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Chemical Control of Septoria Blight of Parsley Caused by *Septoria petroselini*

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Abstract: The effects of various fungicides on spore germination, mycelial growth, seed transmission, and infection by *Septoria petroselini* Desm., the causal agent of Septoria blight of parsley, were determined in this study. Eleven fungicides (azoxystrobin, benomyl, captan, copper oxychloride, kresoxim-methyl mancozeb, maneb, propineb, tebuconazole, thiram, and trifloxystrobin) were tested at various concentrations ranging from 0.025 to 50 mg l^{-1} in spore germination and mycelial growth assays. Tebuconazole was the most effective fungicide in all the experiments, with an EC_{50} value < 0.052 mg l^{-1}, followed by azoxystrobin, captan, and trifloxystrobin, with EC_{50} values of 0.053, 0.06, and 0.066 mg l^{-1}, respectively. Many of the fungicides were effective on spore germination in PDA medium at concentrations ranging from 0.052 mg l^{-1} (Tebuconazole) to 23.032 mg l^{-1} (copper oxychloride). Captan, mancozeb, maneb, and thiram were very effective at reducing spore germination, but were less effective at reducing mycelial growth, for which their EC_{50} values were up to 735-, 192-, 192-, and 191-fold higher, respectively. Seed transmission of Septoria blight of parsley was controlled by tebuconazole, benomyl, azoxystrobin, kresoxim-methyl, and captan, which had inhibition rates of up to 95%, 93%, 93%, and 66%, respectively. Azoxystrobin, benomyl, kresoxim-methyl, trifloxystrobin, and tebuconazole inhibited Septoria blight in vivo, but captan, mancozeb, and maneb were effective at reducing the number of lesions only when they were applied before inoculation. Copper oxychloride, the most extensively used fungicide for controlling Septoria blight by parsley growers, was surprisingly the least effective in all the experiments.

Key Words: Septoria blight, *Septoria petroselini*, parsley, fungicide

Septoria petroselini'nin Maydanozda Neden Olduğu Septoria Yanıklık Hastalığının Kimyasal Mücadelesi

Özet: Bu çalışmada, farklı fungisitlerin maydanozda Septoria yanıklık hastalığı etmeni olan *Septoria petroselini*'nin spor çimlenmesi, miselyal gelişimi, tohumla taşınımı ve enfeksiyonu üzerine etkileri araştırılmıştır. Spor çimlenmesi ve miselyal gelişme denemelerinde 0,025-50 mg l^{-1} arasında değişen dozlarda 11 farklı fungisit (azoxystrobin, benomyl, captan, copper oxychloride, kresoxim – methyl, mancozeb, maneb, propineb, tebuconazole, thiram and trifloxystrobin) kullanılmıştır. Tüm denemelerde en başarılı fungisit olarak bulunan Tebuconazole, spor çimlenmesi denemelerinde 0,052 mg l^{-1}'den daha düşük bir EC_{50} değerinde etkili olmuş, Tebuconazole'den sonra sırasıyla 0,053 EC_{50} değeriyle azoxystrobin, 0,06 ile captan ve 0,066 ile trifloxystrobin etkinlik göstermiştir. PDA ortamında spor çimlenmesi denemesinde kullanılan fungisitlerin pek çoğu 0,052-23,032 EC_{50} değerleri arasında etkili oldukları tespit edilmiştir. Captan, mancozeb, maneb ve thiram'ın spor çimlenmesi üzerinde etkinliklerinin çok yüksek olduğu tespit edilmiştir. Ancak, miselyal gelişimi engelledikleri EC_{50} değerleri spor çimlenmesindeki değerlerinde sırasıyla 735, 192, 192 ve 191kez fazla bulunmuştur. Maydanozda Septoria yanıklığının tohumla taşınımı yaklaşık olarak tebuconazole ile % 95, benomyl ile % 93, azoxstrobin ve kresoxy-methyl ile % 93 ve captan ile % 66 oranında engellenmiştir. Azoxystrobin, benomyl, kresoxim-methyl, trifloxystrobin ve tebuconazole'ün saksı denemelerinde Septoria yanıklığını üzerinde etkili oldukları tespit edilmiştir. Fakat mancozeb ve maneb sadece inokulasyondan once uygulandıklarında etkinlik göstermişlerdir. Akdeniz bölgesinde maydanozda Septoria yanıklığına karşı en çok kullanılan fungisitlerden biri olan Bakır oksiklorür, şaşırtıcı bir şekilde tüm denemelerde başarısız bulunmuştur.

Anahtar Sözcükler: Septoria yanıklığı, *Septoria petroselini*, maydanoz, fungisit

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Introduction

Parsley (*Petroselinum crispum* (Mill.) Nym. ex A.W.Hill) is one of the most extensively grown vegetables in southern Turkey (Tok and Kurt, 2004). Parsley is infected by many fungi, including *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Fusarium* spp., *Pythium* spp., *Alternaria radicina*, *Cercospora* spp., and *Septoria petroselini* Desm (Raid and Roberts, 2004). Septoria blight, caused by *S. petroselini*, is the most important and destructive disease of parsley. The disease has been reported in Canada (Cerkauskas and Uyenaka, 1990), Iraq (Mohammad, 1990), Germany (Scholze et al., 1996), and Turkey (Kurt, 2003). Recently, severe epidemics of Septoria blight have become a problem, particularly in the Mediterranean region of Turkey, causing yield losses of up to 70% (Kurt and Tok, 2006). Septoria blight of parsley is characterized by brown necrotic lesions (3-8 mm in diameter, on average) with dark-brown pycnidia on the foliage. Small oval lesions are observed on petioles in favorable weather conditions (Kurt, 2003). Septoria conidia produced within pycnidia can be extruded in long tendrils (Smith et al., 1988). These conidia are surrounded by a mucilaginous matrix composed of proteins and sugar, which swell when the mucilage absorbs free water or is exposed to relative humidity ≥ 90% (Lacy et al., 1996). Septoria blight is transmitted by seeds, plant debris, or adjacent plants and is disseminated by splash droplets from rainfall or overhead irrigation (Fournet, 1969; Sherf and MacNab, 1986; Smith et al., 1988; Fitt et al., 1989). *Septoria* may survive for up to 2 years on infected seeds. Plant seeds that have been certified as being free of *Septoria* should be stored as if suspected of being infected for a period of 2 or more years. This reduces the viability of the pycnidia, rendering the pathogen incapable of infecting. Avoiding the use of overhead irrigation is a must, as the pathogen is disseminated by rain splash. Additionally, if an outbreak has been detected, movement of equipment or workers through the field while the canopy is wet should be minimized in order to control the spread of Septoria blight of parsley (Raid and Roberts, 2007). The critical feature of disease epidemics worldwide is the transmission of *Septoria petroselini* by infected seeds in broad areas; therefore, the disease is controlled by using uninfected or treated seeds, and by using fungicide sprays on foliage in many countries (Smith et al., 1988). In the USA, the strobilurin group of fungicides is recommended for chemical control of Septoria, Cercospora, and Alternaria blights, as well as powdery mildew of parsley (Raid and Roberts, 2007). Many parsley growers historically have applied fungicides for controlling Septoria blight of parsley, but currently no fungicide is registered for Septoria blight on parsley in Turkey. We have observed that parsley growers in the Hatay region of Turkey have been using copper oxychloride, captan, maneb, and mancozeb for controlling Septoria blight of parsley. Furthermore, the majority of the growers use fungicides for controlling Septoria blight of parsley without knowing if they are effective, which has negative economic and environmental consequences. In addition, the effectiveness of unregistered fungicides for the management of Septoria disease of parsley in Turkey remains unknown. The objectives of the present study were to screen some fungicides for their effects on spore germination and mycelial growth of *S. petroselini*, and to test the effectiveness of available fungicides against the disease as seed and foliar treatments.

Materials and Methods

Fungicides

Eleven fungicides were used in all the experiments. The tested fungicides included azoxystrobin (Quadris 250 g l^{-1}, Syngenta, USA), benomyl (Safomyl 50 WP, Safa Tarım, Turkey), captan (Safa Captan 50 WP, Safa Tarım, Turkey), copper oxychloride (Cupravit Ob 21, Bayer Turk, Bayer), kresoxim-methyl (Candit WG, Basf, Germany), mancozeb (Sakozeb Blue, Safa Tarım, Turkey), maneb (Saneb M 22, Safa Tarım, Turkey), propineb (Antracol WP 70, Bayer Turk, Bayer), tebuconazole (Folicur WP 25, Bayer Turk, Bayer), thiram (Pomarsol Forte 80 WP, Bayer Turk, Bayer), and trifloxystrobin (Flint WG 50, Bayer Turk, Bayer) (Table 1). Fungicides used for in vitro experiments were technical grade, but some used for in vivo experiments were at the label rate for some vegetables. Stock solutions were prepared for each fungicide in sterile distilled water, and aliquots of stock solutions were incorporated into PDA (potato dextrose agar) medium at 45-50 °C to provide concentrations of 0.025-50 mg l^{-1} in spore germination and mycelial growth experiments.

Table 1. Fungicides used in the experiments.

Group	Active Ingredient	Fungicide	Company
Benzimidazole	Benomyl	Safomyl 50 WP	Safa Tarım
Copper	Copper oxychloride	Cupravit Ob 21	Bayer Turk
Dithiocarbamate	Maneb	Saneb M 22	Safa Tarım
	Mancozeb	Sakozeb Blue	Safa Tarım
	Propineb	Antracol WP 70	Bayer Turk
	Thiram	Pomarsol Forte 80	Bayer Turk
Triazole	Tebuconazole	Folicur WP 25	Bayer Turk
Trihalomethylthio	Captan	Safa Captan 50 WP	Safa Tarım
Strobilurin	Azoxystrobin	Quadris 250 g l^{-1}	Syngenta
	Kresoxim-methyl	Candit WG	BASF
	Trifloxystrobin	Flint WG 50	Bayer Turk

Effects of Fungicides on Spore Germination and Mycelial Growth

Spore suspensions at a concentration of 10^4 spores ml^{-1} were prepared in sterilized distilled water from *S. petroselini* cultures grown on PDA medium for 10 days. Then, 100 µl of spore suspension was dropped on water agar medium containing fungicides in 9.0-cm diameter petri dishes and were spread onto the medium surface with a Drigalski spatula. After 24-h incubation at 21 °C, the germination rate of approximately 100 *S. petroselini* spores was evaluated per treatment under a stereoscopic microscope. Three petri dishes were used for each treatment and all tests were repeated at least twice. EC_{50} and minimum inhibition concentration (MIC) values were calculated from the regression equation of each treatment by applying the value of 50% of the mean number of geminated control spores to the formula.

In the mycelial growth experiments, mycelial disks (4 mm in diameter) of *S. petroselini* cultures were transferred to PDA medium amended with fungicides at concentrations of 0.025-50 mg l^{-1}. Petri dishes were incubated at 21 °C for 2 weeks in dark conditions. After 2 weeks of incubation, the diameter of each colony was measured and the mean diameter of 3 colonies was recorded. Mean colony diameters were used to calculate growth rate inhibition for each fungicide, relative to the control plates. Three petri dishes were used for each treatment and all tests were repeated at least twice. EC_{50} and MIC values were calculated from the regression equation of each treatment by applying the value of 50% of the mean diameter of control mycelial growth to the formula.

Effects of Fungicides on Infected Seeds

Fungicide treatment of infected seeds and seed inoculation were carried out according to Vesilescu et al. (2004). Seeds were disinfected by soaking them in 1% sodium hypochlorite solution for 5 min and rinsing with sterile distilled water. Then, the seeds were soaked in a spore suspension of 10^6 conidia ml^{-1} of *S. petroselini* for 1 h, filtered by a cheese-cloth, and then incubated in petri dishes containing water agar medium (2%) for 5-6 days. Coat-infected seeds containing pycnidia of *S. petroselini* were transferred to sterile filter paper and dried at room temperature for 2 days. Following all these processes, coat-infected seeds were treated with the fungicides. One hundred seeds were used for each treatment and all fungicide-treated seeds were sown in plastic pots containing a mixture of soil, sand, and peat in a 1:1:1 (v:v:v) ratio. Seeds treated only with sterile water were sown in 1 pot and labeled as the control treatment. After 15 days of incubation at 21 °C, emerged seedlings were examined under a stereomicroscope for infection by *S. petroselini*. Infected seedlings were counted and the rate of infection calculated for each treatment. Infection rates were compared by Duncan's multiple range test.

In Vivo Effects of Fungicides on Septoria Blight

Parsley seeds were sown in plastic pots containing a sterilized mixture of soil, sand, and peat (1:1:1, v:v:v). Plants were grown in a controlled environment chamber at 21 °C for 4-5 weeks. Parsley plants were inoculated by spraying a spore suspension (10^6 conidia ml^{-1}) of *S. petroselini* from a spray bottle. One group including 3 pots was treated with all fungicides 2 days before inoculation (pre-inoculation), whereas the other group was treated with all fungicides 2 days after inoculation (post-inoculation). Plants treated with sterile distilled water served as the control. Fifteen days after inoculation the number of lesions per plant for each treatment was counted and the mean numbers of lesions for each plant were compared by Duncan's multiple range test.

Data Analysis

Regression analyses and Duncan's multiple range tests were performed to determine EC_{50} values, and for comparing seed transmissions and in vivo effects, respectively, using SPSS v.11.5 for Windows (SPSS Inc., LEAD Technologies, Chicago, IL, USA).

Results

Effects of Fungicides on Spore Germination and Mycelial Growth

Eleven fungicides (azoxystrobin, benomyl, captan, copper oxychloride, kresoxim-methyl, mancozeb, maneb, propineb, tebuconazole, thiram, and trifloxystrobin) were used at various concentrations. Comparing EC_{50} values, tebuconazole was the most effective in reducing spore germination, with an EC_{50} value of 0.052 mg l^{-1} in water agar medium, followed by azoxystrobin, captan, and trifloxystrobin, with EC_{50} values of 0.053 mg l^{-1}, 0.06 mg l^{-1}, and 0.066 mg l^{-1}, respectively. Most of the fungicides had a remarkable effect on reducing spore germination, but copper oxychloride (EC_{50} value: 23.032 mg l^{-1}) did not. The EC_{50} values of all the fungicides ranged between 0.052 mg l^{-1} and 50 mg l^{-1} (Table 2). MIC values were similar to EC_{50} values. All the fungicide MIC values ranged from 0.1 to 50 mg l^{-1}.

In the mycelial growth experiments, EC_{50} values of the fungicides ranged from 0.047 to 45.15 mg l^{-1}. According to the results of the mycelial growth tests, the most effective fungicide was benomyl, with an EC_{50} of 0.047

Table 2. MIC and EC_{50} values of the fungicides against *S. petroselini* mycelial growth and spore germination on PDA medium.

Fungicides	Spore Germination[1]		Mycelial Growth	
	EC_{50}	MIC	EC_{50}	MIC
Azoxystrobin	0.053	> 0.1 < 1.13	0.65	> 1 < 1.5
Benomyl	0.084	> 0.13 < 0.16	0.047	> 0.05 < 0.1
Captan	0.06	> 0.13 < 0.16	44.15	> 50
Copper oxychloride	23.032	> 25 < 50	> 50	> 50
Kresoxim-methyl	0.09	> 0.13 < 0.16	0.05	> 0.05 < 0.1
Mancozeb	0.1	> 0.16 < 2	19.19	> 25 < 50
Maneb	0.1	> 0.16 < 2	19.19	> 25 < 50
Propineb	8.46	> 10 < 25	27.03	> 50
Tebuconazole	0.052	> 0.1 < 1.13	0.63	> 1 < 1.5
Thiram	0.07	> 0.13 < 0.16	45.15	> 50
Trifloxystrobin	0.066	> 0.13 < 0.16	0.71	> 1 < 1.5

[1] Mean effect on spore germination and mycelial growth were calculated from at least 3 petri dishes for each fungicide.

mg l^{-1}, followed by tebuconazole, azoxystrobin, and trifloxystrobin, with EC_{50} values of 0.63 mg l^{-1}, 0.65 mg l^{-1}, and 0.71 mg l^{-1}, respectively (Table 2). Captan, mancozeb, maneb, and thiram were effective at reducing *S. petroselini* spore germination on water agar medium, but were not effective at reducing mycelial growth. Mancozeb, maneb, captan, thiram, and propineb had high MIC values (up to 50 mg l^{-1}). All fungicides tested had a higher MIC value for mycelial growth than for spore germination.

Effects of Fungicides on Infected Seeds

Tebuconazole inhibited disease transmission to seedlings by more than 95%, with an infection rate of 4.66%, followed by azoxystrobin and benomyl, both of which inhibited transmission to seedlings by 93%, and then thiram, captan, and propineb. Mancozeb and maneb significantly suppressed spore germination, but not seed transmission (Table 3). None of the tested fungicides reduced seed germination significantly, as compared to the negative control (non-infected seeds that were not treated with fungicides).

In Vivo Effects of Fungicides on Septoria Blight

Applied both before and after inoculation, tebuconazole was the most effective fungicide for inhibiting the number of lesions (100%), as compared to the control. Tebuconazole, benomyl, azoxystrobin, and kresoxim-methyl were effective when applied both before and after inoculation, while mancozeb was more effective when applied before inoculation. Captan and thiram were also effective at reducing the number of lesions when applied post-inoculation. In fact, captan, mancozeb, maneb, and thiram were effective on spore germination, but had no effect on mycelial growth. Copper oxychloride was surprisingly the least effective fungicide, with an inhibition rate of 3.52% (Tables 4 and 5).

Discussion

The present study's data show that most of the tested fungicides were effective on *S. petroselini* spore germination on PDA medium. On the other hand, some fungicides were effective against spore germination, but not mycelial growth. Additionally, captan, thiram,

Table 3. Effectiveness of the fungicides on seeds artificially infected with *S. petroselini*.[1]

Fungicides	Dose rate	Infection (%)	Inhibition (%)
Tebuconazole	0.5 g kg^{-1}	4.66 ± 0.33 a[2]	95.34
Benomyl	0.4 g kg^{-1}	6.66 ± 0.88 a	93.34
Azoxystrobin	0.4 ml kg^{-1}	7.33 ± 0.80 a	92.67
Kresoxim-methyl	0.4 g kg^{-1}	7.33 ± 0.80 a	92.67
Thiram	3 g kg^{-1}	33 ± 1.80 b	67
Captan	3 g kg^{-1}	33.66 ± 1.85 b	66.34
Propineb	2 g kg^{-1}	46.33 ± 1.85 c	53.67
Trifloxystrobin	0.15 g kg^{-1}	53.00 ± 1.52 d	47
Mancozeb	2.5 g kg^{-1}	64.00 ± 2.08 e	36
Maneb	2.5 g kg^{-1}	65.00 ± 2.00 e	35
Copper oxychloride	4 g kg^{-1}	90.66 ± 1.20 f	9.34
Control	-	100 ± 0.33 g	0

[1] Mean effect on seed transmission was calculated from 100 seeds and 3 replicates for each fungicide.

[2] Values with different letters are significantly different (P = 0.05) according to Duncan's multiple range test.

Table 4. In vivo effects of the fungicides applied pre-inoculation on Septoria blight.

Fungicides	Dose rate	Number of lesions plant^{-1}	Inhibition (%)
Tebuconazole	50 g 100 l^{-1}	0 ± 0.00 a[1]	100
Benomyl	40 g 100 l^{-1}	0.66 ± 0.33 ab	96.52
Azoxystrobin	40 ml 100 l^{-1}	0.7 ± 0.33 ab	96.31
Kresoxim-methyl	40 ml 100 l^{-1}	0.70 ± 0.33 ab	96.31
Trifloxystrobin	15 g 100 l^{-1}	1.66 ± 0.33 b	91.26
Captan	300 g 100 l^{-1}	4.33 ± 0.33 c	77.21
Thiram	300 g 100 l^{-1}	4.33 ± 0.33 c	77.21
Mancozeb	250 g 100 l^{-1}	4.66 ± 0.66 c	75.47
Maneb	250 g 100 l^{-1}	4.88 ± 0.66 c	74.31
Propineb	200 g 100 l^{-1}	10.33 ± 0.33 d	45.63
Copper oxychloride	400 g 100 l^{-1}	17.33 ± 0.33 f	8.78
Control	-	19 ± 0.33 g	0

[1] Values with different letters are significantly different (P = 0.05) according to Duncan's multiple range test.

Table 5. In vivo effects of the fungicides applied post-inoculation on Septoria blight.

Fungicides	Dose rate	Number of lesions plant^{-1}	Inhibition (%)
Tebuconazole	50 g 100 l^{-1}	0 ± 0.00 a[1]	100
Benomyl	40 g 100 l^{-1}	0.33 ± 0.33 a	98.26
Azoxystrobin	40 ml 100 l^{-1}	0.66 ± 0.33 a	96.52
Kresoxim-methyl	40 ml 100 l^{-1}	0.70 ± 0.33 a	96.31
Trifloxystrobin	15 g 100 l^{-1}	4.66 ± 0.33 c	75.47
Thiram	300 g 100 l^{-1}	10.33 ± 0.66 d	45.63
Captan	300 g 100 l^{-1}	10.66 ± 0.66 d	43.89
Mancozeb	250 g 100 l^{-1}	13.66 ± 0.66 e	28.1
Maneb	250 g 100 l^{-1}	13.66 ± 0.66 e	28.1
Propineb	200 g 100 l^{-1}	14.33 ± 0.33 e	24.57
Copper oxychloride	400 g 100 l^{-1}	18.33 ± 0.33 f	3.52
Control	-	19 ± 0.33 g	0

[1] Values with different letters are significantly different (P = 0.05) according to Duncan's multiple range test.

mancozeb, maneb, and propineb were not effective in vivo when applied post-inoculation. Azoxystrobin, tebuconazole, benomyl, kresoxim-methyl, and trifloxystrobin were effective in all treatments. Strobilurins (azoxystrobin, kresoxim-methyl, and trifloxystrobin) have been recommended for controlling Septoria blight of parsley in the USA and UK. Among these, azoxystrobin (Quadris) is the only registered fungicide against Septoria blight of parsley in USA, while azoxystrobin (Quadris) and trifloxystrobin (Cityplast Flint) have been recommended for controlling Septoria, Cercospora, and Alternaria blights, and powdery mildew of parsley in the USA (Hochmuth et al., 1999; Pornezy et al., 2007). Strobilurins can be recommended for controlling Septoria blight of parsley in Turkey; however, benomyl, in addition to its effectiveness, has the potential for residual risk to parsley leaves, which must be seriously considered. Tebuconazole was statistically in the same group as azoxystrobin and kresoxim-methyl in in vivo tests due to its 100% inhibition rate; however, the residual risks and half life of this chemical on parsley leaves have yet to be reported. As such, tebuconazole should only be used for seed treatment and not for spay application on leaves. On the other hand, mancozeb, maneb, captan, and thiram can be valuable protective agents when used before infection, because their demonstrated inhibitive effects in vivo were very strong when used before the infection of parsley plants by *S. petroselini*. It is concluded that these fungicides might be promising if they are used on young parsley plants in the early stages of infection. Copper oxychloride is the most extensively used fungicide for the control of Septoria blight of parsley in southern Turkey. Unexpectedly, copper oxychloride was not effective in any of our experiments. Prior to sowing, fungicide treatment of infected parsley seeds with tebuconazole or benomyl would be the most useful chemical control for Septoria blight, ecologically friendly, and both time and cost effective.

The results of our study show that Septoria blight of parsley can be controlled by using some fungicides as seed and foliar treatments. The fungicides identified as being effective against *S. petroselini* in this investigation may have the potential to protect parsley from the damage of this pathogen. The strategies and methods of fungicide application need to be determined experimentally on parsley of all ages. Moreover, the potential fungicide strategies identified by this study should be investigated in greater detail as the need for effective treatments for Septoria blight of parsley worldwide is critical. To the best of our knowledge, this is the first study on the chemical control of Septoria blight of parsley in Turkey. This is the first step for a chemical control strategy. New fungicides must be added and effective fungicides should be investigated in field conditions. Following effectiveness tests, the most effective spray timing and plant age for application of fungicides should be determined by future experiments.

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