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Ekaterina A. Jeliazkova
University of Wyoming, ekaterinaj.pubs@gmail.com

Clarissa Balbalian
Mississippi State University

Tess Astatkie
Nova Scotia Agricultural College

Pamela Collins
Mississippi State University

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Evaluating Natural Products for Control of Black Spot Disease on Roses

Ekaterina A. Jeliazkova[1]*, Clarissa Balbalian[2], Tess Astatkie[3], and Pamela Collins[1]

[1]Plant and Soil Sciences, Mississippi State University, Mississippi State, MS, 39762
[2]Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS, 39762
[3]Engineering, Nova Scotia Agricultural College, Truro, NS, B2N 5E3, Canada

***Corresponding author:** ekaterinaj.pubs@gmail.com. Present address: University of Wyoming, Sheridan Research and Extension Center, 663 Wyarno Road, Sheridan, WY 82801

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Abstract

Black spot caused by the fungus *Diplocarpon rosea* Wolf. is the most widespread disease in ornamental roses. Frequent applications of environmentally non-benign chemical fungicides are necessary to effectively prevent and control this disease. Ecological and environmental concerns about frequent fungicide applications are growing, and public demand for safer alternatives to fungicides is increasing. This study evaluated water extracts and essential oils from holy basil (*Ocimum gratissimum* L.), sweet basil (*Ocimum basilicum* L.), English thyme (*Thymus vulgaris* L.), and 'Scotch' spearmint (*Mentha* x *gracilis* Sole) for suppression and control of black spot disease in roses as compared with a commonly used fungicide. The results revealed that weekly treatments with essential oils of 'Scotch' spearmint and English thyme produced significantly lower black spot rating as compared with a water control, and the fungicide treatment had lower black spot rating than all treatments, except the 'Scotch' spearmint essential oil and the English thyme essential oil. Our results demonstrate that essential oils of English thyme and 'Scotch' spearmint hold promise as biopesticides for control of black spot on roses as an alternative to chemical fungicides. The use of essential oils for suppression or control of black spot on roses would benefit environmental health, and minimize human exposure to pesticides in general.

Introduction

Black spot (*Diplocarpon rosea* Wolf.) disease is economically the most important and devastating disease in ornamental roses (Horst and Cloyd, 2007), especially in hot and humid climates. Disease outbreaks at the beginning of the growing season are initiated by rain-splashed pathogen spores overwintered on fallen leaves. Infected leaves develop characteristic dark spots, chlorosis, and drop prematurely. When left untreated, the disease can lead to reduced plant vigor, fewer blossoms, compromised aesthetics, and eventual failure of the plant (Henn, 2010). Frequent applications of synthetic fungicides are necessary to effectively prevent and control this disease.

Our 4-year observations at the Mississippi State University Veterans Memorial Rose Garden as well as observations of producers in Mississippi showed that to control black spot, ornamental roses need to be sprayed every week with commercial fungicides. In 2009 for example, 36 fungicide applications (from March to December) were required for the prevention and control of black spot at the Veterans Memorial Rose Garden. Such a rate of pesticide applications in frequently visited public gardens brings not only ecological and environmental problems, but also public health concerns. Among these concerns are possible exposure of the general public to pesticide residues, producer/worker safety, reentry restrictions, residue buildup, as well as development of fungicide-resistant strains of the pathogen. The re-entry interval for the fungicides used to control black spot at the MSU Veterans Memorial Rose Garden is 24 h, which creates disruption of public access and other activities in the garden. Ecological and environmental concerns about frequent fungicide applications are growing and public demand for safer alternatives to chemical fungicides is increasing. Certain plant extracts and natural products have been demonstrated to successfully control fungal diseases in crops. Colpas et al. (2009) reported that basil extract suppressed anthracnose disease (*Colletotrichum lagenarium* Pass.) in cucumber. In a field study with ornamental roses 'Kardinal' conducted by Wagner and Spasowka (2007) applications of English thyme essential oil reduced black spot severity by up to 21%. In an experiment by Gachomo and Kotchoni (2008), treatment of roses with extract from drought-stressed rose leaves, enhanced the disease resistance of the plants. Such studies are missing for the Mississippi climate.

The objective of this study was to evaluate water extracts and essential oils from several plant species for suppression and control of black spot disease in roses and compare the effect against a commonly used fungicide treatment.

Materials and Methods

Plant material and experimental design. A total of 88 bareroot *Rosa hybrida* 'Red Queen' (Kortocrea, Marlena) grafted on 'Dr. Huey' rootstock were planted individually into #3 nursery containers (11 L) on April 23, 2010. Growing media was Sunshine mix #1 (Sun Gro Horticulture, Bellevue, WA) and consisted of 70% to 80% Canadian sphagnum peat moss, 20% to 30% perlite, dolomitic limestone, gypsum and wetting agent. To overcome transplanting shock, roses were maintained for 14 days in a greenhouse (25±3 °C day/18 ±3 °C night) and afterward all roses were grown outside in pots placed on a cement pad at the Rodney Foil Plant Sciences Research Center, Mississippi Agricultural and Forestry Experiment Station in Starkville, MS. Drip irrigation was provided daily by a pressure compensated dribble ring (15 cm diameter, Dramm Corporation, WI) placed in each container. A controlled-release fertilizer 15N-9P-12K (Osmocote, The Scotts Co., Marysville, Ohio) was applied on June 1, 2010 at the rate of 23 g/container as per label recommendations. The experiment was a randomized complete block design with four blocks. Treatments included: (i) Plant extracts at 4 levels and essential oils at 4 levels from the following plant species: holy basil (*Ocimum gratissimum* L.), sweet basil (*Ocimum basilicum* L.), English thyme (*Thymus vulgaris* L.), and 'Scotch' spearmint (*Mentha* x *gracilis* Sole); (ii) fungicide treatment with fungicides used at the MSU Veterans Memorial Rose Garden Pentathlon*LF (a.i. ethylenebisdithiocarbamate ion), a contact fungicide used with every spray application; Banner Maxx® (a.i. propiconazole), a systemic fungicide; and Compass™ (a.i. Trifloxystrobin), a broad-spectrum fungicide, Banner Maxx® alternated with Compass™ every other spray application; and (iii) a water spray control.

Extract preparation. Arrangements were made with the Medicinal Plants Program at the North Mississippi Research and Extensions Center at Verona to obtain certified plant material from several selected essential oil plant species, from which plant extracts were prepared and used in this study. Aqueous extracts of holy basil, sweet basil, English thyme, and 'Scotch' spearmint were prepared from 5 g of dry leaves following a previously described method (Colpas et al., 2009). Leaves were ground in 100 mL distilled water in a blender for 1 min, the homogenate was filtered subsequently through a pre-wetted double-layered cheesecloth. Also, 1% aqueous solutions were prepared using essential oils from holy basil, sweet basil, English thyme, and 'Scotch' spearmint to which 20% ethanol was added as a solvent, because essential oils are not very soluble in aqueous solution. The aqueous solutions were stored at 4 °C for the duration of the experiment. The essential oils in this study were obtained from the Medicinal Plants Program at the North Mississippi Research and Extension Center at Verona, MS and were distilled from the same certified plant material of English thyme, holy basil, 'Scotch' spearmint, and sweet basil. These crops were grown in the same field and under the same growth conditions in Mississippi.

Inoculum production. About 500 black spot infected leaflets with evident pycnidia were collected on June 7, 2010 from a variety of roses on the perimeter of the Veterans Memorial Rose garden at Mississippi State University. The leaves were placed in covered plastic boxes, misted with sterile distilled water and incubated at 4 °C for 15 h. The rose leaves were then removed from the plastic boxes and evenly divided among 4 sterile glass beakers, each filled with approximately 1.25 L of sterile distilled water, and incubated at 4 °C for 11 hours. From the resulting spore solution, 800 mL aliquots were decanted into 946 mL plastic spray bottles (Delta Industries, Ft. Wayne, IN), each of which contained 165 mL of the surfactant in the form of 0.05% Difco agar.

Plant inoculation. All roses were inoculated by misting both the adaxial and abaxial leaf surfaces with the prepared spore solution just before runoff occurred. The temperature at inoculation was approximately 30 °C. A plate of malt extract agar was sprayed with each spray bottle of spore solution used in the inoculation process. Each agar plate was sealed with parafilm and incubated at 30 °C and successful spore germination was observed on each plate.

Treatment application and data collection. Treatment solutions were applied once a week for five weeks on June 17, June 24, July 1, July 8, and July 15 2010. Max/min air temperature on the days of treatments application was 33.3 °C /24.4 °C, 33.8 °C /23.8 °C, 30.5 °C /23.3 °C, 35 °C /23.8 °C, 36.6 °C /25 °C, respectively. Treatment solutions were applied between 9:00 AM and 11:00 AM, using 946 mL plastic spray bottles (Delta Industries, Ft. Wayne, IN). Data were collected once a week on June 16, June 23, June 30, July 7, July 14, and July 20 2010. The following data were collected weekly: (1) visual rating of entire plant for black spot development using the 1-12 rating scale of Horsfall and Barratt (1945); (2) black spot lesion number per leaflet on 6 randomly selected and fully expanded terminal leaflets per plant; (3) percentage of infected leaflets by counting the number of leaflets infected with black spot on 20 randomly selected axillary leaflets per plant. The data were simultaneously collected by two individuals and their readings were averaged.

Statistical analysis. Black spot rating and number of lesions responses, collected weekly for six weeks, were analyzed as a randomized complete blocks design with four blocks and ten treatments. Repeated measures analysis was completed using the Mixed Procedure of SAS (SAS Institute, Inc. 2008), and further multiple means comparison was completed for significant (p-value < 0.05) and marginally significant (p-value between 0.05 and 0.1) effects by comparing the least squares means of the corresponding treatment combinations using the LSmeans statement of Proc Mixed with PDIFF option to produce p-values for all pair wise differences. Letter groupings were generated using a 5% level of significance to compare the levels Week which was a significant main effect; where-as a 1% level of significance was used to compare the treatment combinations from the marginally significant Treatment by Week interaction effect to protect Type I experiment-wise error rate from over inflation. Since the total number of combinations of Treatment and Week is very large, separate letter groupings were done within each week, and within each treatment. For each response, the validity of model assumptions on the error terms was verified by examining the residuals as described in Montgomery (2009). While normality assumption on Black spot rating response was satisfied with the original values, the assumption for number of lesions response was satisfied with a square root transformation. The mean values reported are back-transformed to facilitate easy reading.

Results

Statistical analysis indicated that Treatment and Week had a marginally significant interaction effect on the black spot rating while only the main effect of Week was significant on the number of lesions (Table 1). The number of lesions was highest in weeks 3 and 4, and then declined in weeks 5 and 6 (Table 2). The lowest number of lesions were observed in weeks 1 and 2 (Table 2). None of the treatments affected the percentage of infected leaflets (20 randomly selected axillary leaflets per plant, data not

Table 1. Main and interaction effect of treatment and week.

Source of variation	Black spot rating	Number of lesions
	(ANOVA p-values)	
Block	0.077	0.006
Treatment	0.005	0.105
Week	0.001	**0.001**[1]
Treatment*Week	**0.089**[1]	0.422

[1] Effects that require further multiple means comparison.

Table 2. Lesions formed during data collection period.

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Fungicide	1.50 BC[1]	1.25 C	2.56 B b[2]	4.00 A ab	3.75 A b	2.44 B d
Holy basil extract	1.81 CD	1.31 D	2.63 BC b	3.50 AB ab	4.00 A ab	3.56 AB bc
Holy basil oil	1.94 B	1.69 B	3.94 A a	4.38 A ab	4.44 A ab	4.19 A abc
Scotch spearmint ext	1.69 B	1.31 B	3.63 A ab	4.19 A ab	4.56 A ab	4.06 A abc
Scotch spearmint oil	1.56 B	1.25 B	2.88 A b	3.38 A b	3.81 A b	3.19 A cd
Sweet basil extract	1.88 B	1.94 B	3.13 A ab	3.63 A ab	4.19 A ab	3.75 A abc
Sweet basil oil	1.50 D	1.44 D	2.81 C b	4.25 AB ab	4.88 A a	3.63 BC abc
English thyme extract	1.75 C	1.75 C	3.50 B ab	4.31 AB ab	4.75 A ab	4.50 AB ab
English thyme oil	1.56 B	1.63 B	3.19 A ab	3.81A ab	4.13 A ab	3.31 A cd
Water	1.31 C	1.31 C	3.44 B ab	4.56 A a	4.63 A ab	4.63 A a
Sliced by week P	0.913	0.726	0.016	0.062	0.079	0.001

[1]Within each treatment, means sharing the same upper case letter are not significantly different.
[2]Within each week, means sharing the same lower case letter are not significantly different.

shown), indicating that under the hot humid conditions of Mississippi, black spot infestation rating of the whole plant would be a more reliable response than the percent of the infected leaflets.

Further analysis of the interaction effect on black spot rating showed that none of the treatments affected black spot ratings in weeks 1 and 2 (Table 3). However, in week 3, while neither of the treatments was different from the water control, the fungicide treatment and holy basil extract had lower black spot rating than the holy basil essential oil. In week 4, ‘Scotch’ spearmint essential oil was lower than the water control in terms of black spot rating. The fungicide treatment in week 4 was not different from any of the other treatments. In week 5, neither of the treatments was different from the water control, however, the fungicide treatment and the ‘Scotch’ spearmint essential oil had lower incidence of black spot than the sweet basil essential oil treatment. In week 6, the fungicide, the holy basil extract, the ‘Scotch’ spearmint essential oil, and the English thyme essential oil treatments had significantly lower black spot rating compared to the water control, and also the fungicide treatment was lower than all treatments except the ‘Scotch’ spearmint essential oil and the English thyme essential oil.

Table 3. Weekly black spot rating for each treatment.

Week	Number of lesions
1 (Jun-16-10)	0.049 c[1]
2 (Jun-23-10)	0.049 c
3 (Jun-30-10)	0.538 a
4 (Jul-7-10)	0.697 a
5 (Jul-14-10)	0.299 b
6 (Jul-20-10)	0.376 b

[1]Means sharing the same letter are not significantly different at $P \leq 0.05$.

Discussion

The results suggest that ‘Scotch’ spearmint essential oil and the English thyme essential oil have an ability, similar to the fungicide treatment, to suppress black spot disease on roses. Further concentration gradient and time frequency research with ‘Scotch’ spearmint essential oil and English thyme essential oil may be needed to establish the minimum inhibitory concentrations of these two essential oils against the organism causing black spot disease on roses. The treatments were not rated for phytotoxicity, but during the course of the experiment, brown or necrotic spots were observed on some upper leaf surfaces of plants across all treatments. We do not know whether this response was due to the treatments alone, the air temperature alone, or to a combination of both. Results from this study are consistent with previous research in showing that thyme essential oil has a potential to control black spot in roses (Wagner and Spasowka, 2007). Previous reports have shown that spearmint essential oil possesses antifungal properties (Soliman and Badeaa, 2002). These oils have wide range of bioactivity. Antifungal activities of essential oils from *Thymus* and *Mentha* species have been reported in other studies as well (Chao et al., 2000; Sokovic et al., 2009).

'Scotch' spearmint is grown as essential oil crop in the Midwest and northwestern United States (Lawrence, 2007; MIRC, 2009). The US yearly production of 'Scotch' spearmint essential oil is over 1.0 million kg (NASS, 2009), making the oil easily available. Recently, Zheljazkov et al. (2010) demonstrated that spearmints could be viable essential oil crops in the southeastern United States as well. In addition, basil, spearmint, and English thyme are popular garden herbs in the Southeastern US. Our results show that English thyme essential oil and 'Scotch' spearmint essential oil show promise as biopesticides for control of black spot on roses as an alternative to chemical fungicides. The use of a natural product for suppression or control of black spot on roses would benefit environmental health, and minimize human exposure to pesticides in general.

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