population of *R. solanacearum* can be determined. In this study, the pathogens were artificially applied to 15-cm-deep holes in the field. In naturally infested fields, they may be present at different soil depths and it would be interesting to determine how deep in the soil the effect of thymol can reach. Previous studies indicated that ASM provided significant protection against bacterial wilt on a susceptible tomato cultivar when relatively lower concentrations of *R. solanacearum* were used (2). It is expected that *R. solanacearum* populations in some natural soils are not as high as those used in this study. Hence, further studies to evaluate the efficacy of ASM in controlling bacterial wilt under natural field conditions are warranted. In addition, the efficacy of thymol for control of other soilborne plant pathogens under field conditions remains to be evaluated.

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